Lecture 6 &7

Physiology of microorganisms •

Fourth year students •

Botany /Chemistry •

Site-specific inhibitors target individual sites within the fungal cell.

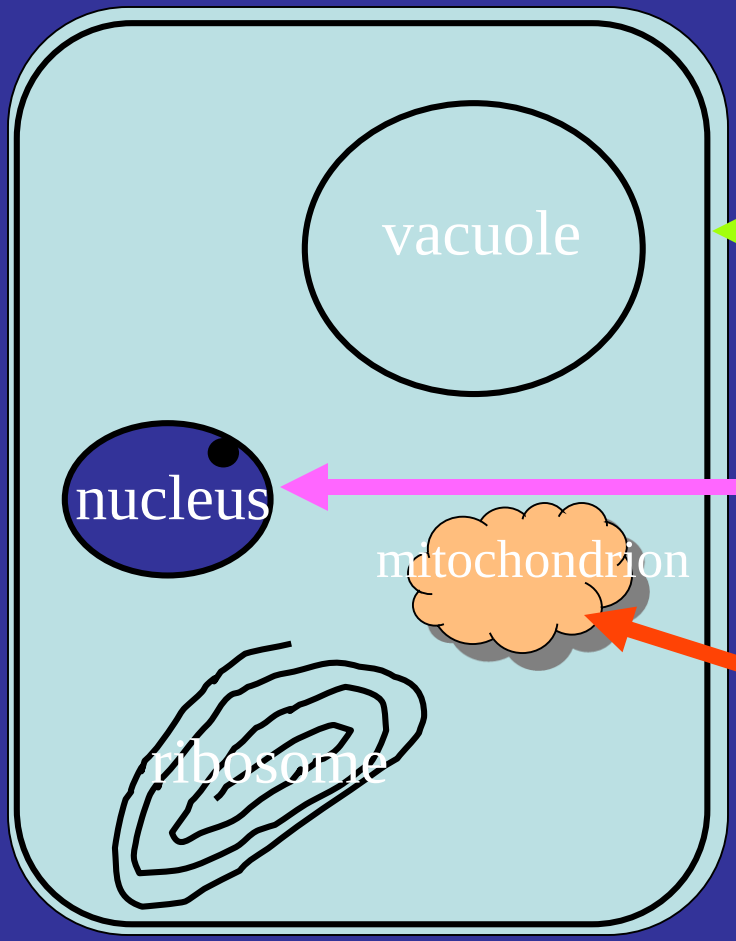
1-The **DMI** (DeMethylation Inhibitors) or SBI (Sterol Biosynthesis inhibiting) fungicides which include the triazoles and imidazoles..

2-benzimidazoles – inhibit DNA synthesis

3-carboxamides – inhibit respiration

4-Tricyclazole inhibit melanin biosynthesis.

Site-specific inhibitors target individual sites within the fungal cell.



Examples

DMI fungicides – inhibit sterol synthesis in membranes

benzimidazoles – inhibit DNA Synthesis

carboxamides – inhibit respiration

- 1928 – Fleming discovered penicillin, produced by *Penicillium*.
- 1940 – Howard Florey and Ernst Chain performed first clinical trials of penicillin.

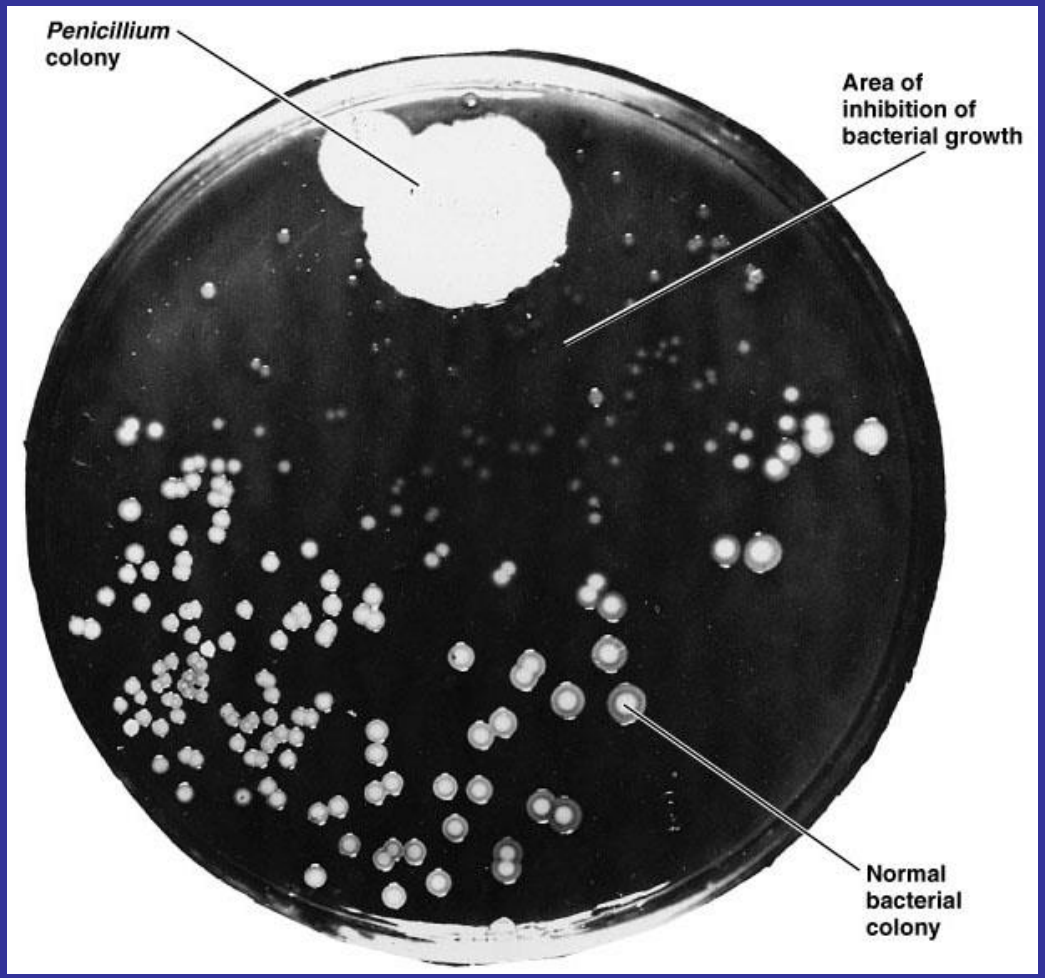
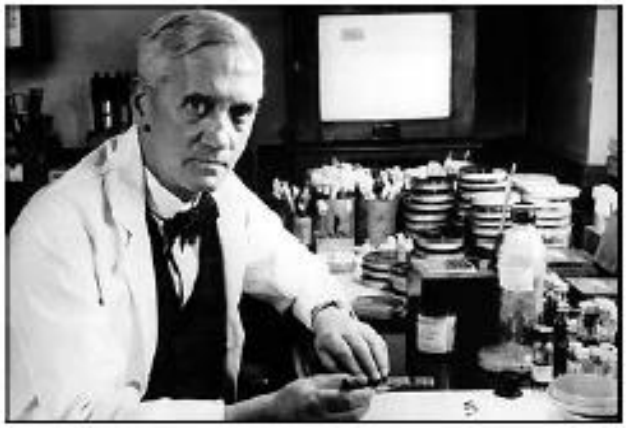


Figure 20.1

Penicillin

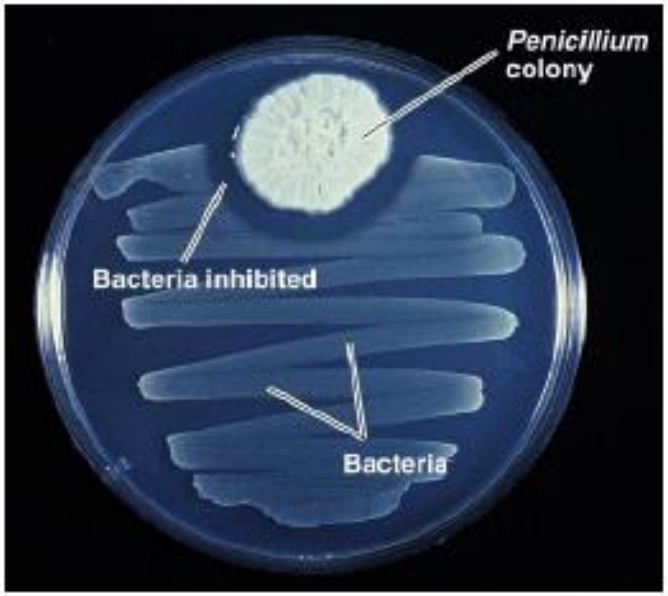
Antibacterial activity in *Penicillin notatum* by Alexander Fleming in 1928



Research continued by Howard Florey and Ernst Chain

Mass production of penicillin in 1940s

Nobel Prize 1945



Inhibition of bacterial growth by a contaminating colony of *Penicillium notatum*

What are antibiotics?

- Drugs that prevent the growth of bacteria
- Attack prokaryotic cellular processes
- Do not affect eukaryotic cells
  - Do not harm human cells
  - Cannot be used for fungal or parasitic diseases
- Are not effective against viruses
- Characterised based on target specificity
  - Narrow or broad spectrum

Modes of action

- Disruption of cell membrane function
  - Polymyxins –
- Inhibition of protein synthesis
  - Chloramphenicol –
  - Erythromycin –
  - Tetracycline –
  - Streptomycin –

Inhibitors of cell wall Synthesis

Ampicillin

Cephalosporin

Bacitracin

Vancomycin

- Inhibition of nucleic acid synthesis
  - Rifamycin –
- Inhibitors of enzymatic function of primary metabolism
  - Competitive inhibition –
  - Noncompetitive inhibition –

How Do Bacteria Develop Resistance?

- Presence of antibiotics provides selection pressure for spontaneous mutants (1 in 10^6) with increased resistance
- High population density → efficient gene transfer
- Short generation time → rapid evolution

How Does it work?

- Inactivating enzymes
- Alter antibiotic target
- Pump antibiotics out of the cell

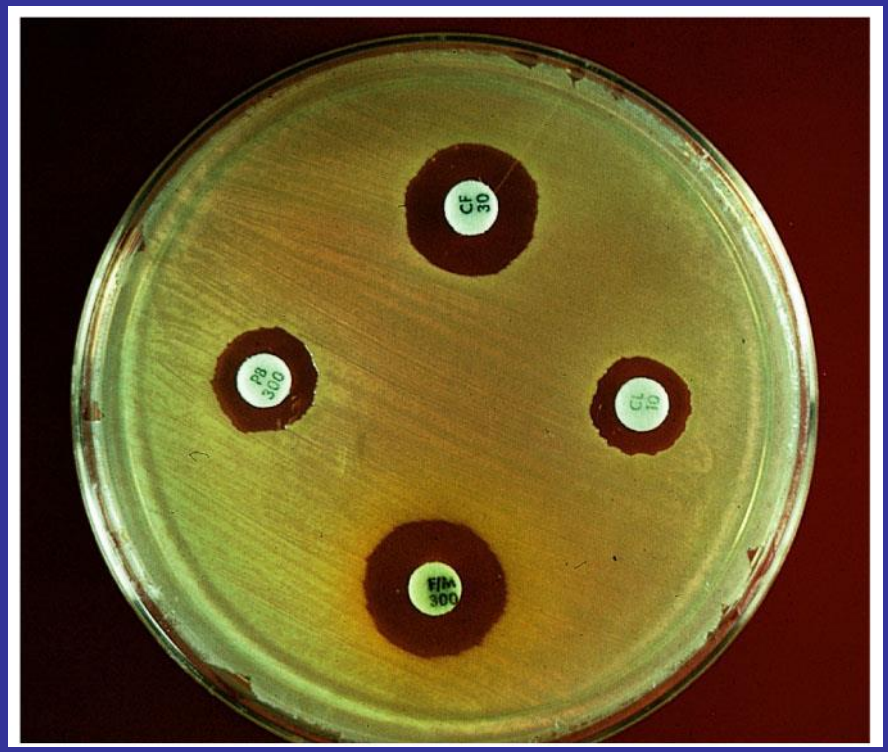
Antibiotic Resistance

- A variety of mutations can lead to antibiotic resistance.
- Mechanisms of antibiotic resistance

1. Enzymatic destruction of drug
2. Prevention of penetration of drug
3. Alteration of drug's target site
4. Rapid ejection of the drug

- Resistance genes are often on plasmids or transposons that can be transferred between bacteria.

- We humans will always have to find or create new antibiotics as microbes become resistant



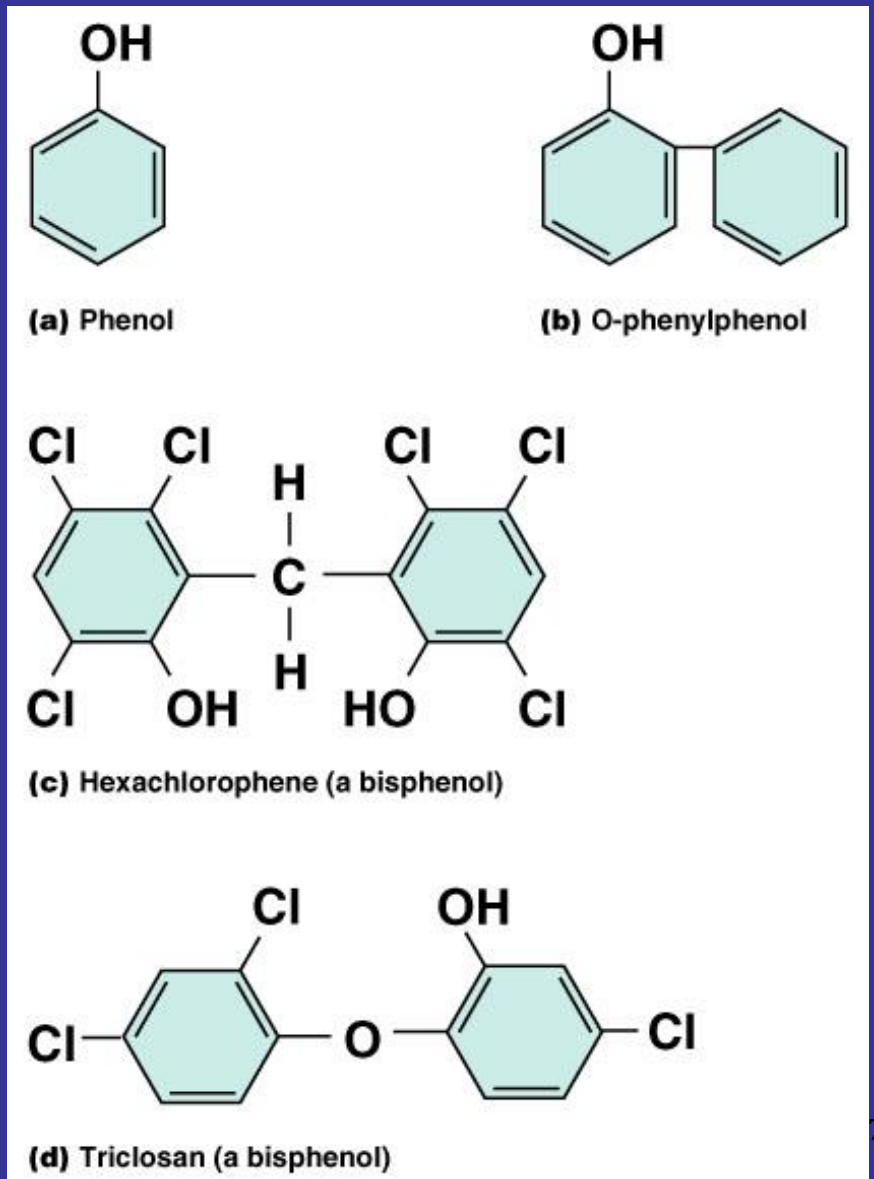
Attributes of an ideal antimicrobial agent

1- Solubility in body fluids
2- Selectively toxic
3- Toxicity not easily altered
4- Not allergenic
5- Stability in body
6- Resistance not easily acquired
7- Long shelf life
8- Reasonable cost

Types of Disinfectants

- Phenol
- Phenolics. Lysol
- Bisphenols. Hexachlorophene, Triclosan

Disrupt plasma – membranes



For example for phenol η is 6

Therefore if concentration halved

then contact time is increased

2^6 or 64 times

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard, and the corresponding rating system is called the "**Phenol coefficient**". The disinfectant to be tested is compared with phenol on a standard microbe (usually *Salmonella typhi* or *Staphylococcus aureus*). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

Phenol and other phenolics – The active ingredient in most bottles of "household disinfectant". It is also to found in some mouthwashes and in disinfectant soap and handwashes. Phenol is probably the oldest disinfectant (used by Lister) and was called carbolic acid in the early days of antiseptics. Phenol is rather corrosive to the skin and sometimes toxic to sensitive people, so the somewhat less corrosive substitute phenol.

- *o*-phenylphenol is often used as part of a disinfectant formula. Hexachlorophene is a phenolic which was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.

Common disinfectants

Chlorine – Used to disinfect swimming pools, and is added in small quantities to drinking water to reduce waterborne diseases.

Chloramine – Used in drinking water treatment instead of chlorine because it produces less disinfection byproducts.

Chlorine dioxide – Used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts.

- Sodium chlorite, sodium chlorate, and potassium chlorate have little disinfection effect but are used as precursors for generating chlorine dioxide.

- Ozone – a gas that can be added to water for sanitation.

Dettol –**Chloroxylenol**, Used to disinfect surfaces at home. It kills the majority of bacteria. It is one of the few disinfectants useful against viruses.

Alcohol – Usually ethanol or isopropanol – Wiped over benches and skin and allowed to evaporate for quick disinfection. Alcohols are more effective combined with water, 70% alcohol is more active than 95% alcohol. Alcohol is not effective against bacterial spores.

Hydrogen peroxide – Used in hospitals to disinfect surfaces. It is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.

When hydrogen peroxide comes into contact with the catalase enzyme in cells it is broken down into water and oxygen. It is the oxygen that kills bacteria. However, as recent studies have show hydrogen peroxide to be toxic to growing cells as well as bacteria, its use as an antiseptic is no longer recommended.

Iodine – Usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. Iodine is rapidly neutralised by the presence of organic material, so surfaces must be cleaned prior to disinfection. Although no longer recommended because it increases scar tissue formation and increases healing time, tincture of iodine has also been used as an antiseptic for skin cuts and scrapes.

Potassium permanganate – Formula $KMnO_4$. Red Crystalline powder. Colours everything it touches. Used to disinfect aquariums. It is also used widely in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of $KMnO_4$/water solution is kept near the pool ladder.

Participants are required to step in the basin and then go into the pool. It is also used widely to disinfect community water ponds and wells in Tropical countires. It is also used to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution. $KMnO_4$ is a very useful Disinfectant.

Quaternary ammonium salts (quats) such as benzalkonium chloride are a large group of related compounds. Some have been used as a low level disinfectant. They are effective against bacteria, but not against spores or viruses. Nor are they effective against some species of Pseudomonas bacteria. Quats are biocides which also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

Hypochlorites – Sodium hypochlorite, often in the form of common household bleach, is used in the home to disinfect drains, and toilets. A dilute form is used under the brand name Milton to disinfect baby bottles. Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorite gives off free chlorine and it is the chlorine that is the true disinfectant. Hypobromite solutions are also sometimes used.

Objectives, by the end of this lecture you should be able to:

- **Define the growth**
- **Methods of measuring the growth of microorganisms their advantages and disadvantages**
- **Detection the pollution with microorganisms**

The growth

The growth of a cell is culmination of all of the physiological activities of the cell.

It is a complex process involving:

1- Entrance of basic nutrients into the cell.

2- Conversion of these compounds into energy and vital cell constituents.

3- Replication of the chromosomes.

4- Increase in size & mass of the cell.

5- Division of cell into 2 daughter cells each containing a copy of genome and other vital components

Microbial Growth

Microbial growth = increase in number of cells, not cell size

Measurement of Growth

- Change in cell number
  - Microscopic counts
  - Viable plate counts
- Change in turbidity or light scattering
  - Spectrophotometer
- Change in the amount of a cell component
  - Dry weight
  - DNA/RNA
  - Protein

Direct Measurements of Microbial Growth

Breed method:

1-Spread a known volume of the suspension (0.01 ml) over a $1cm^2$ of slide

2-Dry, fix & stain

3-Count the number in many microscopic field. (calculate the mean)

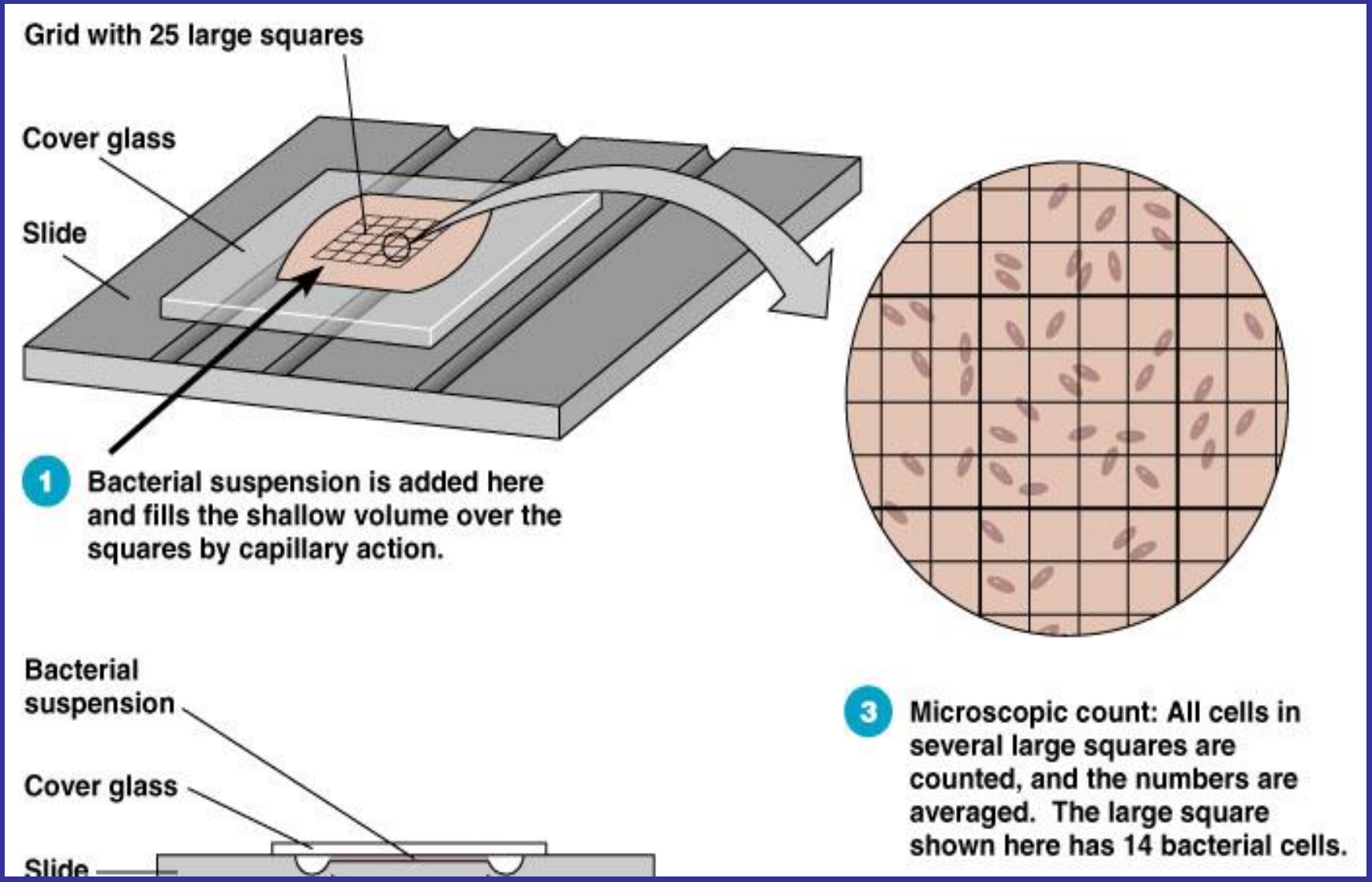
4-Calculate the area of microscopic field πr^2

5- The number of fields = 1/field area

Number of cells in 1 ml =mean number of cells in one field x number of fields x 100

- Diameter of fields=0.16
- Radius=0.08
- Area=3.14x0.08x0.08=0.02mm^2 =0.0002cm^2
- Fields number in 1 $cm^2$1/0.0002=5000
- 1cell represents 500000/ml

Direct Measurements of Microbial Growth



Direct Measurements of Microbial Growth

- Direct Microscopic Count

$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17{,}500{,}000$$

- Advantages:
- simple
- Quick method
- Morphology of cells is observed
- Disadvantages:
- Dead cells are not distinguished from viable cells.
- Fatigue for eyes
- It is difficult in condensed suspension

Direct Measurements of Microbial Growth

1- Plate Counts: Perform serial dilutions of a sample

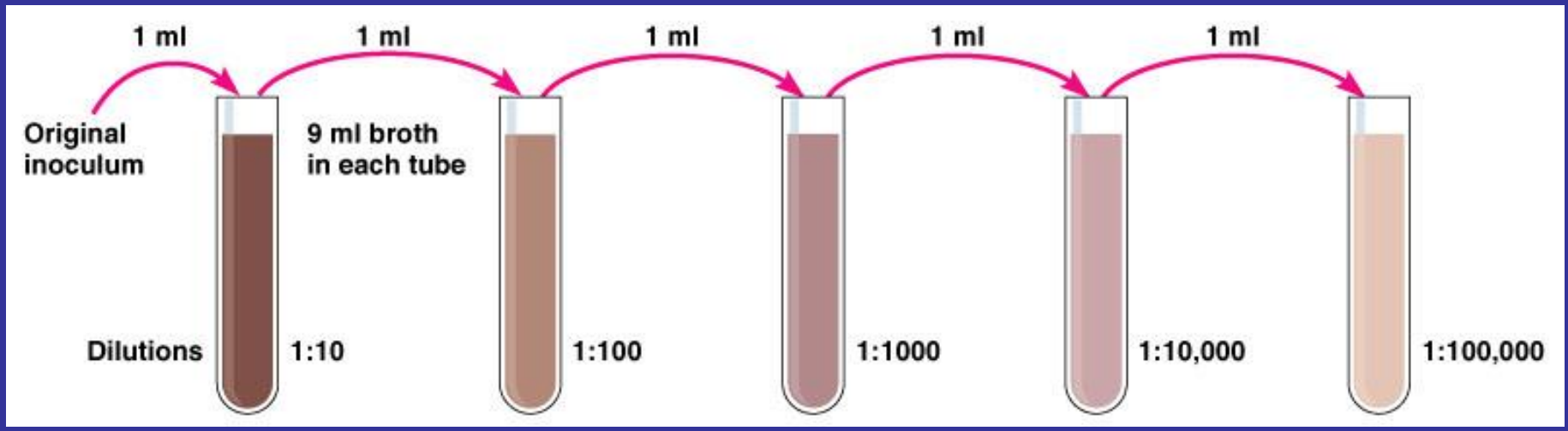


Plate Count

Inoculate Petri plates from serial dilutions

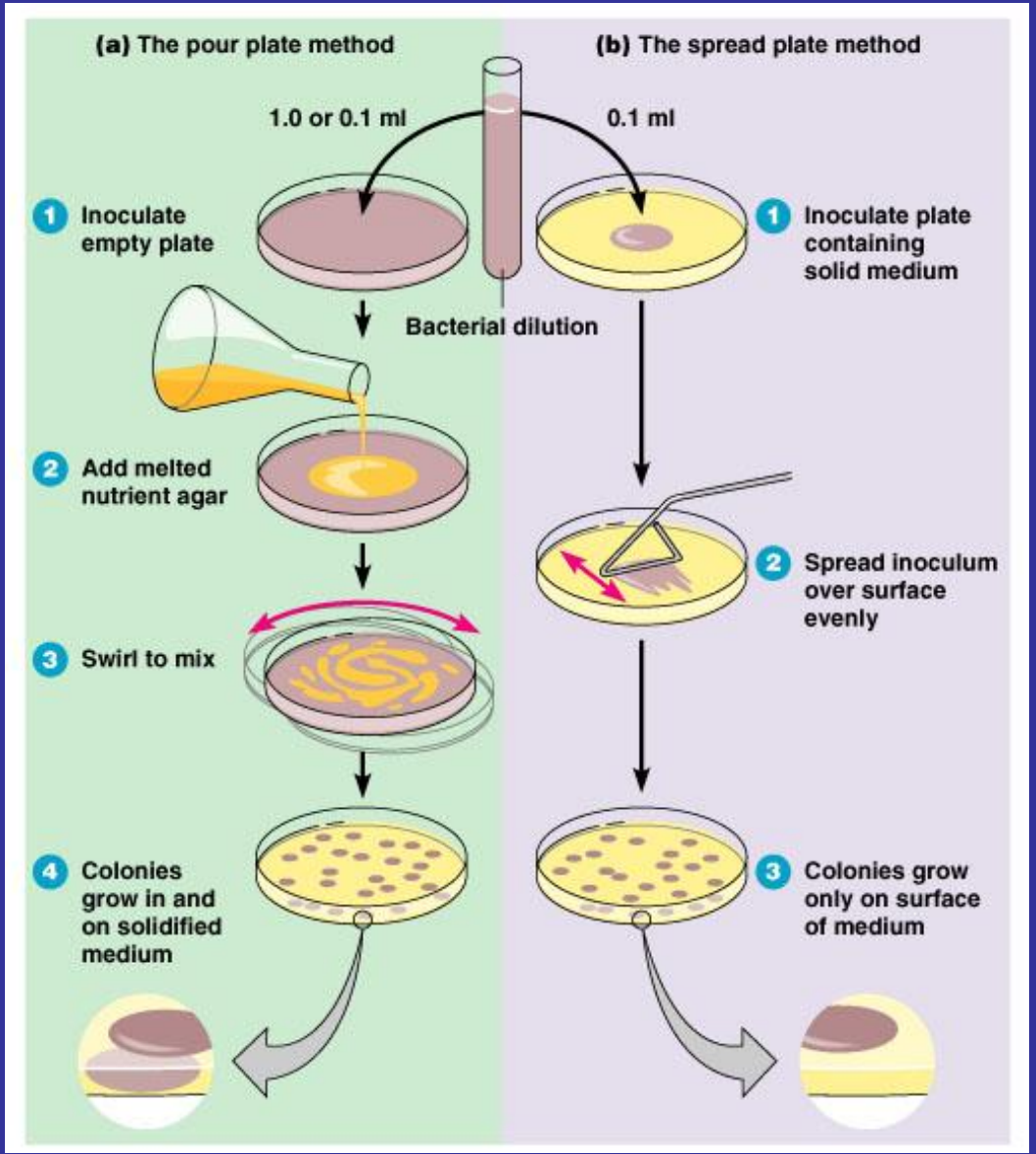
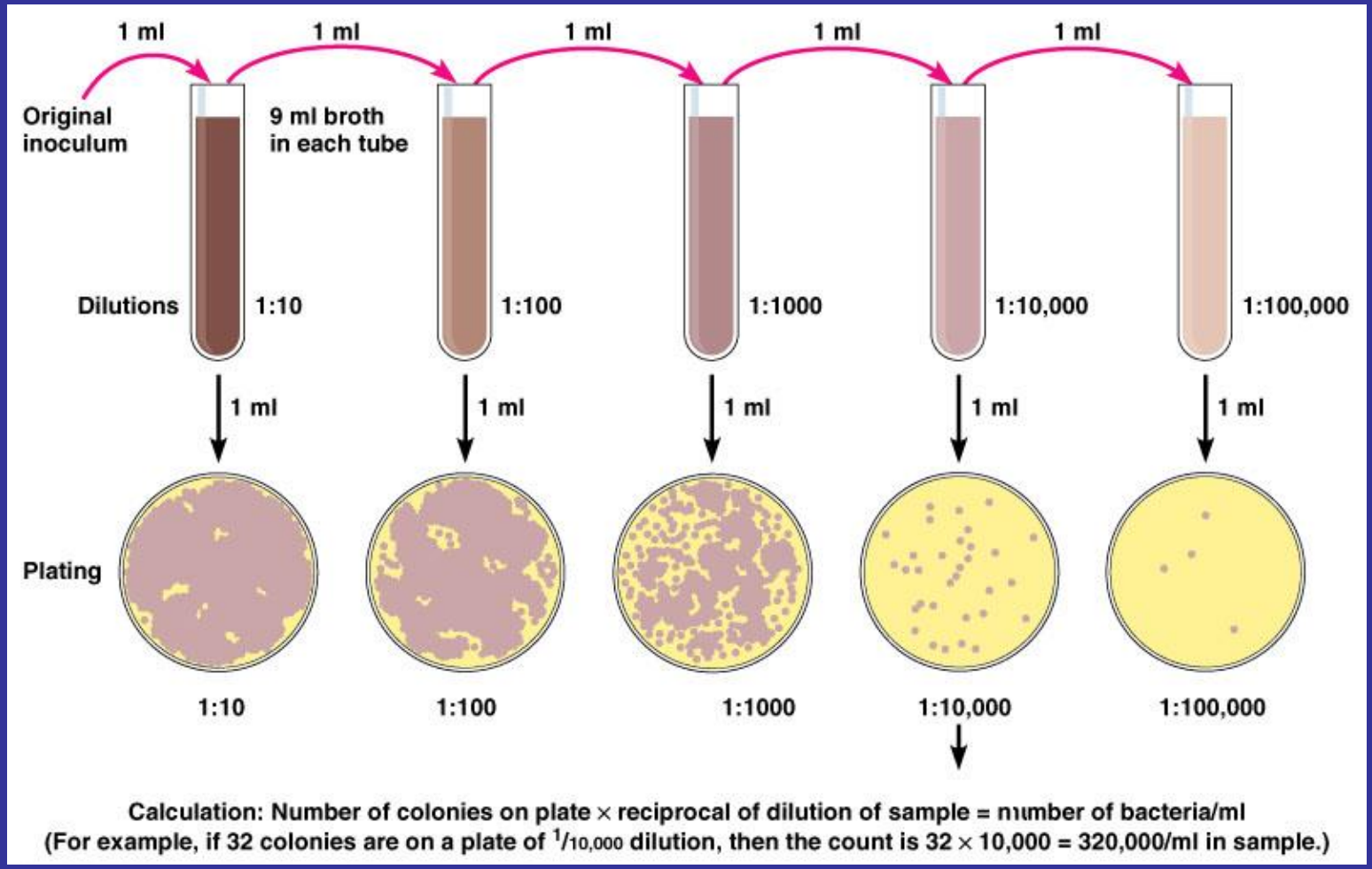
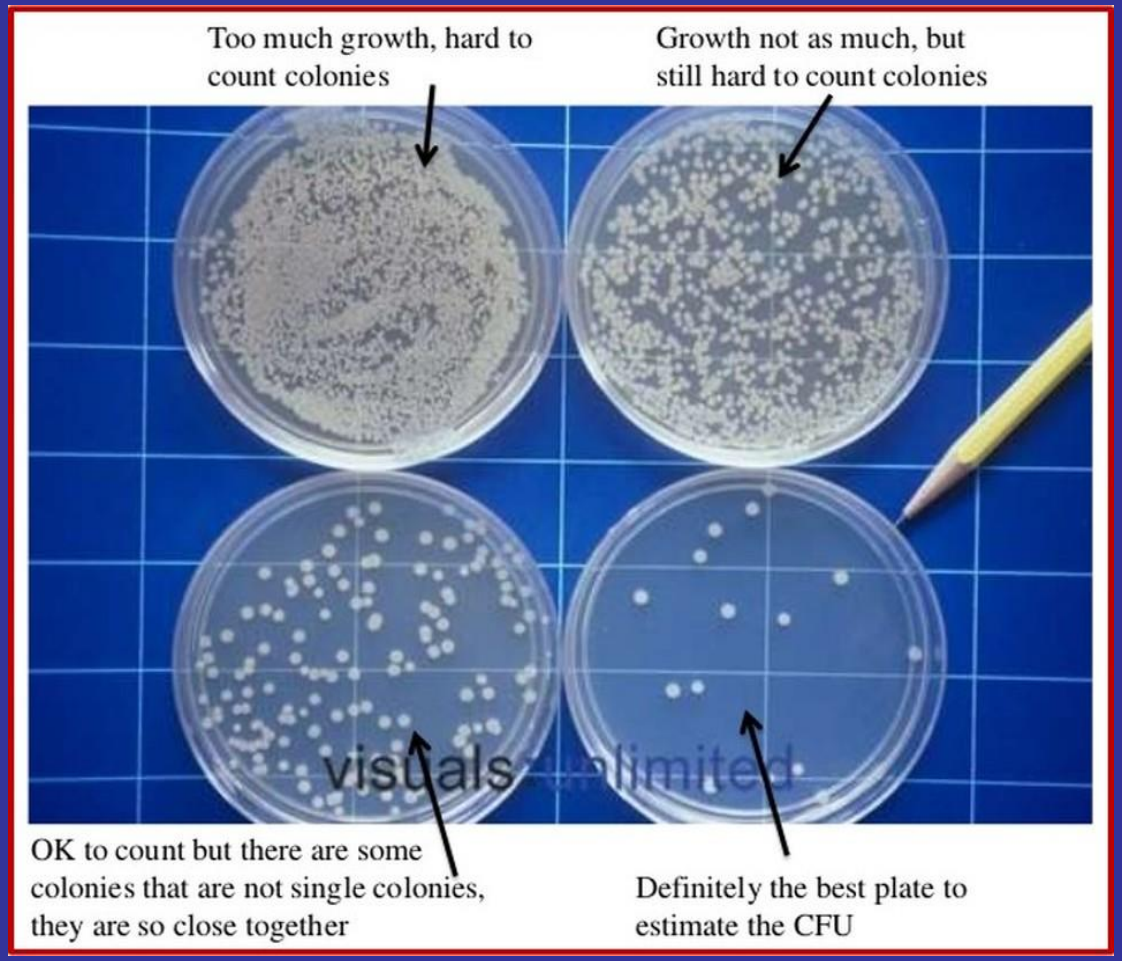


Plate Count

After incubation, count colonies on plates that have 25-250 colonies (CFUs)




Too much growth, hard to count colonies
Growth not as much, but still hard to count colonies
visuals unlimited
OK to count but there are some colonies that are not single colonies, they are so close together
Definitely the best plate to estimate the CFU

Viable count

This is typically carried out by **CFU (colony forming units)**

assay:

1- carry out dilution series

2- plate known volumes on plates

3- count only plates with 30-300 colonies (best statistical accuracy)

4- extrapolate to undiluted cell conc.

- CFU may or may not be same as number of cells --
- Method is accurate, but requires time for incubation.
- Two ways to carry out viable count:

Spread plate: bacteria are spread on the surface of agar using some sterile spreading device.

Advantages: if properly carried out, all colonies should be easily counted.

Disadvantages:

♦takes some time♦ not always reliable in inexperienced hands ♦ cells with low tolerance to oxygen won't grow.

If "spreaders" are present, may overgrow plate surface.

Pour plate: bacteria are mixed with melted agar and cooled; colonies grow throughout the agar.

Advantages: colonies well separated.

Can allow growth of organisms with lower oxygen tolerance in agar.

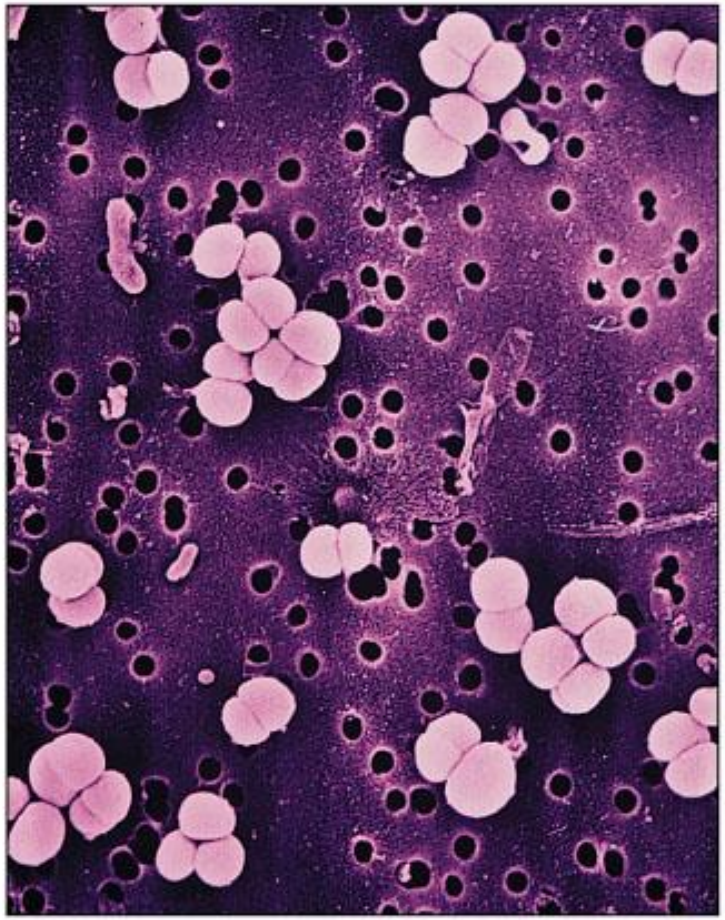
Disadvantages: colonies variable size, harder to see similarity in colony

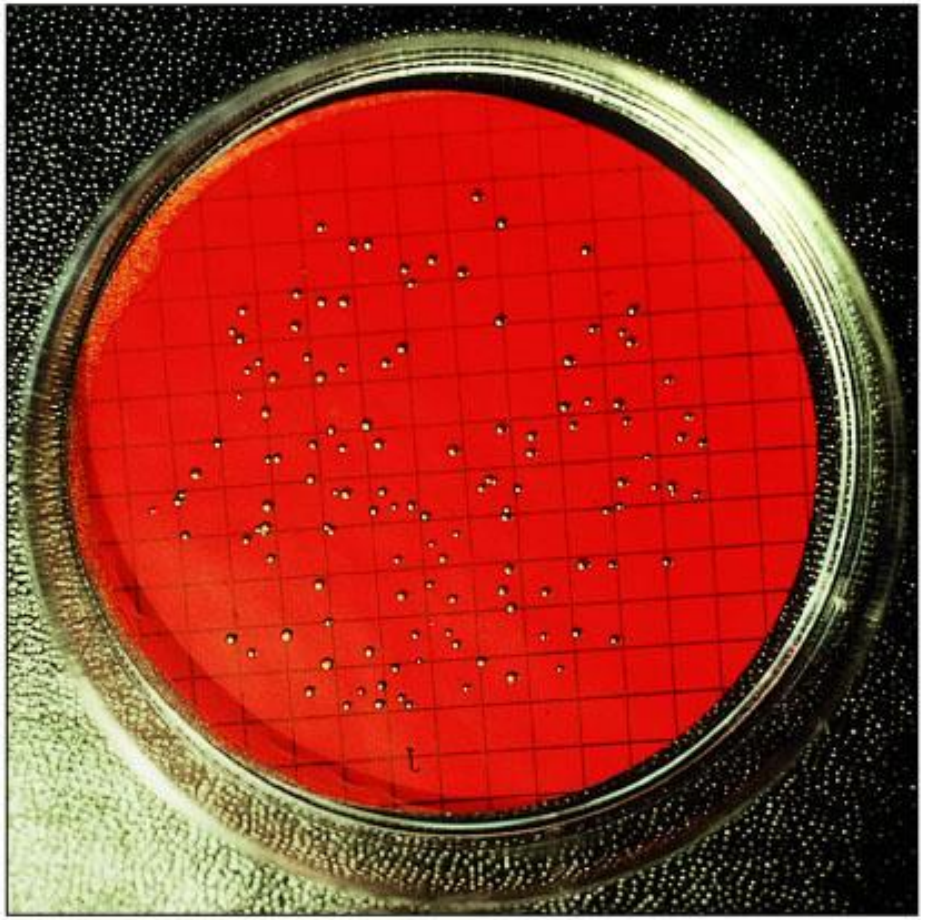
morphology between those on surface and in agar. Counting may be more

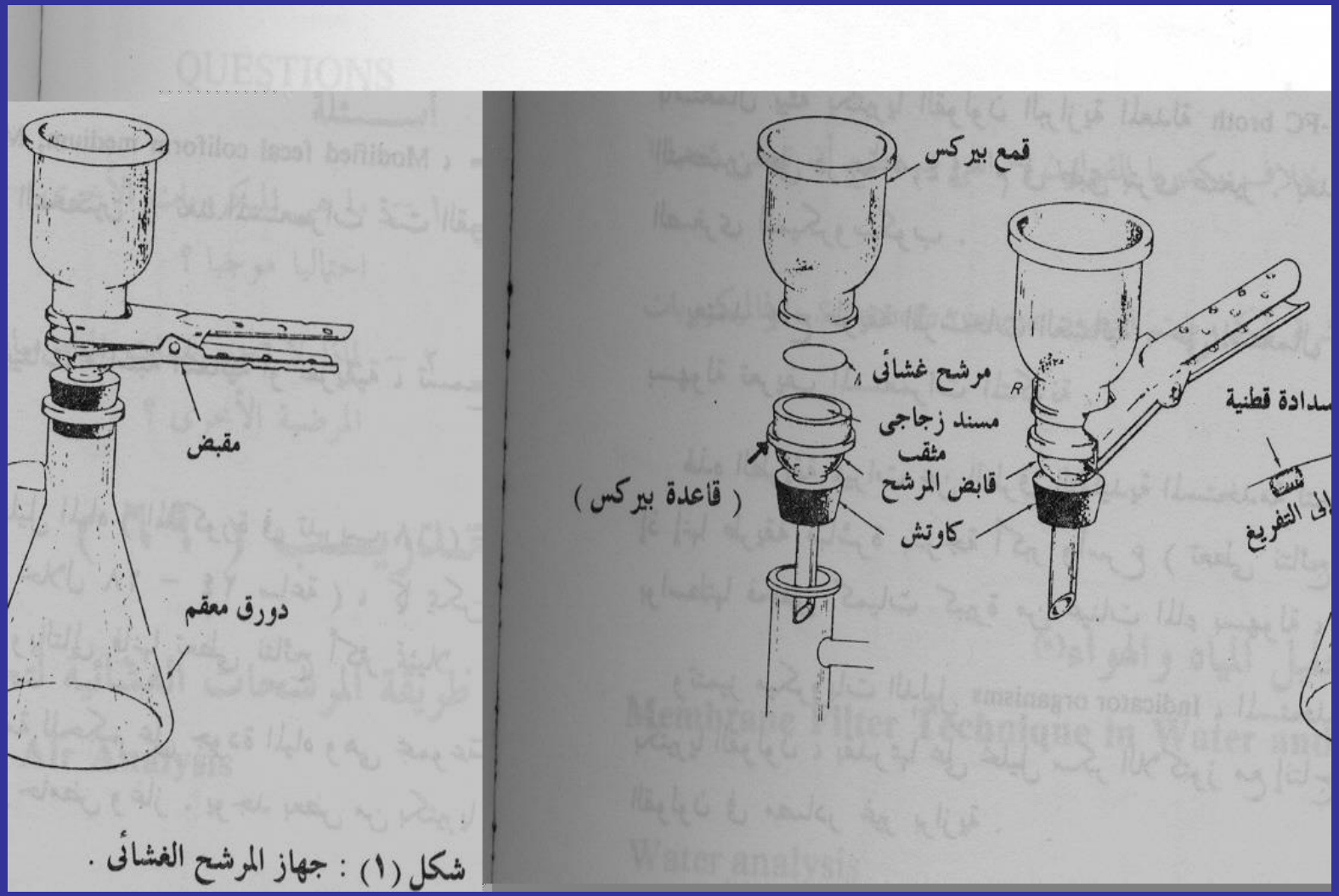
difficult. Heat may kill some cells before agar cools and gels.

Direct Measurements of Microbial Growth

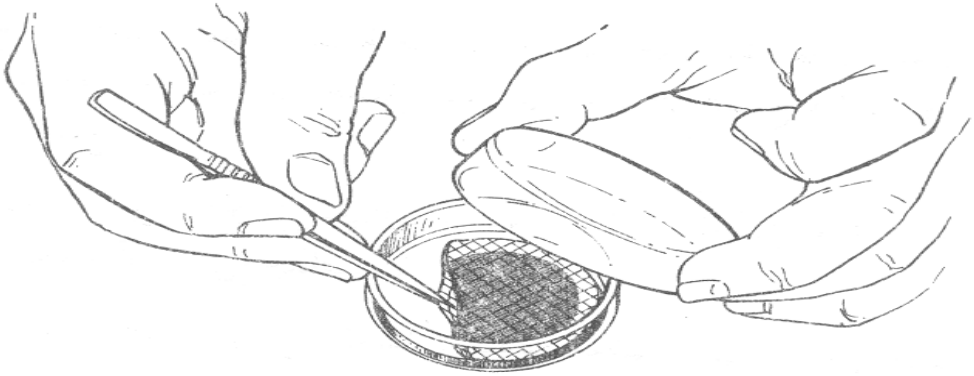
2- Filtration



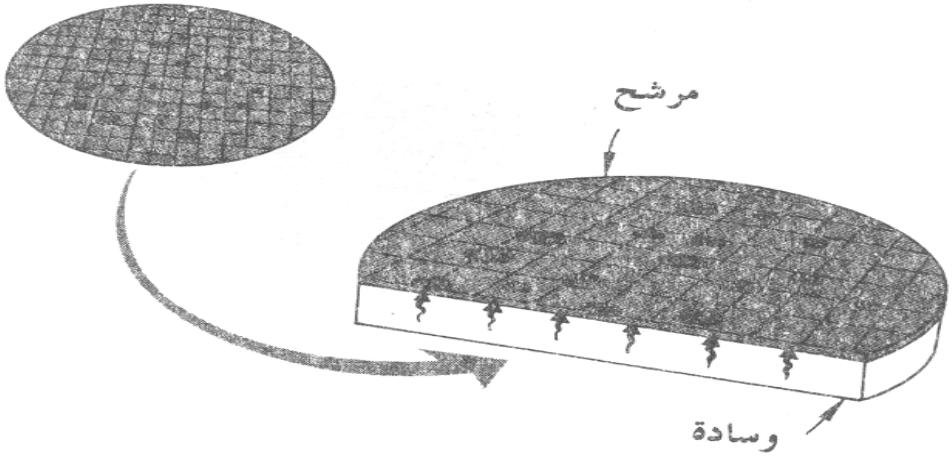




شكل (١) : جهاز المرشح الغشائى .



شكل (٣) : وضع المرشح على الوسادة فى طبق بترى .



شكل (٤) : انتشار سائل المرق من الوسادة إلى المرشح لتدعيم النمو .

Membrane filter technique:

1-Filter a known volume of solution through membrane filter (Bacteria - retaining) with pores diameter of 0.45Mm.

2-Bacteria are retained on its surface.

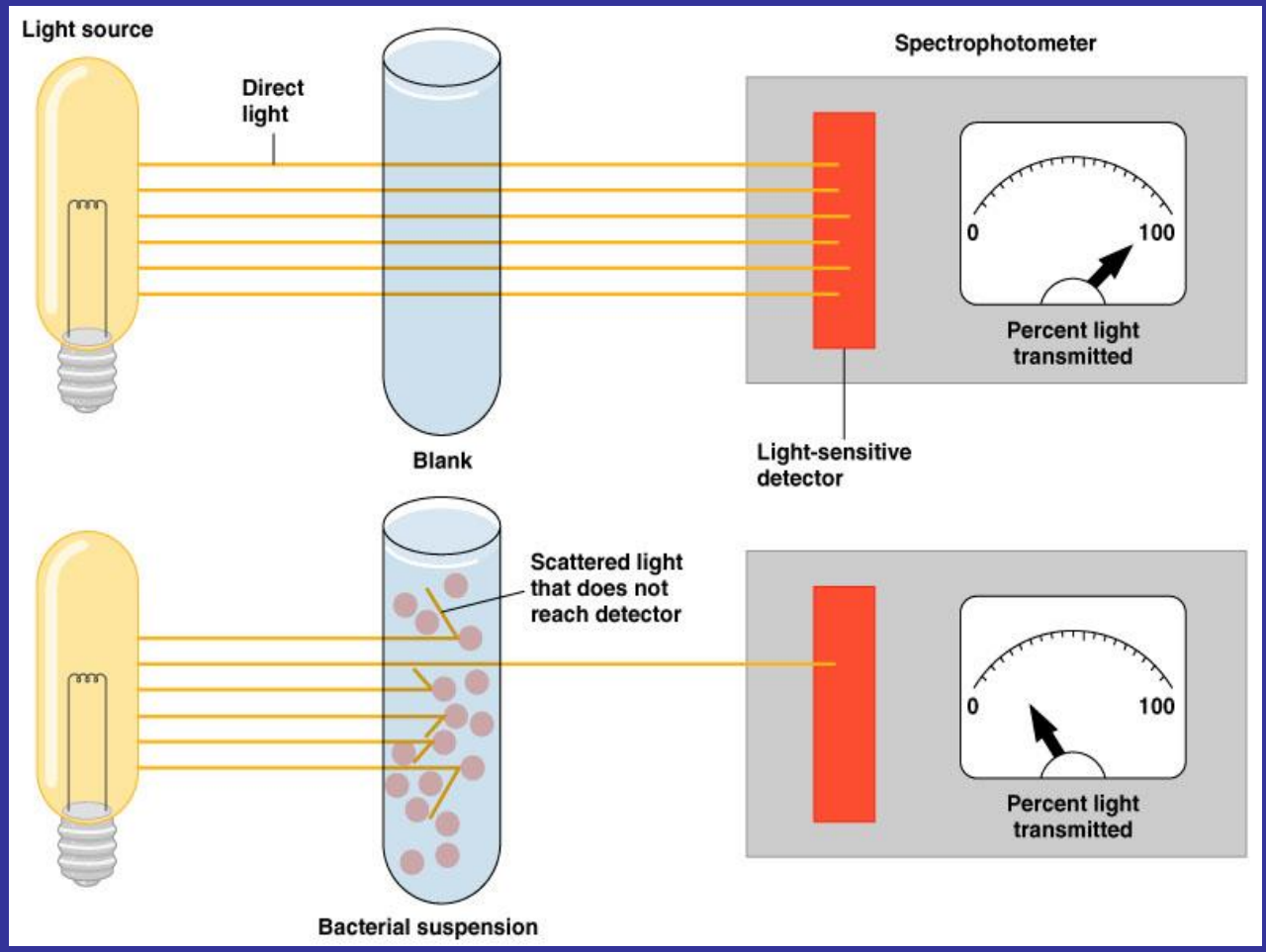
3-Put the membrane on the surface of thin absorbent pad that is saturated with growth suitable medium.

4-After incubation in Petri dish the colonies are counted under microscope at low power.

Estimating Bacterial Numbers by Indirect Methods

Turbidity

Often, can estimate cell numbers accurately by measuring visible turbidity.
Light scattered is proportional to number of cells.
This **only** works above cell densities of 107 in pure cultures.
With less than 107 cells/ml, cannot detect bacteria.



Optical technique:

The bacterial culture is considered as colloidal suspension prevents and reflects light, so the absorbed or reflected light is proportional to cells concentration.

Turbidimetre measures absorbed light.

spectrophotometer is used to measure the reflected or transmitted light.

Turbidity is expressed as absorbance.

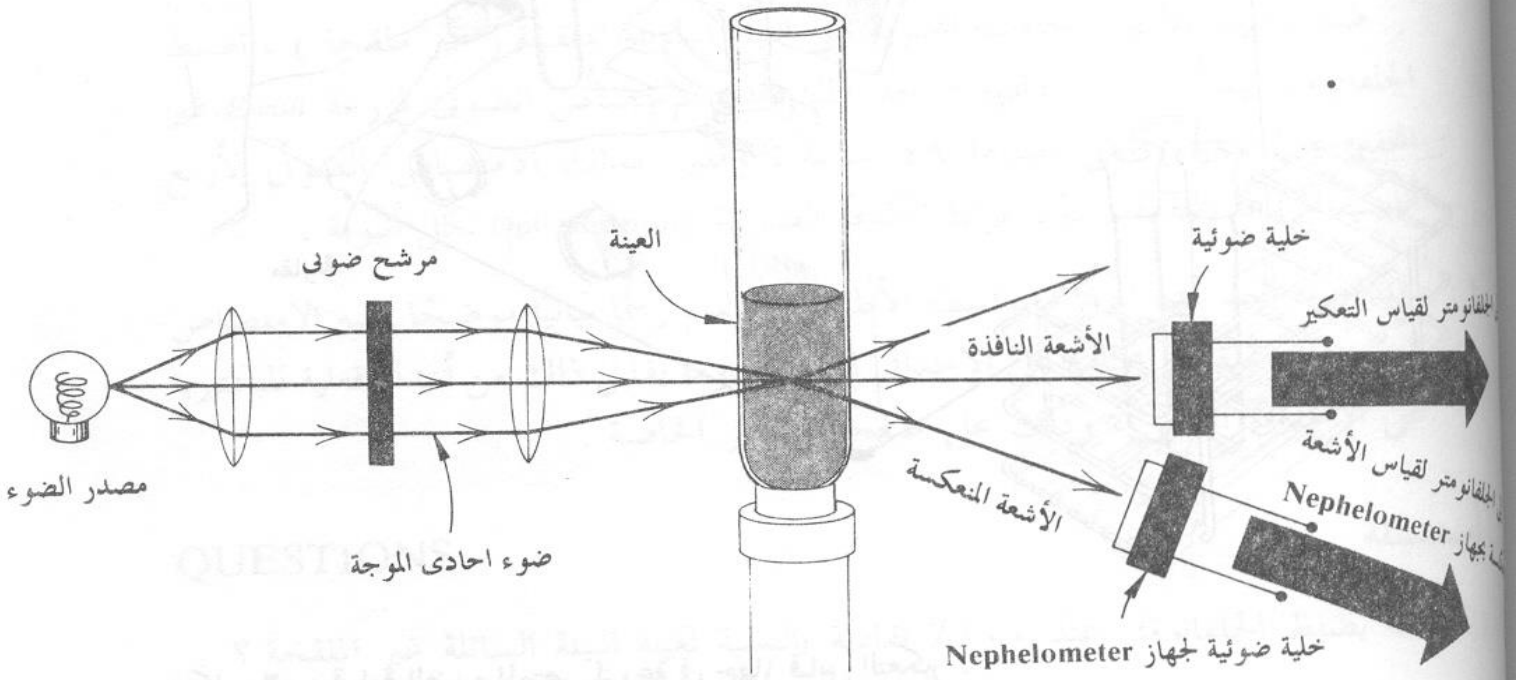
Absorbance method

Use a spectrophotometer to accurately measure

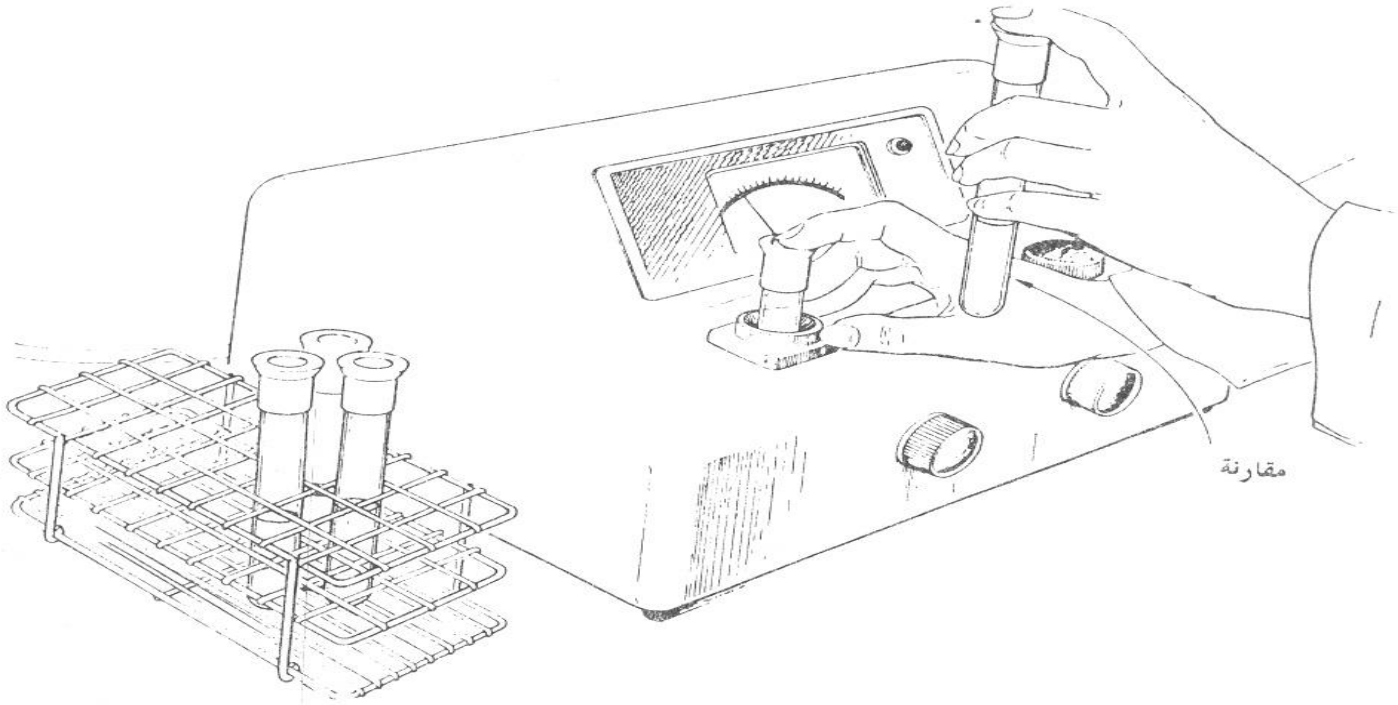
absorbance, usually at wavelengths around 400-600 nm.

Accurate measure of cells when concentration not too high.

Easy and quick to measure (can sample in less than a minute)



شكل (١) : رسم تخطيطى لجهاز قياس الألوان الضوئى الذى يمكن أن يستعمل كجهاز لقياس التعكير أو كجهاز لقياس الأشعة المنعكسة .



شكل (٢) : قراءة الضوء الممتص لمزرعة فى جهاز قياس التعكير .

Measuring the volume

- Special graduated centrifuge tubes are used.
- A known volume of the culture is put into the tube and after centrifugation the volume of deposited cells is measured.

Dry weight

- The cells are collected dried and weighed in crucible.

Growth Measurement of Filamentous fungi

Dry weight:

Weighing the dried fungus is the most widely used and often the most convenient method of measuring growth. Tissue is placed in a tarred pan, heated for 24 hr at 80^{o}C, allowed to cool, and then weighed.

Advantages:

Accurate (not neglected growth density)- Metabolites can be measured

Limitations of this method are

that bulk quantities of tissue are required,

and it is mostly c.w materials that is measured.

In addition when solid media are used, it is often a tedious, if not impossible, process to separate the tissue from the medium.

Obviously, continuous change in the growth of a specimen cannot be observed.

Linear extension

This method involves assessment of the linear increase of either individual hyphae or colony diameter per unit time.

Advantages

Linear extension is nondestructive measure of growth, and thus continued observation can be made.

It is the most rapid method when solid media are used.

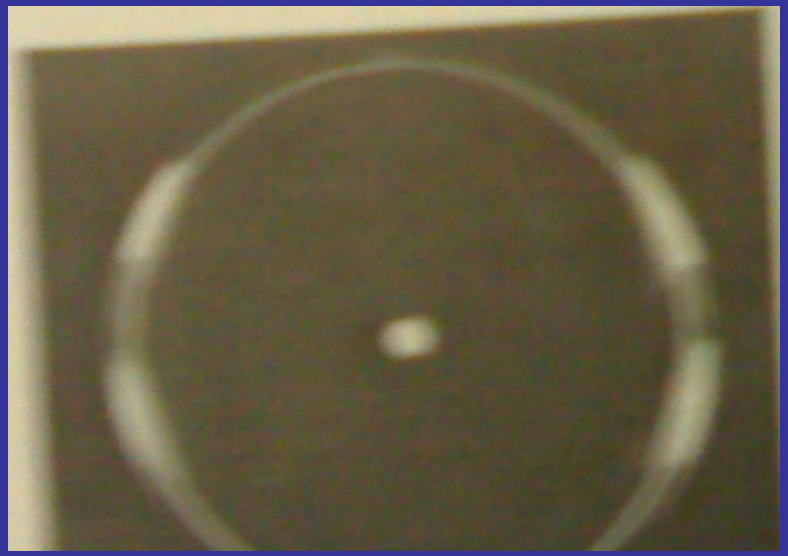
Easy to transport.

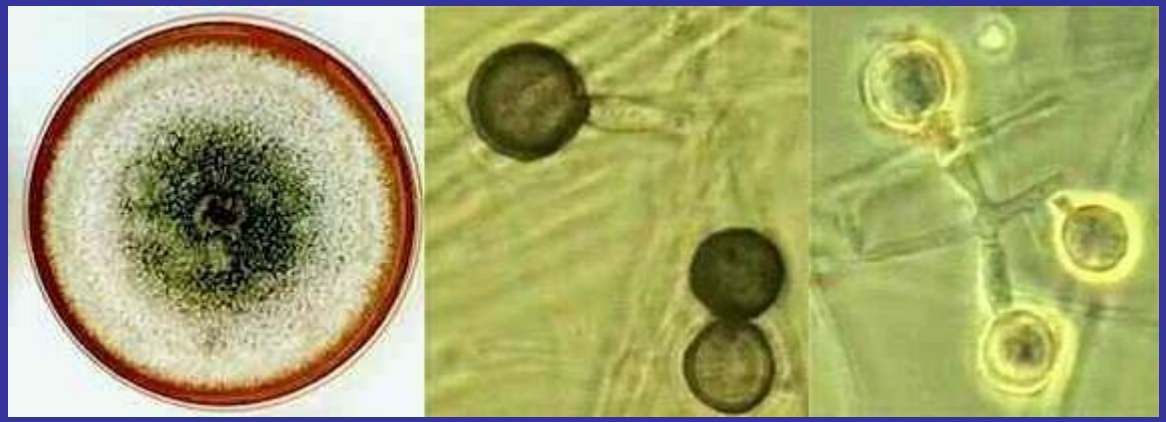
Useful to study morphology

Disadvantages

It neglects the vertical growth of hyphae.

Metabolites cannot be measured.





Cell component:

- The changes in concentration of certain cellular constituents such as chitin and glucosamine have been used as a measure of fungal growth.
- Acid hydrolysis releases glucosamine which can be separated chromatographically, and the amount recovered correlates well with fungal dry weight.
- Sterols such as ergosterols and cholesterol are also good indicators of fungal growth.

Metabolism

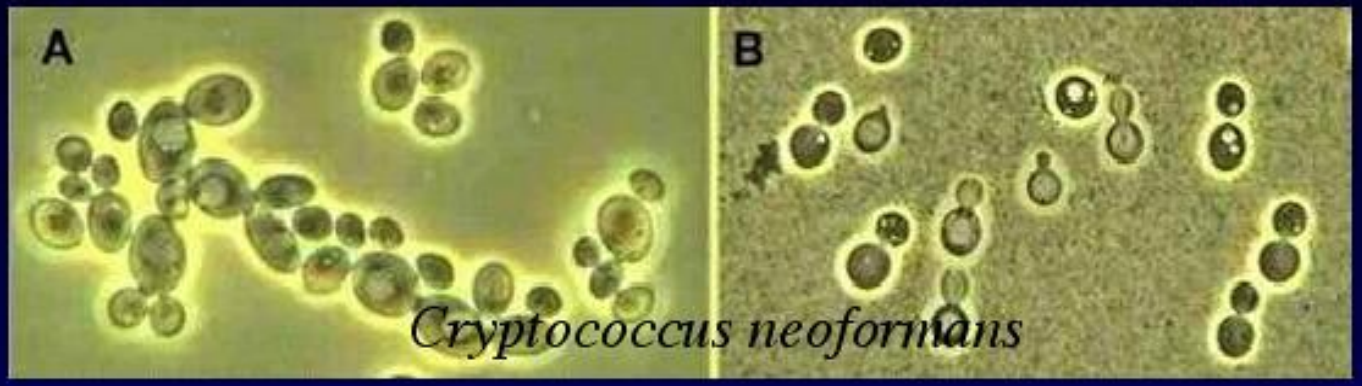
- Estimates of metabolic activity are often used to measure fungal growth.
- CO2 production can be used to determine the growth kinetics.
- C^{14}-glucose incorporation into cell constituents can be used for measuring growth.

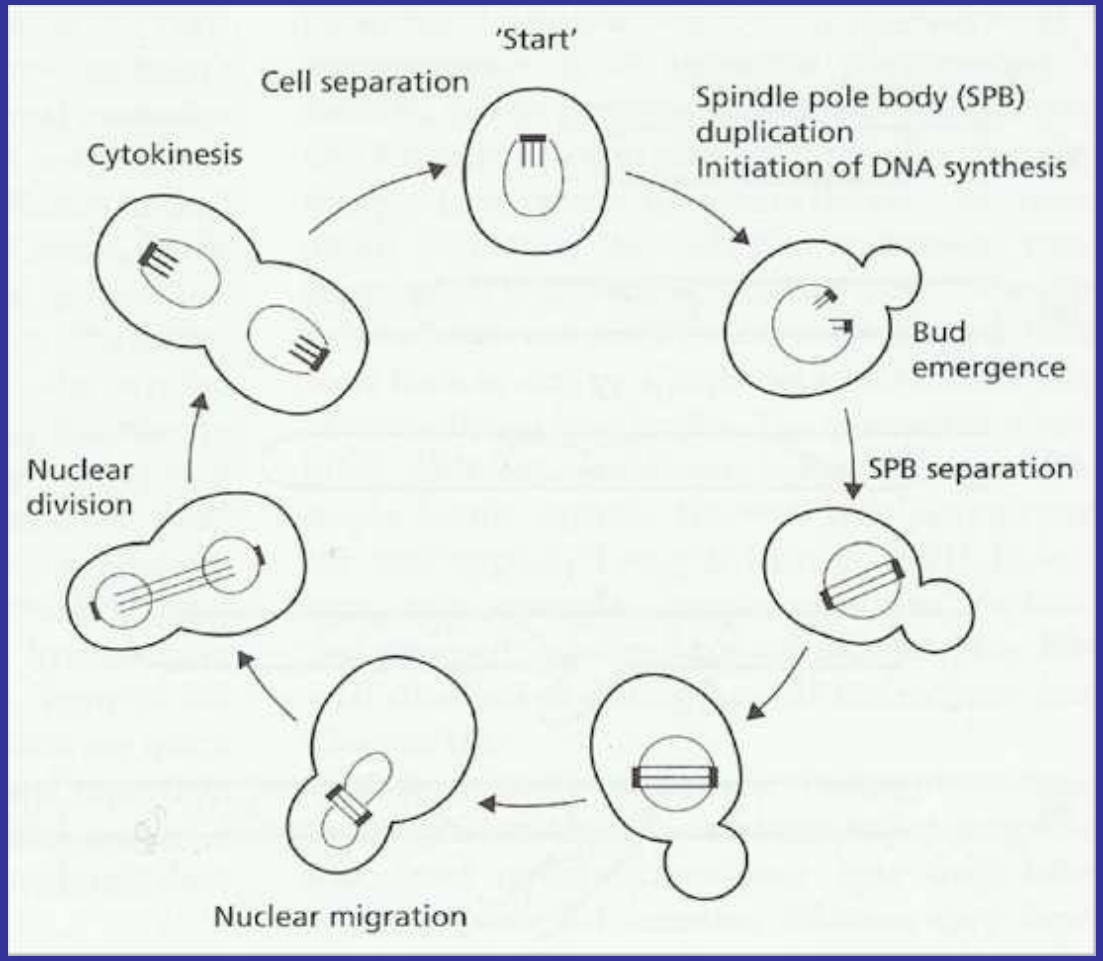
Types of growth in fungi

1- Yeast type growth:

Recorded in yeast and is characterized by budding. The growing protoplast leads to the formation of a bud, which after increase in size separates to form a new cell.

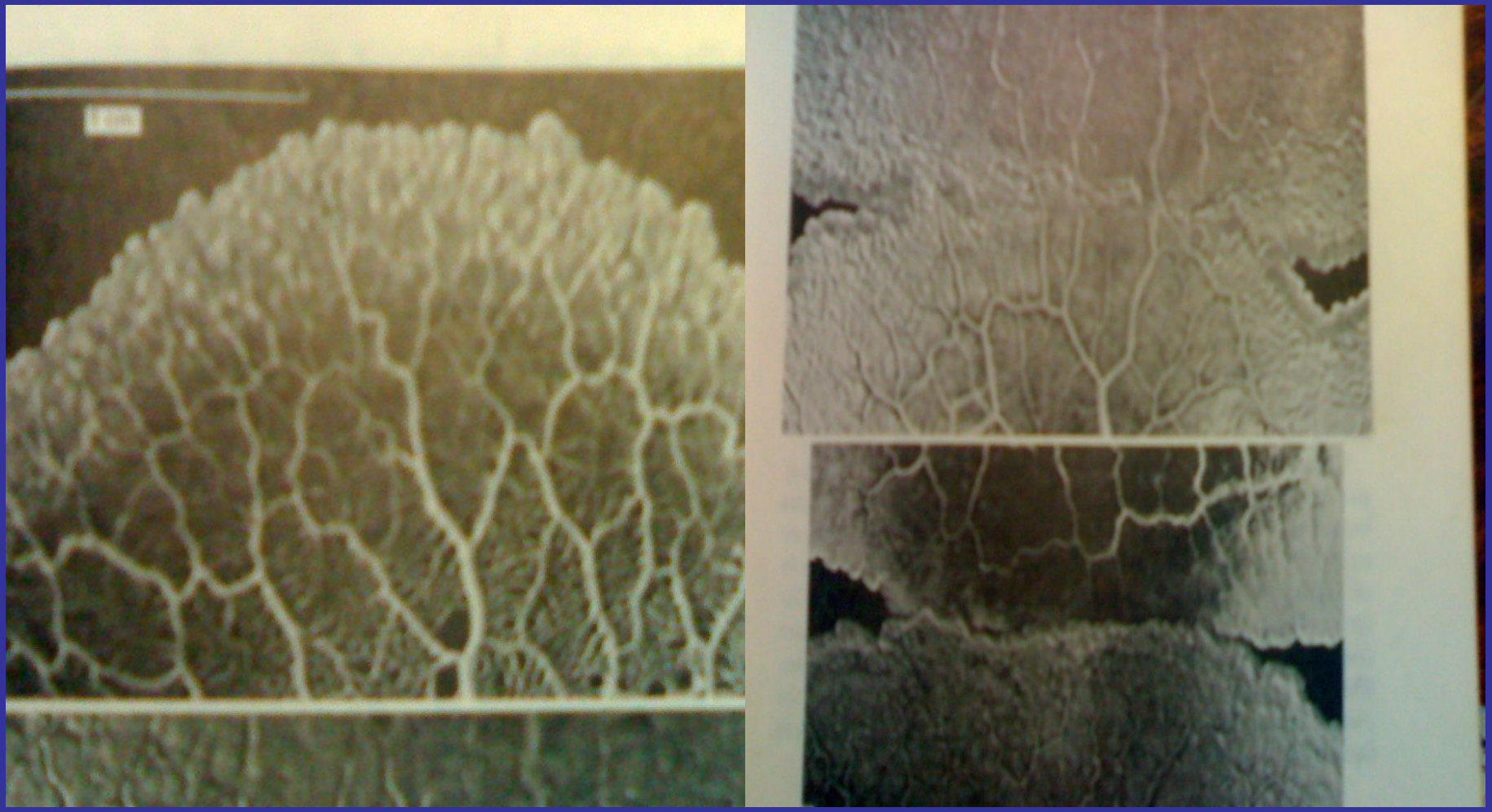
Yeasts and Yeast-like fungi




'Start'
Cell separation
Spindle pole body (SPB) duplication
Initiation of DNA synthesis
Cytokinesis
Bud emergence
SPB separation
Nuclear division
Nuclear migration

2- Plasmodial growth:

The protoplast replicates anywhere in the plasmodium. Different portions show different density and inclusions and thus the replication capacity may remain confined to one or the other part of the plasmodium.



Apical growth :

In filamentous fungi growth is affected at the tip of the hyphae into which new protoplast continuously streams.

Site of cellular extension:

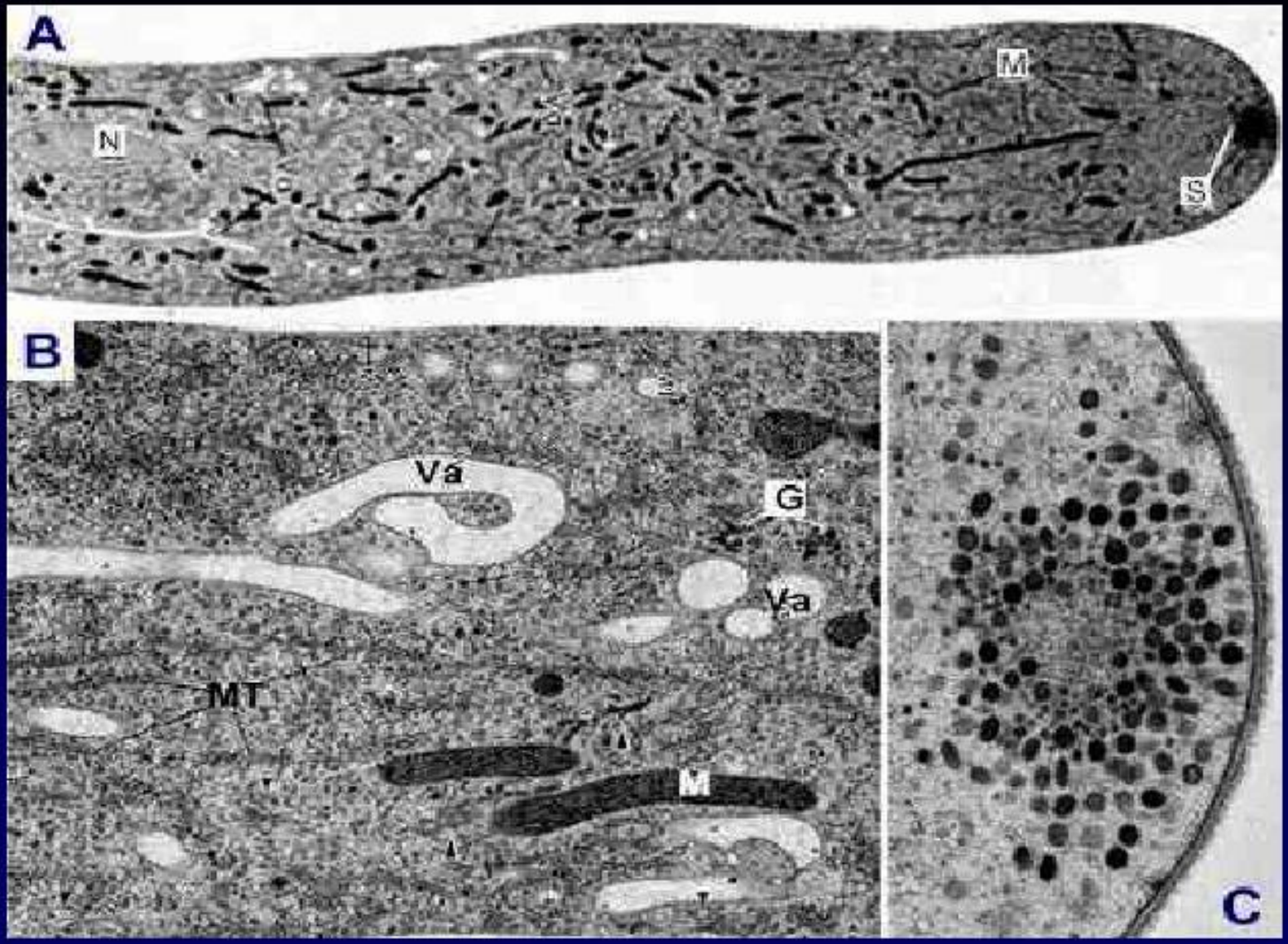
It is restricted to the hyphal tip, comprising 50-100 microns of apical portion

The apical portion has been distinguished into three zones:

i-The extreme tip or apical zone, which is concerned with cell extension and wall synthesis due to incorporation of new materials.

ii- A sub-apical zone, rich in cytoplasm and inclusions, perform other activities concerned with growth, besides translocation of requisite materials to the tip.

iii- The distal portion, which in between sub-apical and the rest of the hypha and it is very much vacuolated to supply


A
N
M
S
B
Va
G
Va
MT
M
C

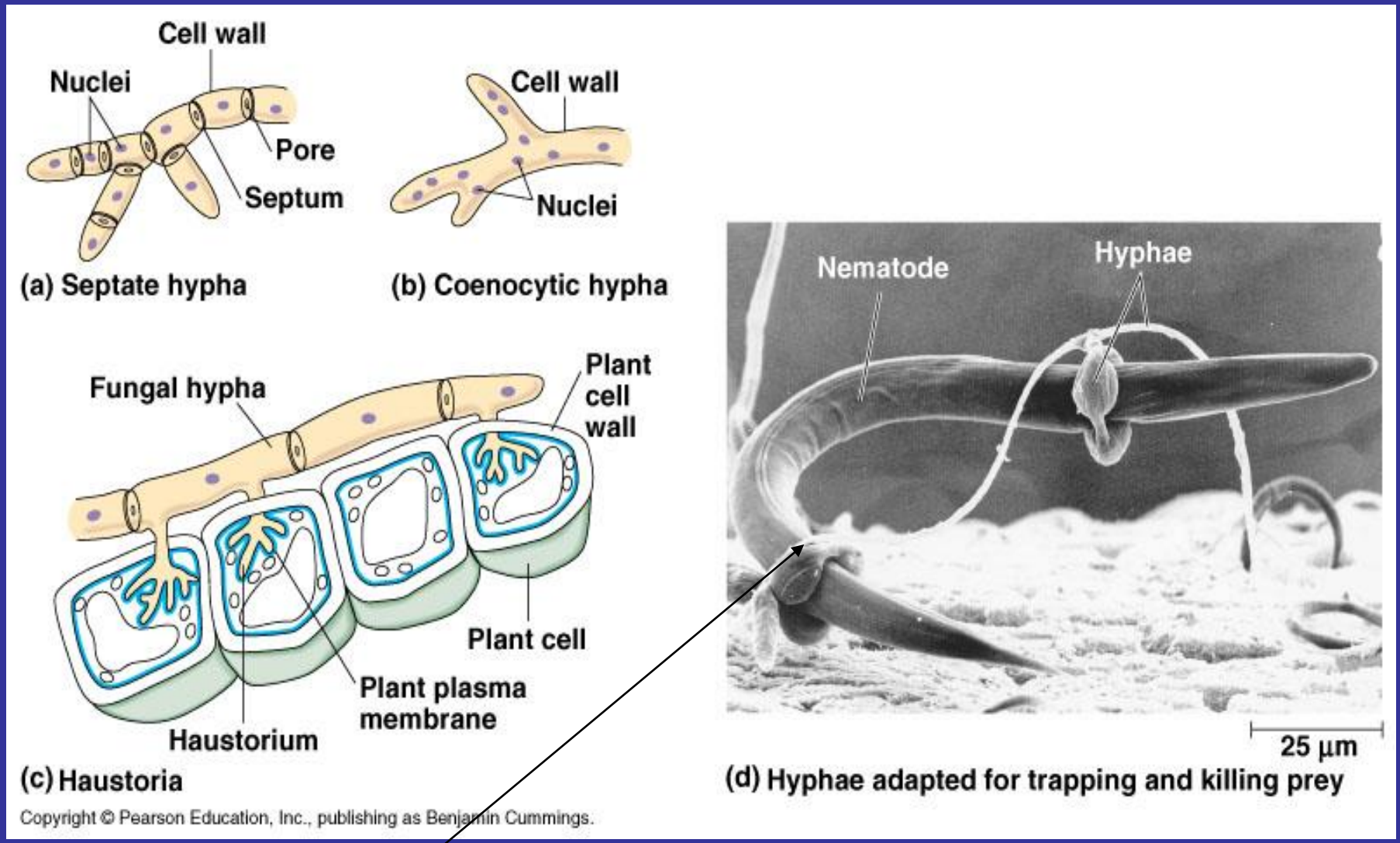
Fungal Growth
Rhizomorphs

- Rhizomorphs = mycelial cords
- These are important components of the disease caused by *Armillaria mellea*!

Fungal Growth

Stromata and Sclerotia

- Stromata (plural), stroma (singular)
  - a mass or matrix of vegetative hyphae
  - a compact, somatic "cushion" on which fruiting bodies are formed
- Sclerotia (plural), sclerotium (singular)
  - are hard resting bodies resistant to unfavorable conditions
  - LONG dormancy periods
  - germinate when favorable conditions return



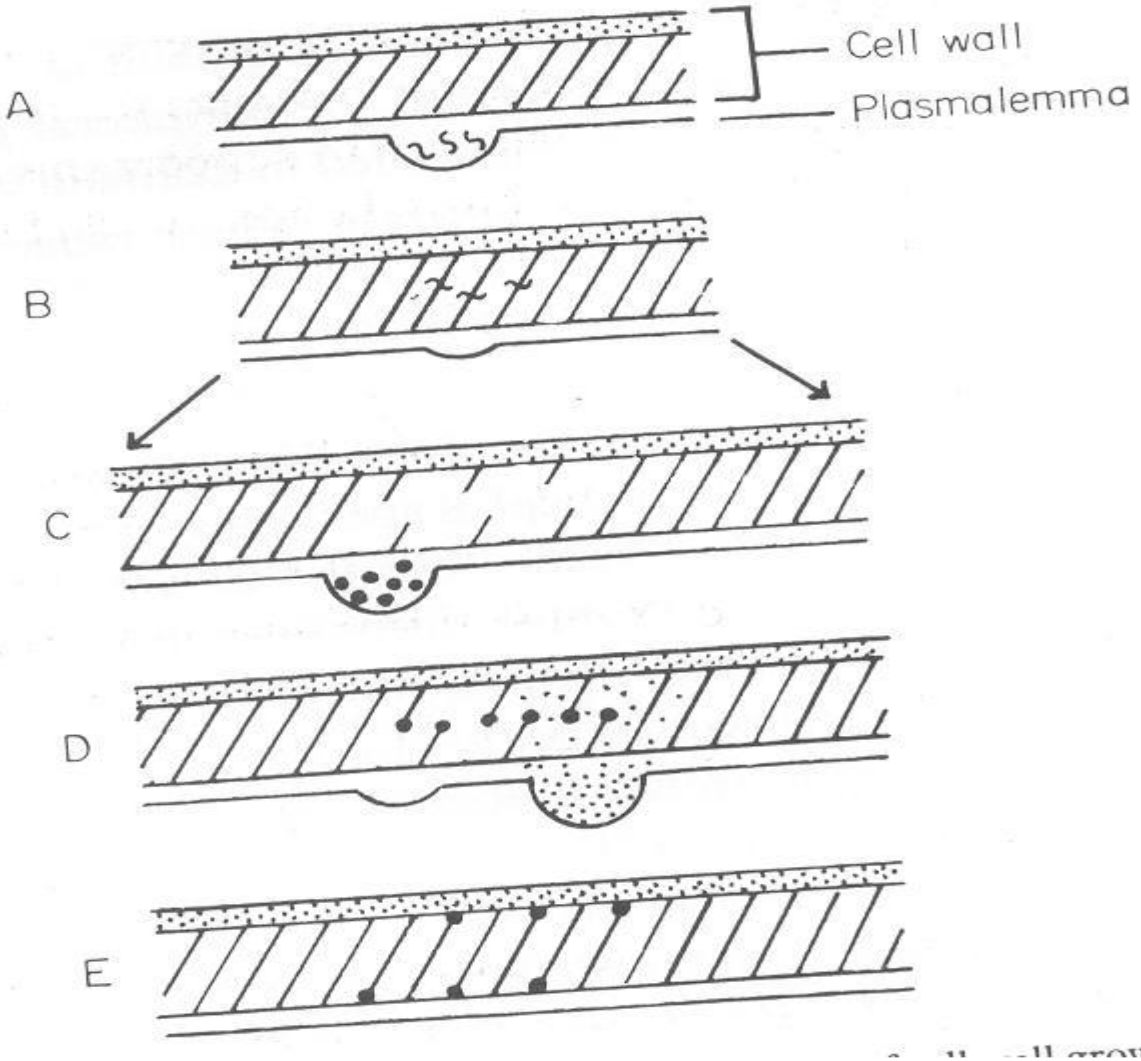
Constricting rings

Hyphal tip growth

- One theory of hyphal tip is supported by Wessels (1986-1988) and is referred to as the steady-state hypothesis.
- It suggests the hyphal apex is inherently viscoelastic and expandable and that the newly synthesized wall at the apex consists of a mixture of non-crystalline chitin and beta-glucan.
- As a result of subsequent cross-linking of polymers of the wall, viscoelastic mixture then gradually develops rigidity.

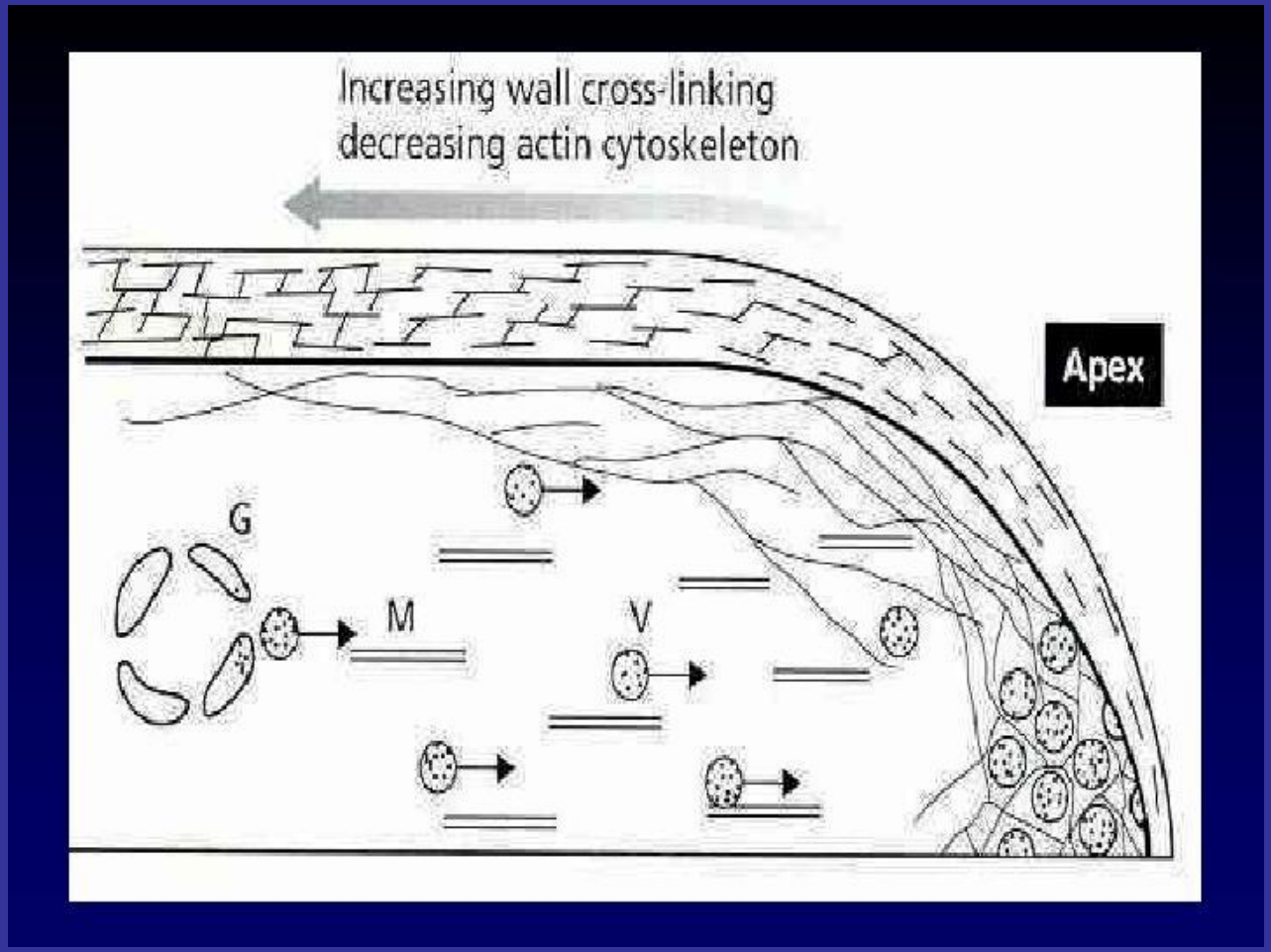
- The second hypothesis (Bartnicki-Garcia, 1973) suggests that the wall is inherently rigid and that for growth to occur there must be a permanent delicate balance between the lysis of the wall followed by synthesis of wall polymers and the pushing out and mending of the wall.

- In either of these two hypotheses, it has become apparent that the sub-apical region of a growing hypha provides the energy, enzymes, wall precursors, and membranes necessary for hyphal tip growth.
- At this point there is strong evidence that many of the raw materials needed by growing hyphal tip are delivered to the tip by mem-bound vesicles.



Hypothetical representation of the events in a unit of cell wall growth

a) Vesicles containing wall lytic enzymes fuse with the plasmalemma.
b) Bonds between existing wall components are broken.
c) Wall stretches as a result of internal pressure; vesicles containing wall synthesizing enzymes fuse with the plasmalemma
d) New wall components arise in vesicles or are synthesized from precursors that cross the plasmalemma
e) A new unit of cell wall has been synthesized.


Increasing wall cross-linking
decreasing actin cytoskeleton
Apex
G
M
V

Function of vesicles

- 1- To transport enzymes that break the bonds between the existing wall components so that the wall stretches as a result of turgor pressure and to transport enzymes involved in further wall synthesis.
- 2- To transport new wall materials, either as precursors or as preformed units, for incorporation into the wall.
- 3- To increase the surface area of the plasmalemma during growth when the mem of the vesicle fuses with the plasmalemma.

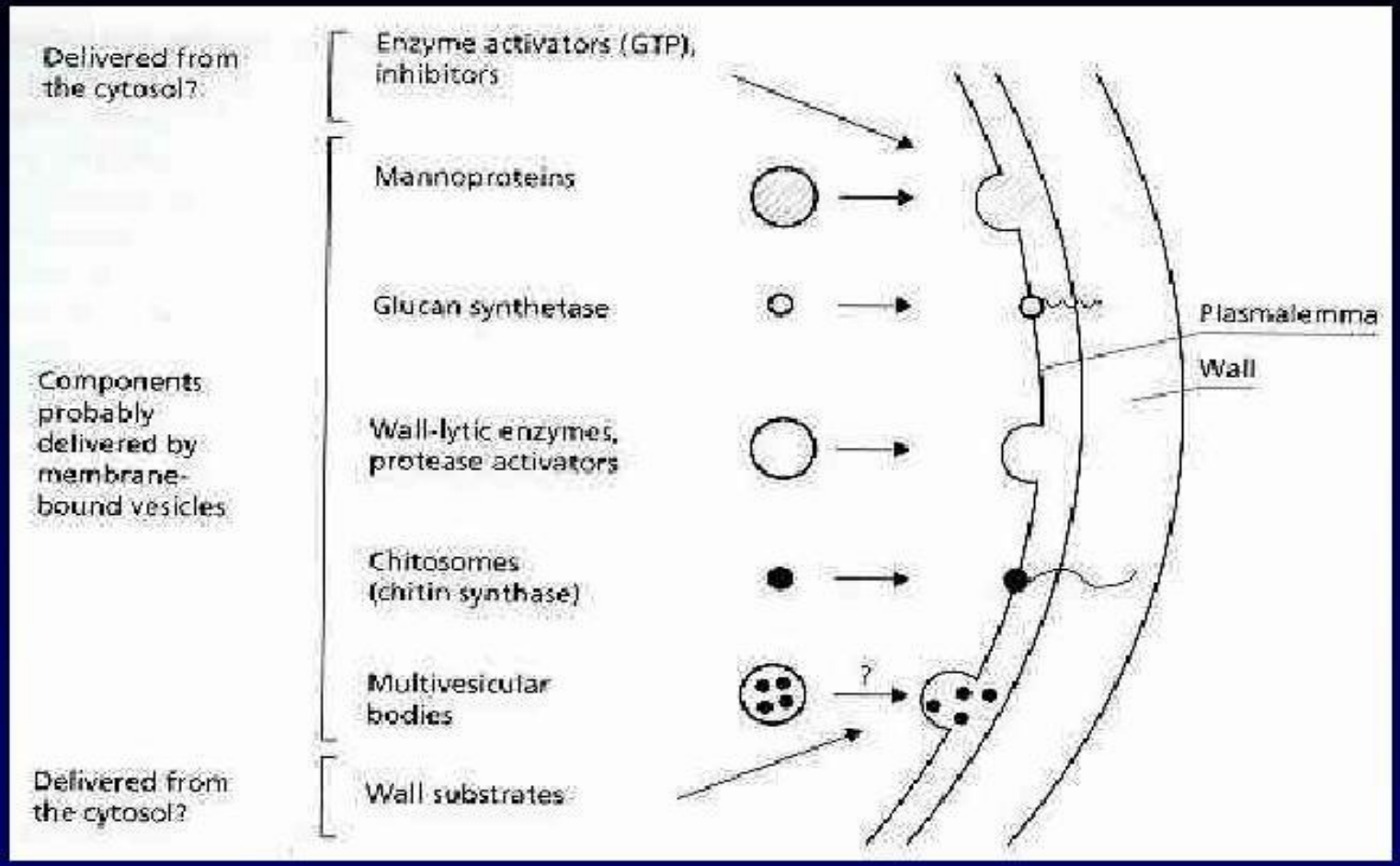
How do vesicles move to the apex?

Number of distinct hypotheses have been advanced:

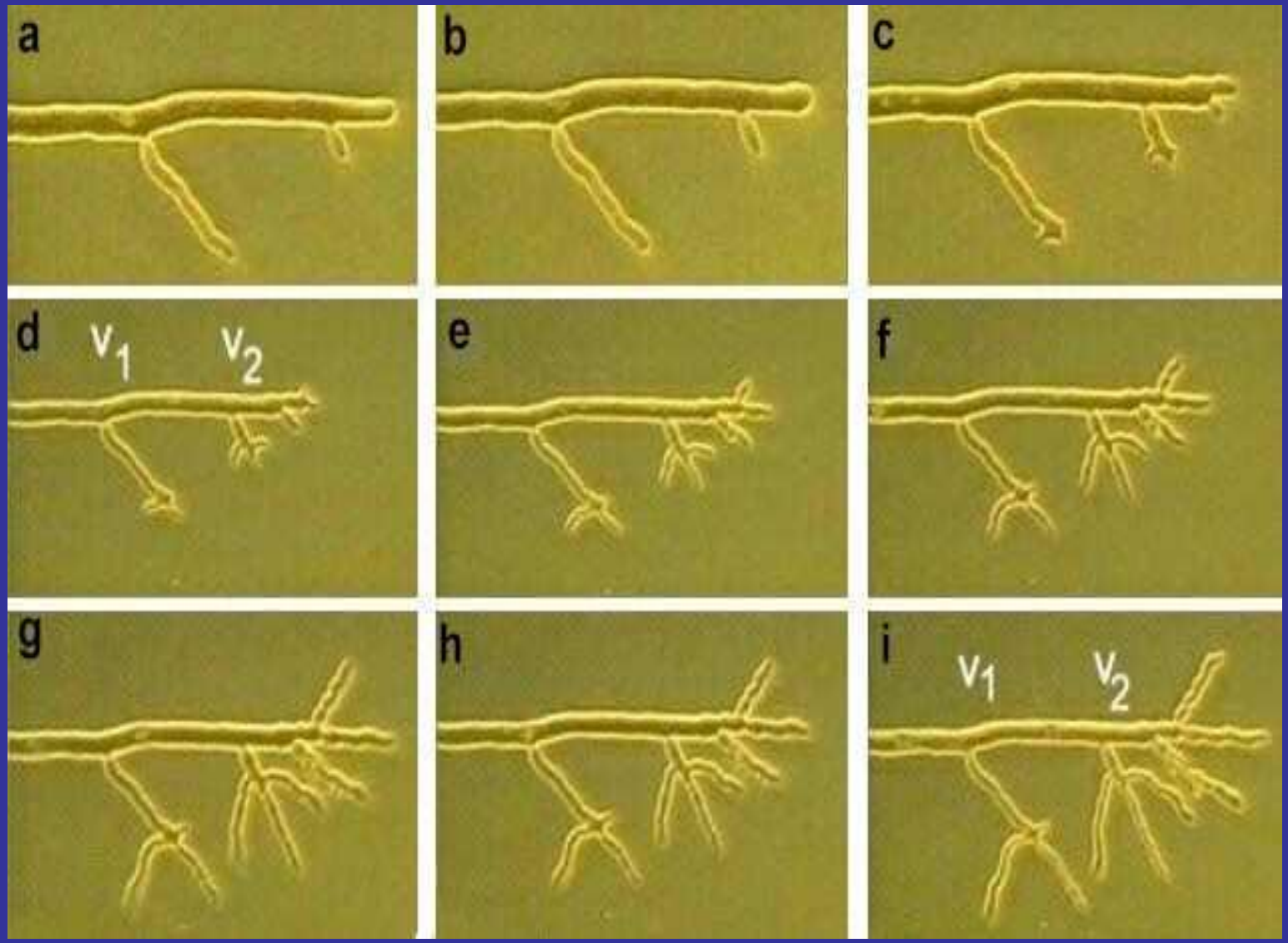
1- Difference of membrane potential between the tip and regions behind the tip, related to a difference in numbers or activities of proton pumps in the membrane.This electrogenic gradient along the hypha might move vesicles by electrophoresis.

2- Difference in the number or activity of ion pumps along the hypha, such that K+ ions are taken up in exchange for H+ ions to a greater degree behind the apex, then water also would be taken up and the flow of ions towards the apex would be accompanied by a flow of water which might move the vesicles. In short, electro-osmotic flow of water towards the apex, brought about by uptake of K+ in subapical regions of the hypha and movement of these K+ ions towards the apex. Vesicles then carried in the water stream.

3- Some contractile system involving microtubules or microfilaments helps to move the vesicles; there is no direct evidence to support this idea, but apical growth of pollen tubes can be halted by applying cytochalasins (group of compound that interfere with the contractile proteins in the cytoplasm.


Delivered from the cytosol?
Enzyme activators (GTP), inhibitors
Mannoproteins
Glucan synthetase
Components probably delivered by membrane-bound vesicles
Wall-lytic enzymes, protease activators
Chitosomes (chitin synthase)
Multivesicular bodies
?
Delivered from the cytosol?
Wall substrates
Plasmalemma
Wall

Loss of plasticity of the wall behind apex


a
b
c
d
v_1
v_2
e
f
g
h
i
v_1
v_2

The Figure illustrates part of the plasticity, when hyphae of *Neurospora crassa* are observed by placing a coverslip over the margin of a colony on an agar plate. The sequence of 9 frames was taken over a one-hour period, starting from the time when the coverslip was added.

In the first frame (a) the hyphal tip was growing normally, and two lateral branches had arisen behind the growing tip - the normal behaviour of a fungal hypha.

Soon afterwards (b and c) the hyphal tips began to swell (a response to disturbance caused by the coverslip) and then branched repeatedly from the tips.

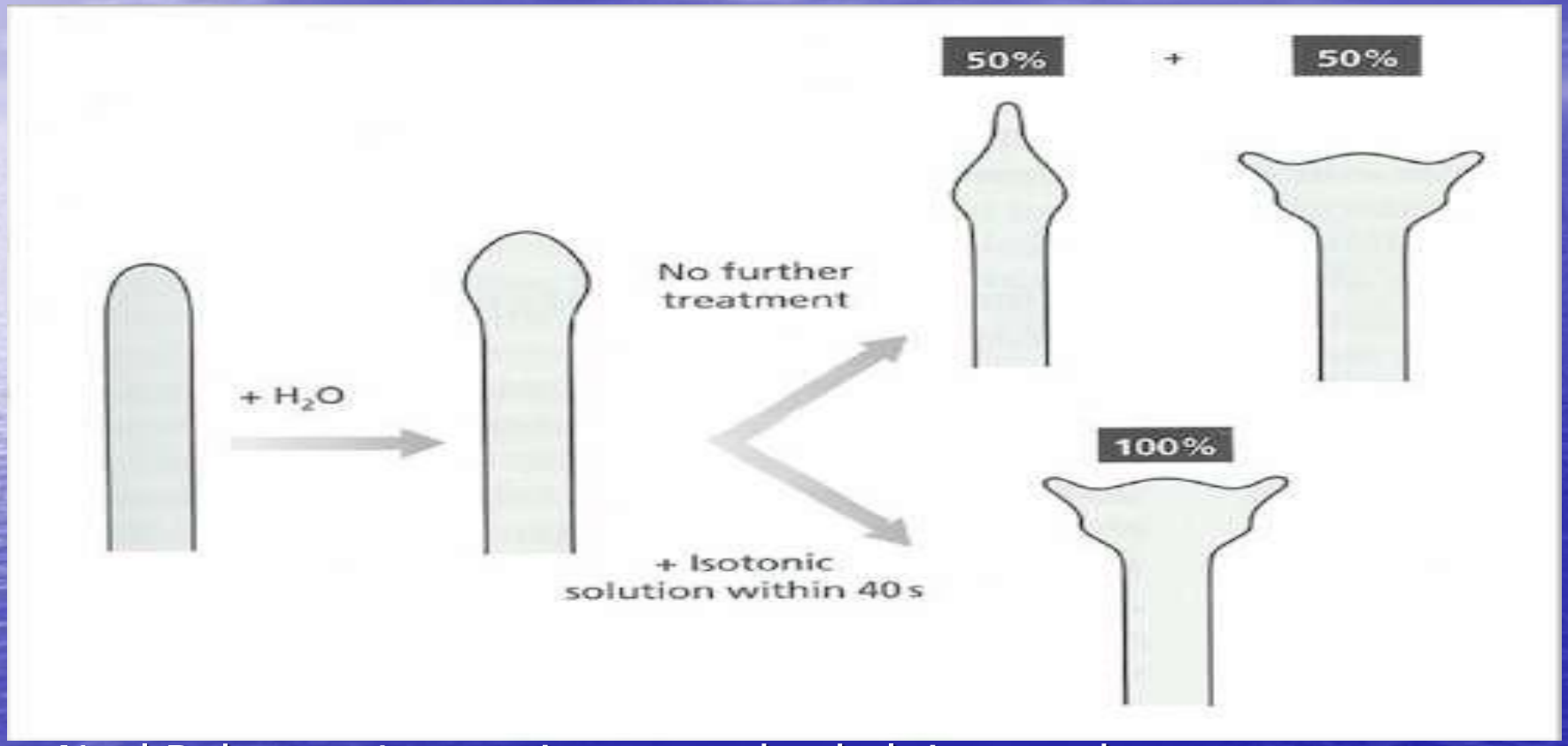
There are 3 points to note:

(1) The disturbance caused by adding the coverslip caused an immediate change in behaviour - the hyphae started to branch at their tips, not behind their tips.

(2) The hyphae originally had a smooth profile, but adding the coverslip caused the hyphae to grow irregularly (perhaps because of the lower oxygen availability).

(3) Fungal hyphae grow only at their extreme tips, and the hyphal wall rigidifies quite rapidly behind the tip. We can see this by comparing frames d and i - the distance between points 1 and 2 remained constant.

Mechanism of apical growth



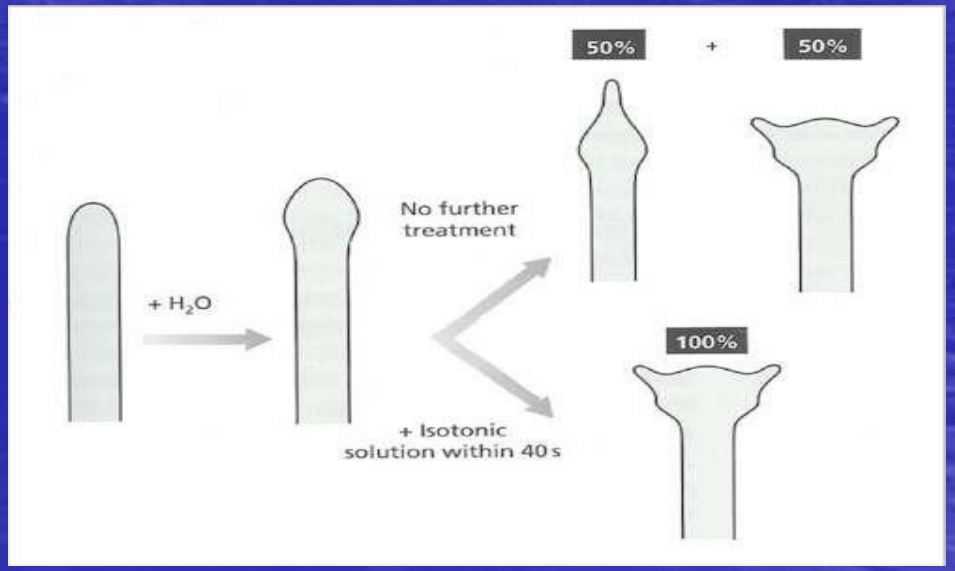
. Noel Robertson's experiments on hyphal tip growth.

Robertson flooded colonies of *Fusarium* with water and observed that apices behaved in two ways: Some stopped growing but recovered within a minute and continued growth from a thinner region of the apex than before.

Others stopped growth for several min. but swelled in meantime and formed one or two new apices

If, next , the colonies were flooded with water but this was replaced within a min. by an isotonic soln, then all apices stopped, swelled and branched instead of growing on from a thinner region of the original apex.

Because the tips would need to make two separate osmotic adjustment and could not do so before the apex had rigidified.



How does an apex form?

- An extremely interesting approach to the study of apical growth has come from observation on spore germination.
- In several fungi, spore germination involves an initial phase of swelling as a result of hydration followed by a further phase of swelling that depend on metabolic activity. During this swelling phase the fungus can be shown to insert new wall material over its inner surface.

Later a young hypha, termed a germ tube, emerges from a localized site on the spore, and at this stage the majority of wall components are inserted locally at what will become the tip of the germ tube.In other wards, spore germination involves an initial phase of non-polar growth of the wall, followed by a phase of polar growth.

Ex. If spores of A. niger are icubated at 44^{o}C they continue to swell and form giant rounded cells.

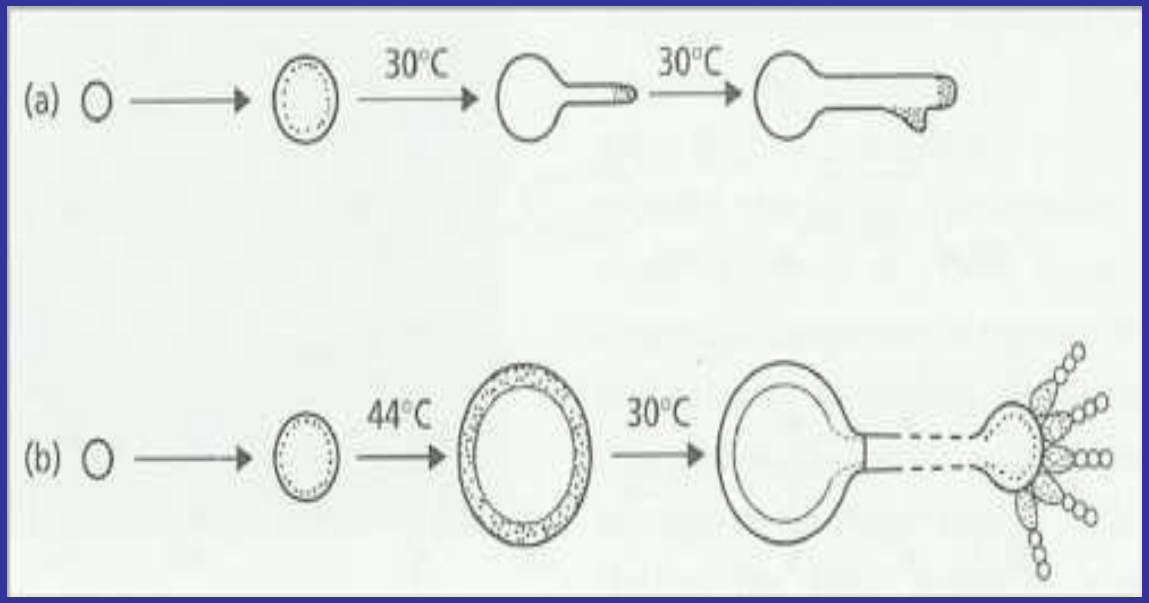
If spores are incubated at 30^{o}C they show a normal early stage of spore swelling with non-polar growth, followed by development of germ tubes.

Temperature inhibits the switch from non-polar topolar growth.

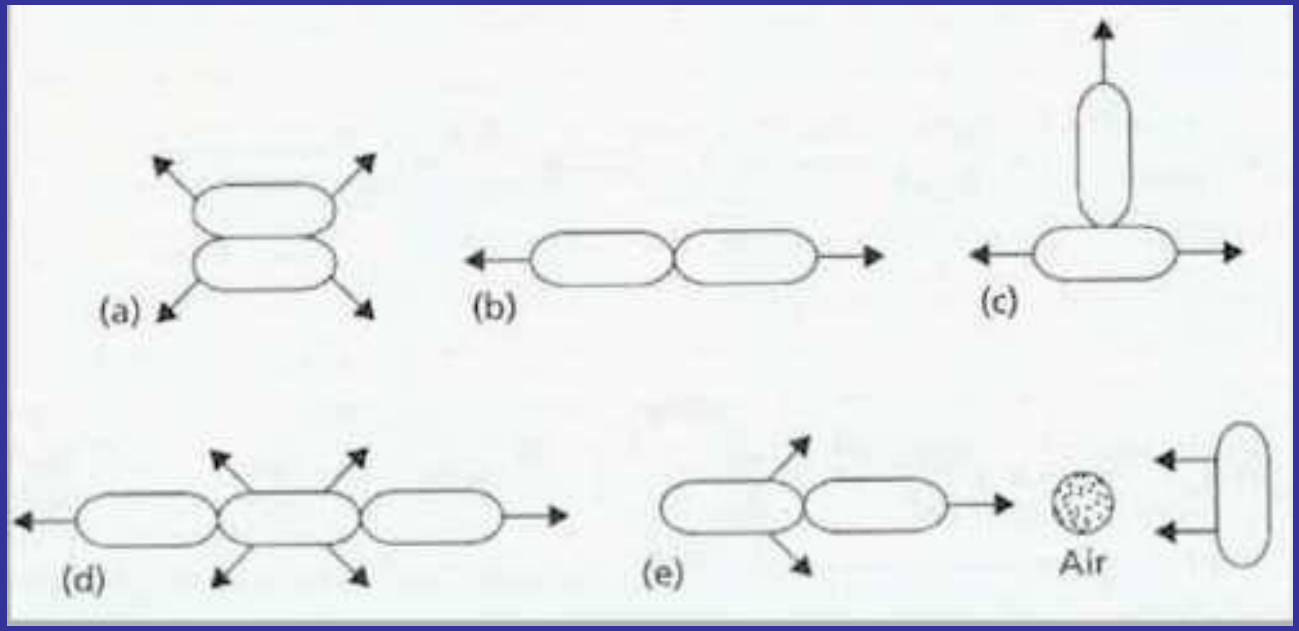
If net, the giant cells formed at 44^{o}C are brought down to 30^{o}C they initiate an apex but this behaves in remarkable way.

Instead of growing into a normal hypha it immediately develops into a sporing structure and produces a further batch of spores.

Microcycle conidiation



Stages in germination of spores of *Aspergillus niger*
(a) In normal conditions (e.g. 30oC) the spore swells and incorporates new wall material over the whole of the cell surface (shown by stippling), then a germ-tube emerges and all new wall incorporation is localised to the hyphal tip. **(b)** At 44oC the spore continues to swell and incorporates wall material in a non-polar manner, producing a giant cell with a thick wall. If the temperature is lowered to 30oC this cell produces an outgrowth, which immediately differentiates to produce a spore-bearing head.



Germination behaviour of spores of *Geotrichum candidum*,
when incubated in a thin water film beneath a cover-slip.
The spores always germinate from positions near their poles.
Arrows indicate the positions of germ-tube outgrowth in different conditions.
a – d) Negative autotropism of spores touching in pairs or in groups –
the spores always germinate from a position furthest from a touching spore.
(e) The presence of oxygen (a small hole in the coverslip) negates the negative autotropism –the spores germinate from a point closest to the oxygen source.

Fungal branching

1- Fungi show apical dominance. Most hyphae branch only at some distance behind their tips and the hyphal system thus resemble a Christmas tree, the branches becoming longer as we move back from the main hyphal apex.

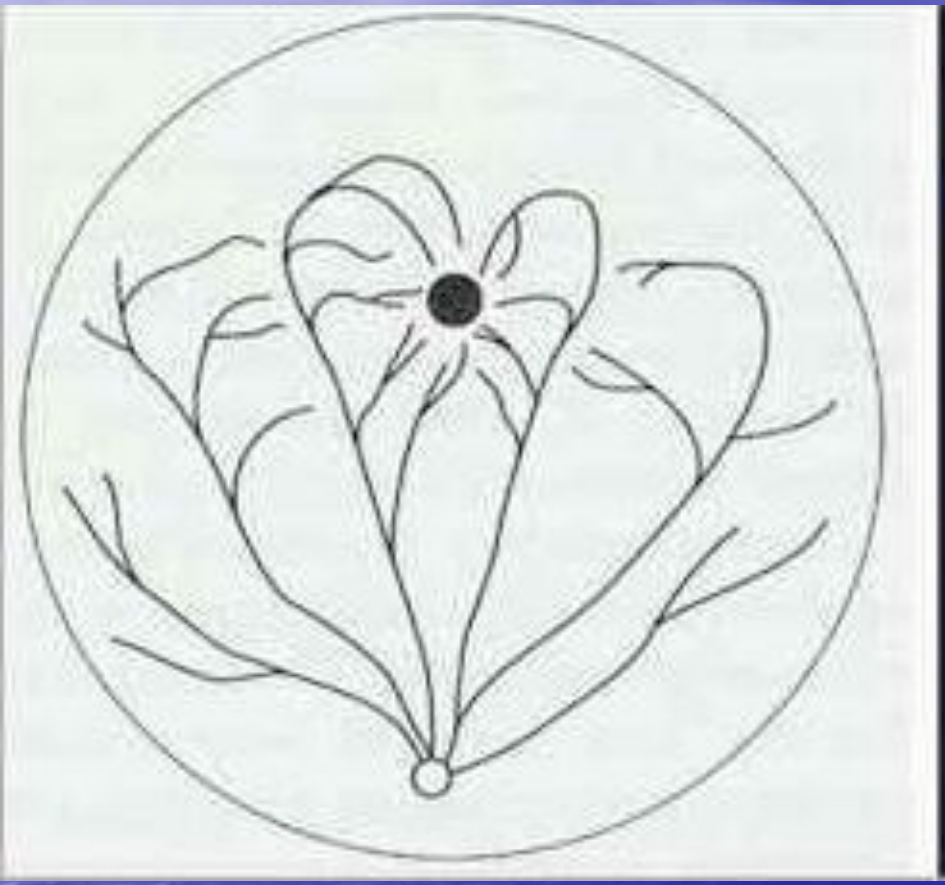
2- Branches tend to diverge from one another, filling the gaps between existing hyphae or that they respond negatively to metabolic by-product of other hyphae, moving out of regions (staled) by growth of existing hyphae.

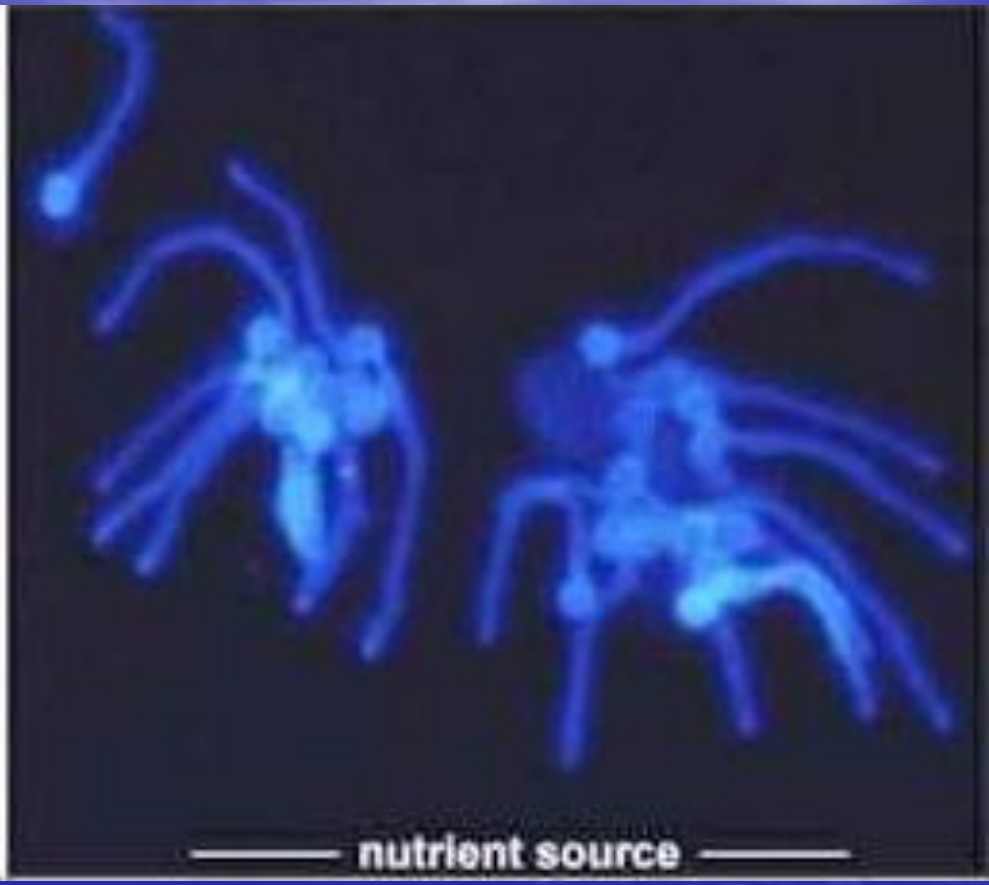
Tropism

Tropism: is defined as a directional growth response of an organism to an external stimulus.

I-Hyphal tropism: is a bending response that orientes a hypha in a particular direction for ex, towards a nutrient source or away from a potential inhibitor.

Ex 1: *Saprolegnia* when grows on very weak agar e.g water agar and placing a disc of nutrient-rich agar inside the colony margin the hyphal tips grow towards the nutrient-rich disc.




nutrient source

2- The density of a fungal colony is related to the nutrient level in the medium; hence branched on very week media and densely branched on rich media.

The existing hyphal apices at the colony margin seem to have a prior call on nutrients, and any nutrients over and above the needs of the existing apices are available to support branching.

Hyphae of many fungi show tropic responses to non-nutrient factors of potential ecological relevance. Ex. Germ tube of mycorrhizal fungi grow towards volatile metabolites from roots.

Some wood –rotting fungi orientate towards volatile compounds from freshly cut wood blocks.

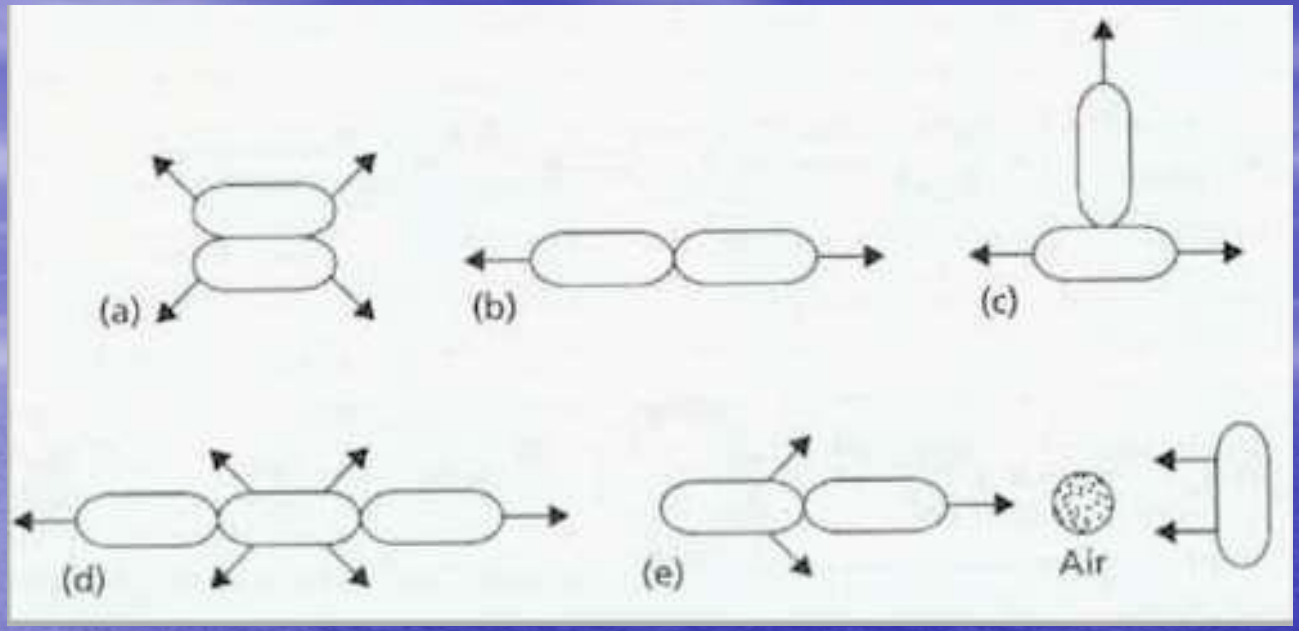
Sexual pheromones elicit orientation responses.

II-Spore germination tropism:

1- Spores can show orientation responses to electrical fields. Ex., spores of N. crassa & M. mucedo were found to germinate towards the anode.

2-Spore can show orientation responses to O2. Ex., Spores of *Geotrichum candidum*

(yeast-like fungus which is common cause of spoliage of dairy products) are seeded densely onto agar so some spore are touching, their germ tubes arise from opposite poles of the spore pairs. It is termed (**negative autotropism**).



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the spores always germinate from a position furthest from a touching spore.

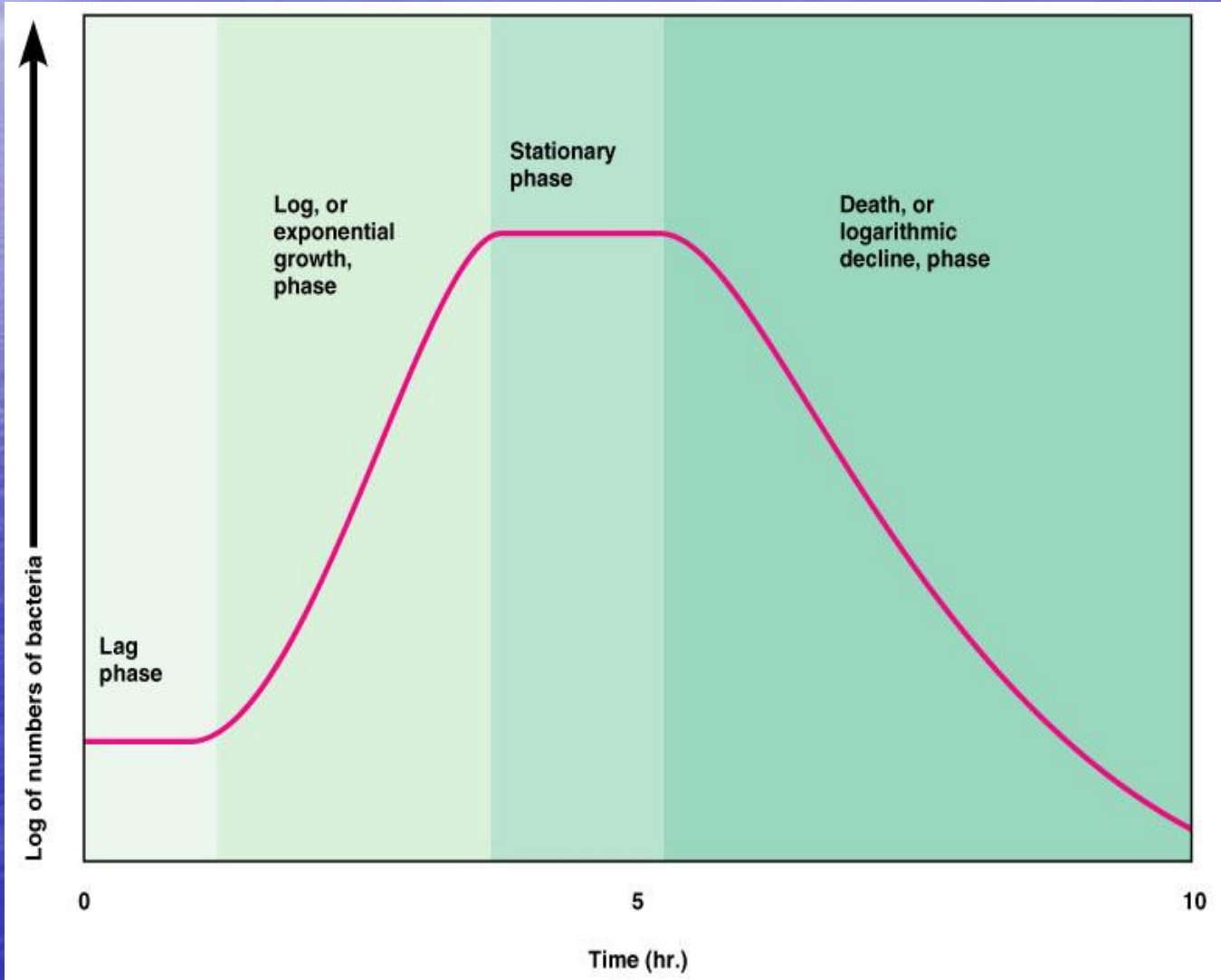
(e) The presence of oxygen (a small hole in the cover slip) negates the negative auto tropism –the spores germinate from a point closest to the oxygen source.

Reasons:

1-release of auto-inhibitors, which would accumulate max. in the zone of contact of 2 spores but diffuse away from free ends, leading to germination there.

2- O_2 depletion in the zone of spore contact because spore always germinate towards an O_2 source and this +ve tropism to O_2 could overcome the negative autotropism of touching spore pairs

Kinetics of growth



Why lag phase?

Previous cells ran out of food, shut down many metabolic pathways needed for active growth, made adaptations necessary for dormancy and protection. Before growth can resume need to:

regenerate pools of essential nutrients

requires new enzyme synthesis

time for pathways to function.

Can reduce or eliminate lag phase by using cells that are not in stationary phase.

Elongation of the period of lag phase depends on :

1- Number of the cells in the inoculum:

Increase number decrease the period due to

RNA or its precursor found in previous medium or released from dead cells after their autolysis.

2- The age of the cells in the inoculum:

Old and sporulated cells increases the period because they grow slowly.

3-The contents of the medium:

If it is not suitable the growth will be slow.

In Fungi amm. acetate increases the period of lag phase and the high concent. of glucose makes the same effect.

Glutamic acid decreases the period of lag phase.

Both fructose and glucose increases the period of lag phase but addition of glutamic acid decreases this period in some fungi.

Gibbrella don't make lag phase but sometimes it can make lag phase if the medium contains amm. acetate.

Why exponential phase?

Cells in optimum growth state, divide repeatedly by binary fission at maximal rate.

Note: useful to calculate doubling time; can vary from 20 min to several days

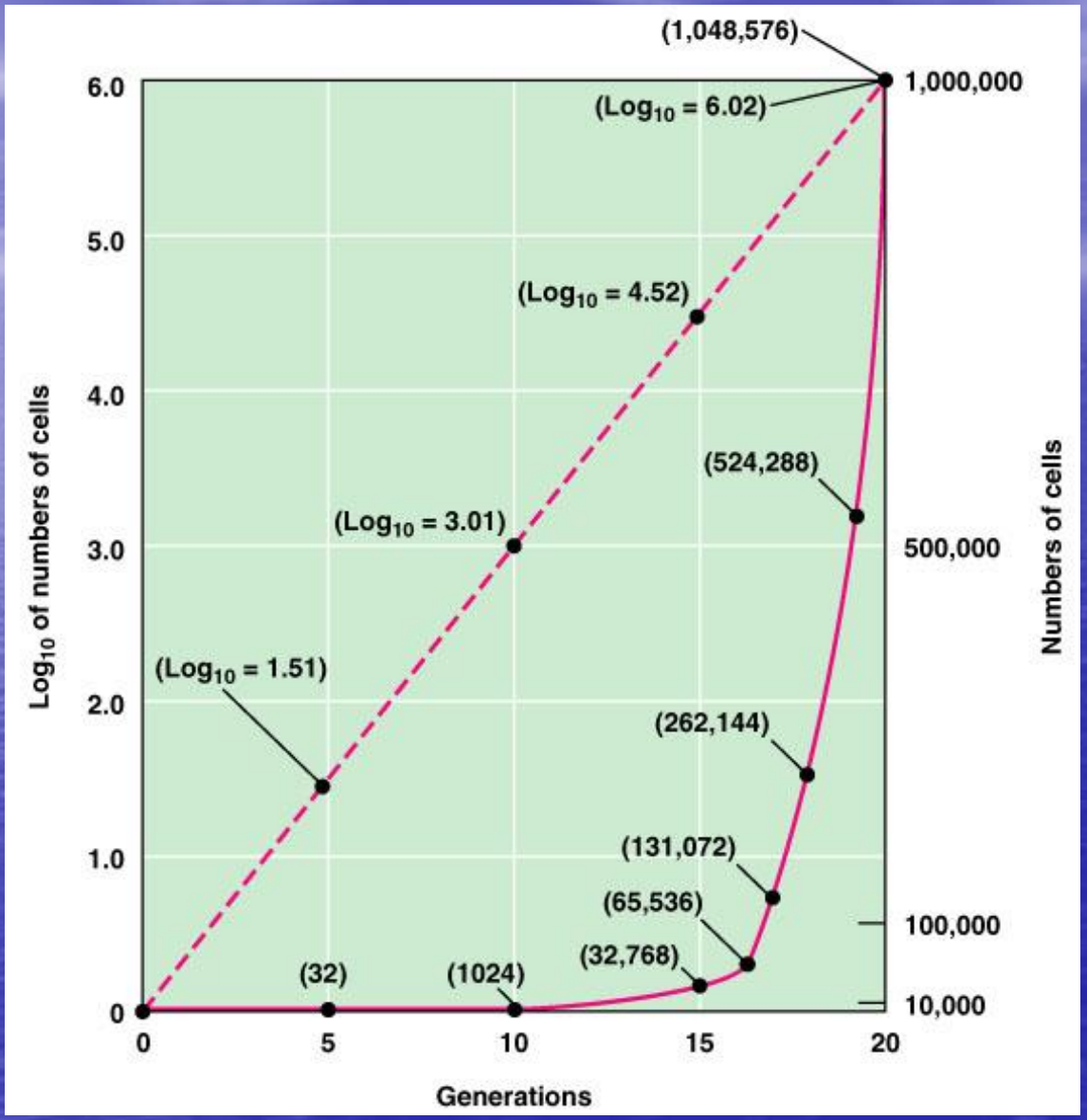
Why stationary phase?

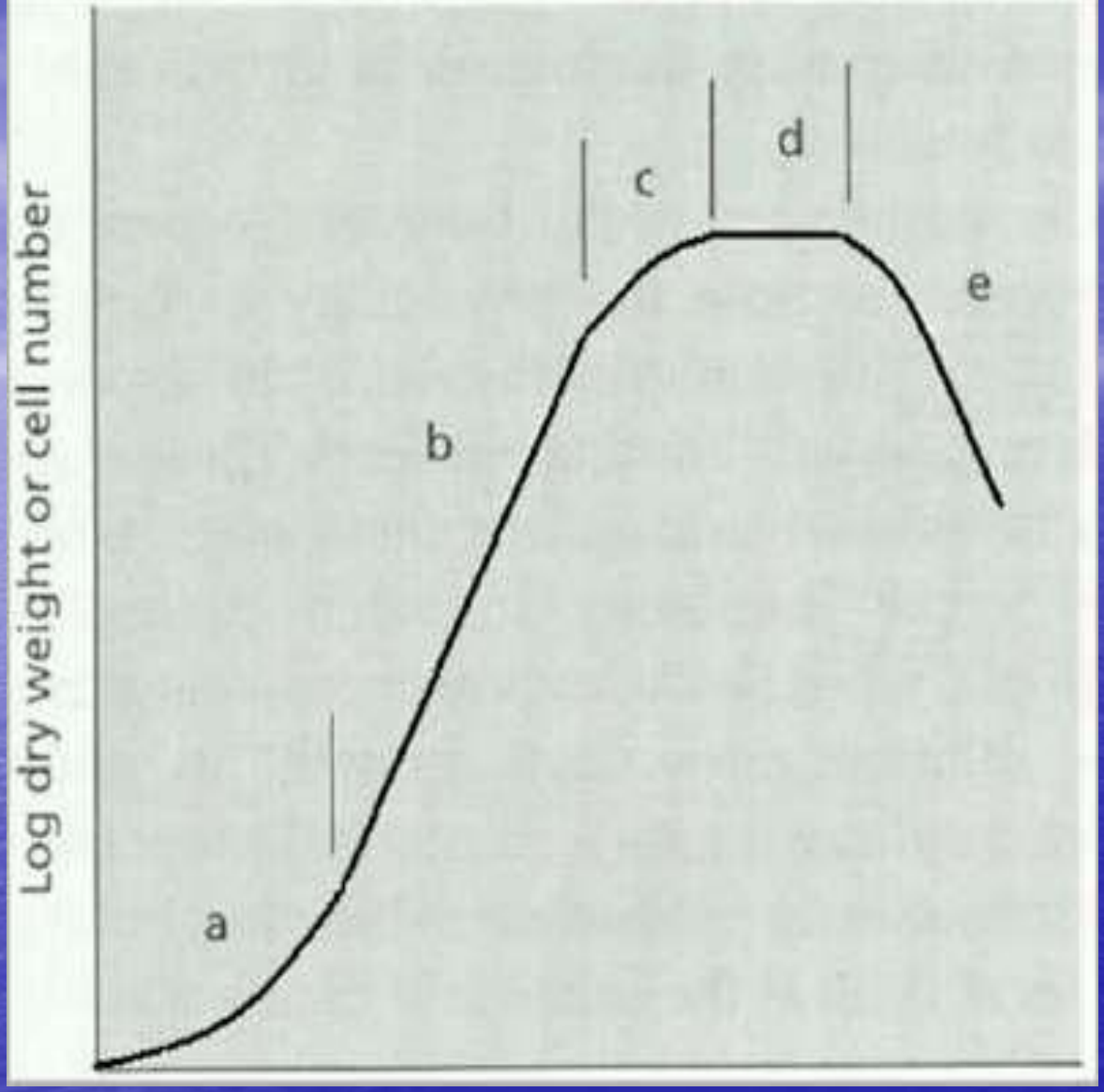
Can be due to **exhaustion of some critical nutrient**, or to **accumulation of waste products** that slow down growth (e.g. acid buildup from fermentation).

Why death phase?

Continued accumulation of wastes, exposure to oxygen, loss of cell's ability to detoxify toxins, etc. Note that death is exponential; 90% of cells die in certain time, another 90% in same time period, etc.

Note: in practice, try to prolong stationary phase, reduce death phase. Don't store cultures at room temperature on plates (ready exposure to oxygen, desiccation, high temperature speeds oxidation reactions). Better, transfer to slants (tubes), store capped in refrigerator once grown. Still better, transfer to stab tube (soft nutrient agar), stopper and seal with airtight seal. Can recover viable culture even after a year of sitting on shelf!


(1,048,576)
1,000,000
(Log10 = 6.02)
6.0
5.0
(Log10 = 4.52)
4.0
(524,288)
(Log10 = 3.01)
3.0
500,000
(Log10 = 1.51)
2.0
(262,144)
(131,072)
1.0
(65,536)
100,000
(32,768)
(32)
(1024)
0
10,000
0
5
10
15
20
Generations
Log10 of numbers of cells
Numbers of cells



Typical growth curve of a batch culture. (a) Lag phase;
(b) exponential or logarithmic growth phase;
(c) deceleration phase; (d) stationary phase; (e) phase of autolysis.

Phases of growth

Growth in the fungi as in other organisms following a definite pattern. The shape of the curve depends upon the species and the environmental and nutritional condition.

(a) Lag phase

(b) exponential or logarithmic growth phase

(c) deceleration phase (declining acceleration) : As nutrients become exhausted or as toxic by-products accumulate, the rate of growth is lessened.

(d) stationary phase: The death of all cells is balanced by new growth. The duration of this phase is dependent upon the organism and upon the composition of the medium at this time.

(e) phase of autolysis

Specific growth rate (μ):

The rate of growth during the log phase is termed Specific growth rate (μ) of the organism.

The numerical value of μ is calculated by measuring $\log_{10}$ of the number of cells (N_0) at any one time (t_0) and $\log_{10}$ of the number of cells (N_t) at some time (t) later, according to the equation:

$$\log_{10} N_t - \log_{10} N_0 = \frac{\mu}{2.303} (t-t_0)$$

where 2.303 is the base of natural logarithm.

$$\mu = \frac{(\log_{10} N_t - \log_{10} N_0)2.303}{(t-t_0)}$$

Ex: $N_0 = 10^3$ cells per ml and $N_t = 10^5$ cells per ml , 4 h later, then:

$$\mu = \frac{(5-3)2.303}{4} = \frac{2.303}{2} = 1.15/h$$

From this we can compute the mean doubling time, or generating time (g), of the organism as the time needed for a doubling of the natural logarithm, according to the equation:

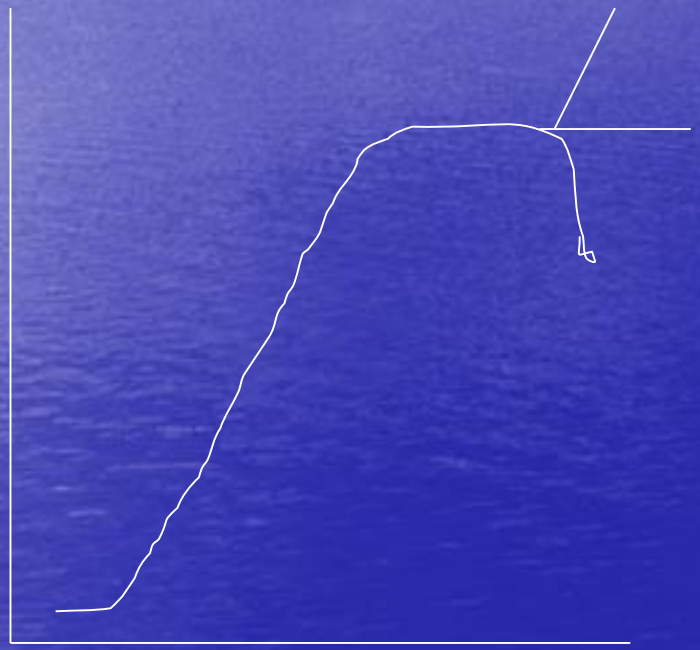
$$G = \frac{\log_e 2}{\mu} = \frac{0.693}{1.15}$$

In this ex. G= 0.6h. For S. cerevisiae at 30^{o}C, μ (0.45/h) and g (1.54h)

The Constant phase could be either:

1- Non staling type:

Where the constant phase is represented by a straight line and growth is still increasing.



2- Staling type:

Where the constant phase does not proceed but depression occur in growth. This is due to production of certain metabolites and toxic substances by the fungus which are suppressive for fungal growth.

3- Promoting type

In which the line of the constant phase becomes successively elevated with lapse of time in this case the metabolic products by fungus are promoting in nature.

Staling in fungi

1- shift in pH

2- Break down of N & C

3- Nutrients (loss of medium)

Some fungi can produce certain metabolites which are suppressive for their growth, these metabolites are known as staling products and the fungi are known as staling type of fungi while the phenomenon is known as staleness.

Staleness my be due to one or more of the following factors:

1- pH shift towards alkalinity

2- Production of suppressive metabolites as a result of the breakdown of complex C & N compounds

3- Nutritional factor due to consumption of nutrients of the medium.

The increase in alka. Is due to the fact that N source in Rhichard's medium is KNO_3. The fungus utilizes NO_3 as N source with liberation K^+ that combin with CO_2 evolved from respiration to form $KHCO_3$ which increases the alka. of the medium

$$KNO_3 \longrightarrow K^+ + NO_3^-$$

$$K^+ + CO_2 + H_2O \longrightarrow KHCO_3$$

It must be noticed that staleness is not an inherited character for any fungus but depend upon the nutritional constituents of the medium as well as the prevailing environmental and physiological conditions.

Physical properties of staling products

Staling products	Physical properties
Fusarium	1- removable by filtration through collodion membrane 2- Partialy deactivation & ppt with alco.
Cladosporium	Destroy by Adsorption
Trichoderma	Destroy by Heating for 10 min Oxidation