Chapter Ⅶ
The Growth and Reproduction of Microorganisms

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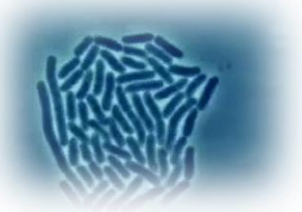
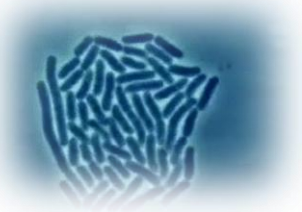
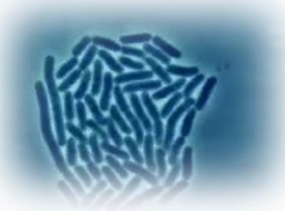


Outline

- **1. Bacterial Reproduction**
- **2. Bacterial Growth**
  - ❖**Generation Time (增代时间)**
  - ❖**Bacterial Growth Curve**
  - ❖**Batch and Continuous Growth (分批生长及连续生长)**
  - ❖**Synchronous culture（同步培养）**

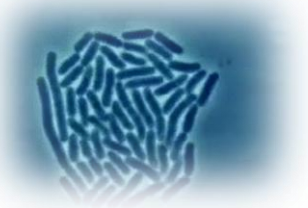


- **3. Enumeration of Bacteria**
  - ❖Viable Count Procedures (活菌计数法)
  - ❖Direct Count Procedures (直接计数法)
  - ❖Most Probable Number (MPN) Procedures (最大可能数量法)
- **4. Factors Influencing Bacterial Growth**
  - ❖Temperature
  - ❖Oxygen
  - ❖Salinity
  - ❖pH
  - ❖Pressure
  - ❖Light Radiation



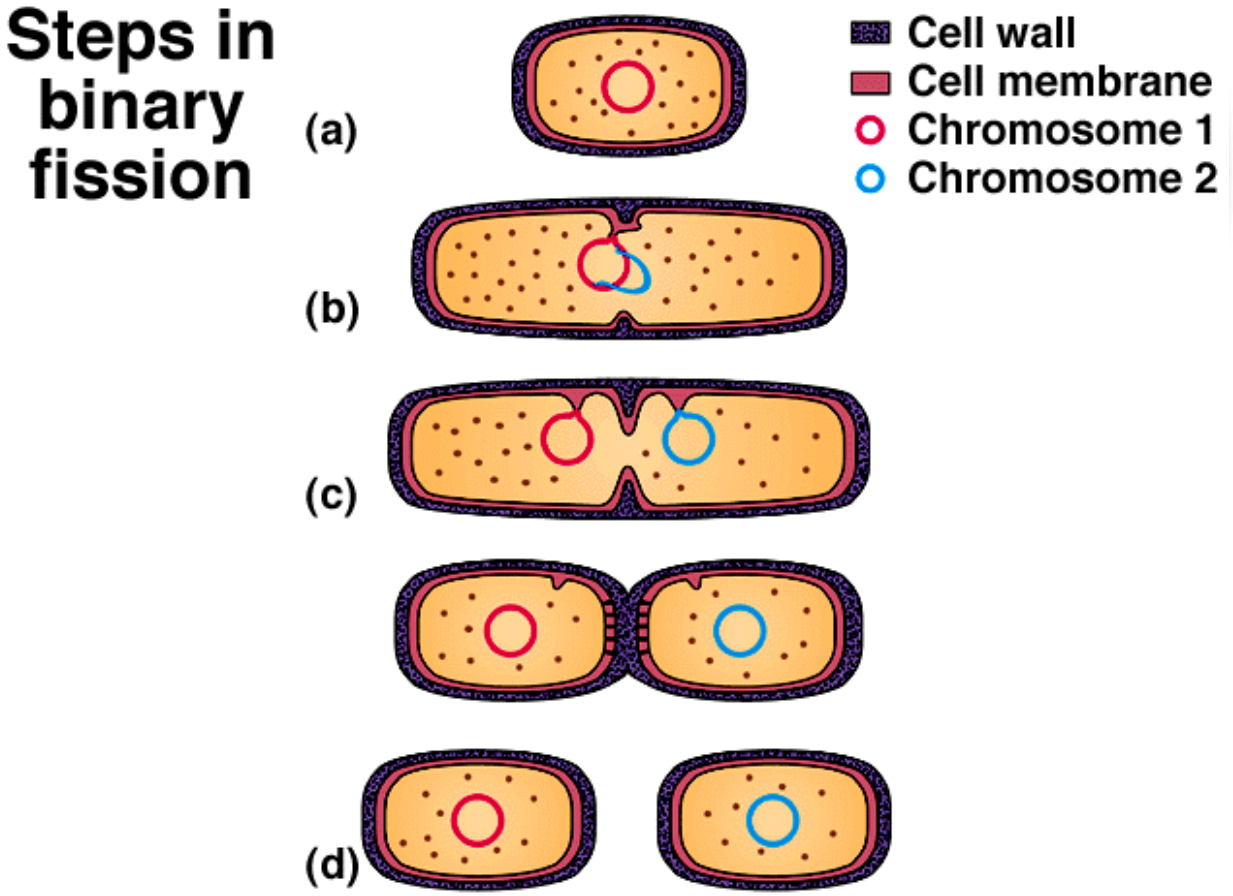
- **Growth - an increase in cellular constituents**
- **Reproduction-increase of numbers of organisms**
- **To most bacteria, growth and reproduction are synonymous.**

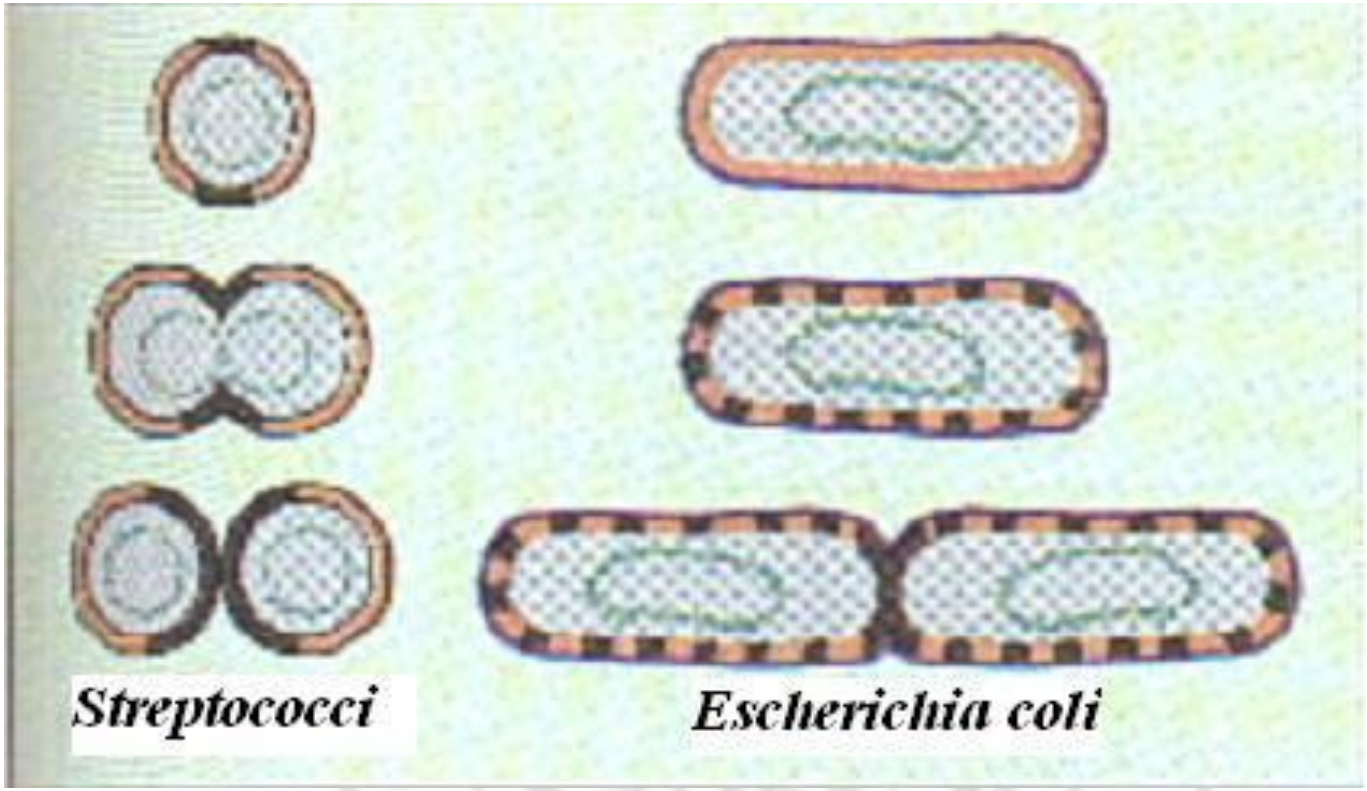
微生物学中提到的“生长”，一般指群体生长，这一点与研究大生物有所不同。



Bacterial Reproduction

- **Binary fission (二分分裂) is the most common means of bacterial reproduction.**

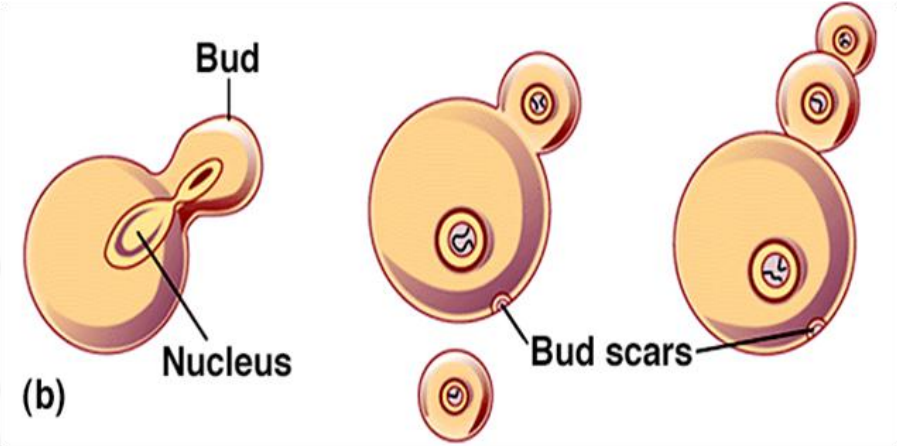




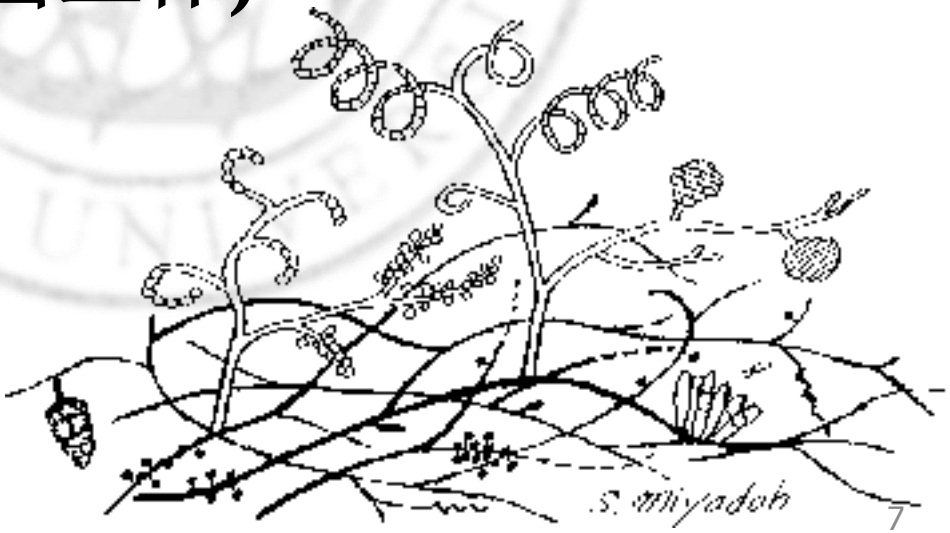
binary fission -- two equal-size cell

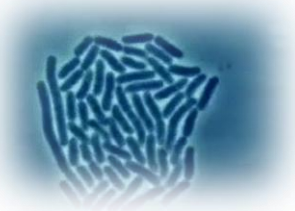
杆菌:新合成的肽聚糖均匀分布

球菌:在赤道板插入，固定位置。

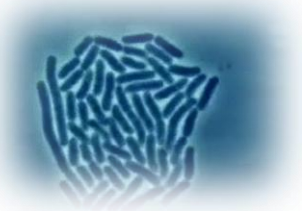


- **Budding (出芽)** -- **unequal division**
- ***actinomycetes* (放线菌) -- formation and rupture of hyphae (菌丝体)**



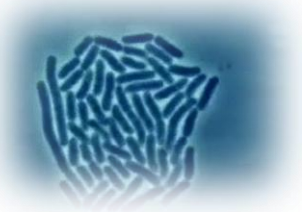


- **Generation Time（代时) or doubling time (倍增时间) is the unit of measure of bacterial growth.**
- **It is the time required to achieve a doubling of the population of bacteria.**
- **$g = （ t_1 - t_0 ） / [3.3 (\log N_t - \log N_0)]$**



Bacterial Growth Curve

- **Growth curve is the curve of (log) concentration/number of bacteria versus time when bacteria are inoculated into a new liquid medium.**



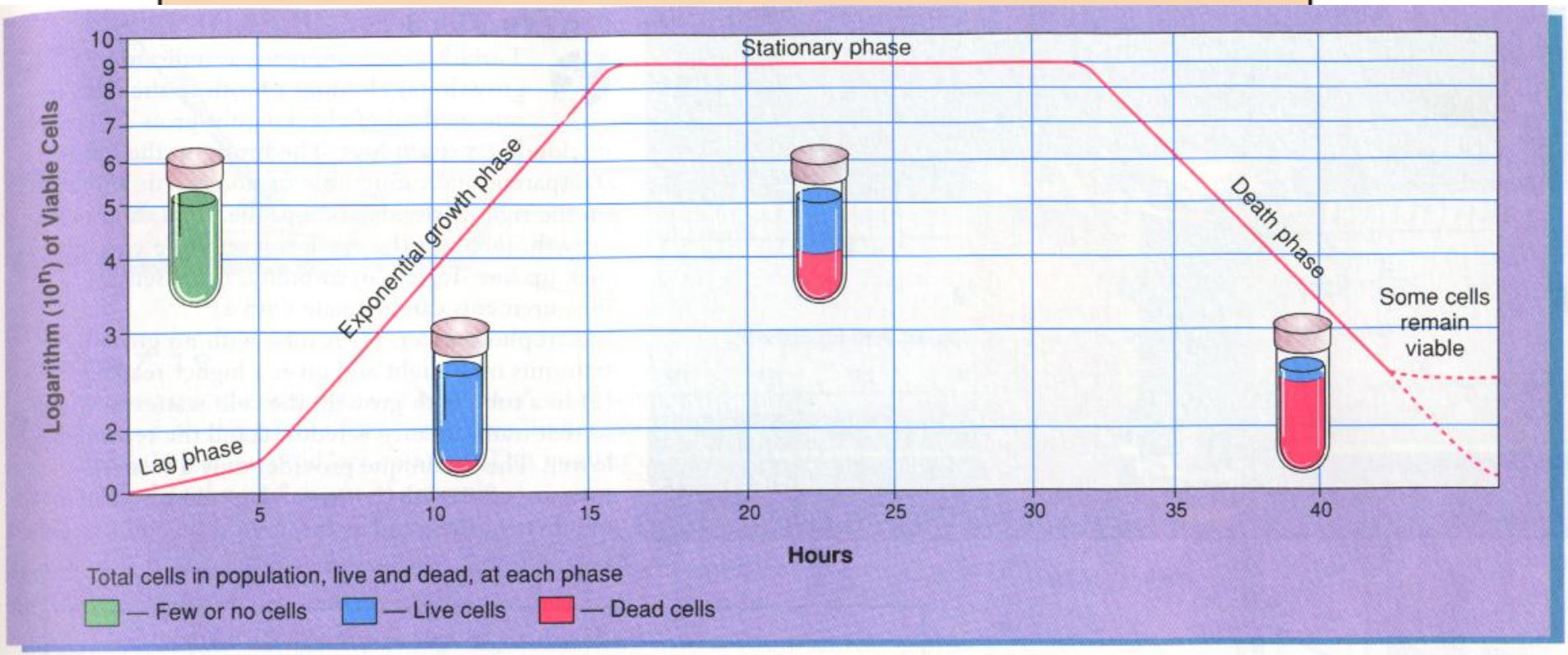


Figure 7.17

The growth curve in a bacterial culture. On this graph, the number of viable cells expressed as a logarithm (log) is plotted against time. See text for discussion of the various phases. Note that with a generation time of 30 minutes, the population has risen from 10 (10^1) cells to 1,000,000,000 (10^9) cells in only 16 hours. Cells are multiplying and dying all along the curve, but the relative rates of these events change as the curve proceeds.

Four phases: lag (延滞), exponential (指数), stationary (稳定) and death (decline, 衰亡).

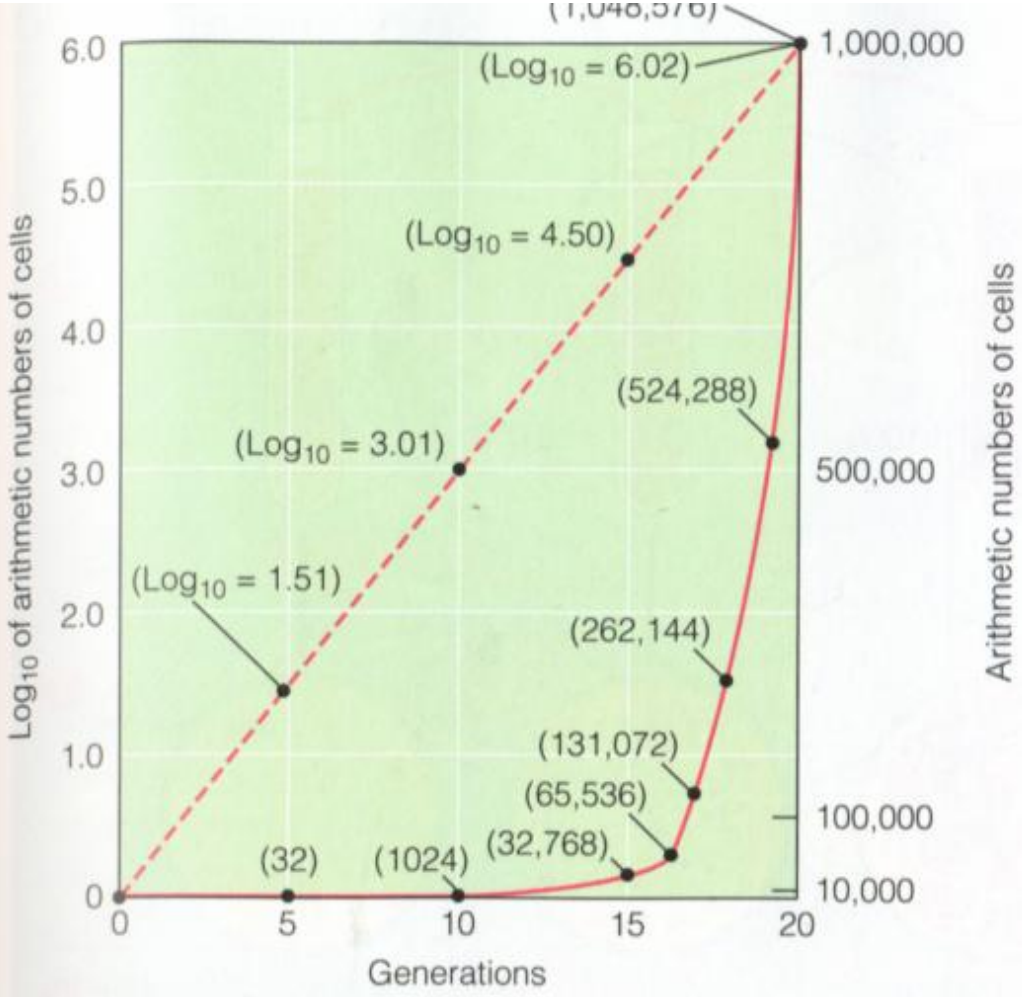


Figure 6.13 A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line).

If the arithmetic line were plotted for two more generations, would it still be on the page?

Generation Number	Arithmetic Number of Cells	Log_{10} of Arithmetic Number of Cells
0	1	0
5 (2^5) =	32	1.51
10 (2^{10}) =	1,024	3.01
15 (2^{15}) =	32,768	4.52
16 (2^{16}) =	65,536	4.82
17 (2^{17}) =	131,072	5.12
18 (2^{18}) =	262,144	5.42
19 (2^{19}) =	524,288	5.72
20 (2^{20}) =	1,048,576	6.02

lag phase（适应期，又称延滞期、调整期)

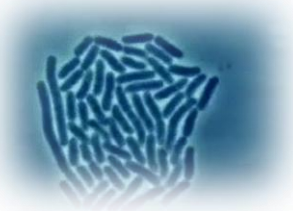
(1) 生长繁殖的速度几乎等于零

(2) 细胞形态增大，杆菌的长度增加。

(3) 细胞内核酸含量增加，原生质呈嗜碱性。

(4) 合成代谢活跃，核糖体、酶类合成加快，易产生诱导酶。

(5) 对外界不良条件，温度和抗生素敏感。



影响适应期因素：

（1）菌种的种类

（2）菌种的菌龄

（3）接种量

（4）培养基成分

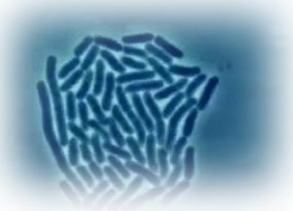
缩短适应期措施：

（1）遗传学方法改变种的遗传特性

（2）用对数生长期的做种子

（3）接种前后培养基成分不要相差太大

（4）适当扩大接种量



exponential phase（对数增长期）

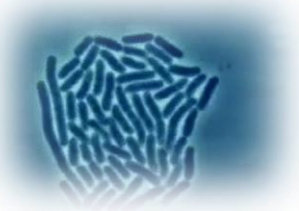
（1）生长繁殖速度快，呈对数增长，世代时间最短。

（2）细菌体内各种成分最为均匀。

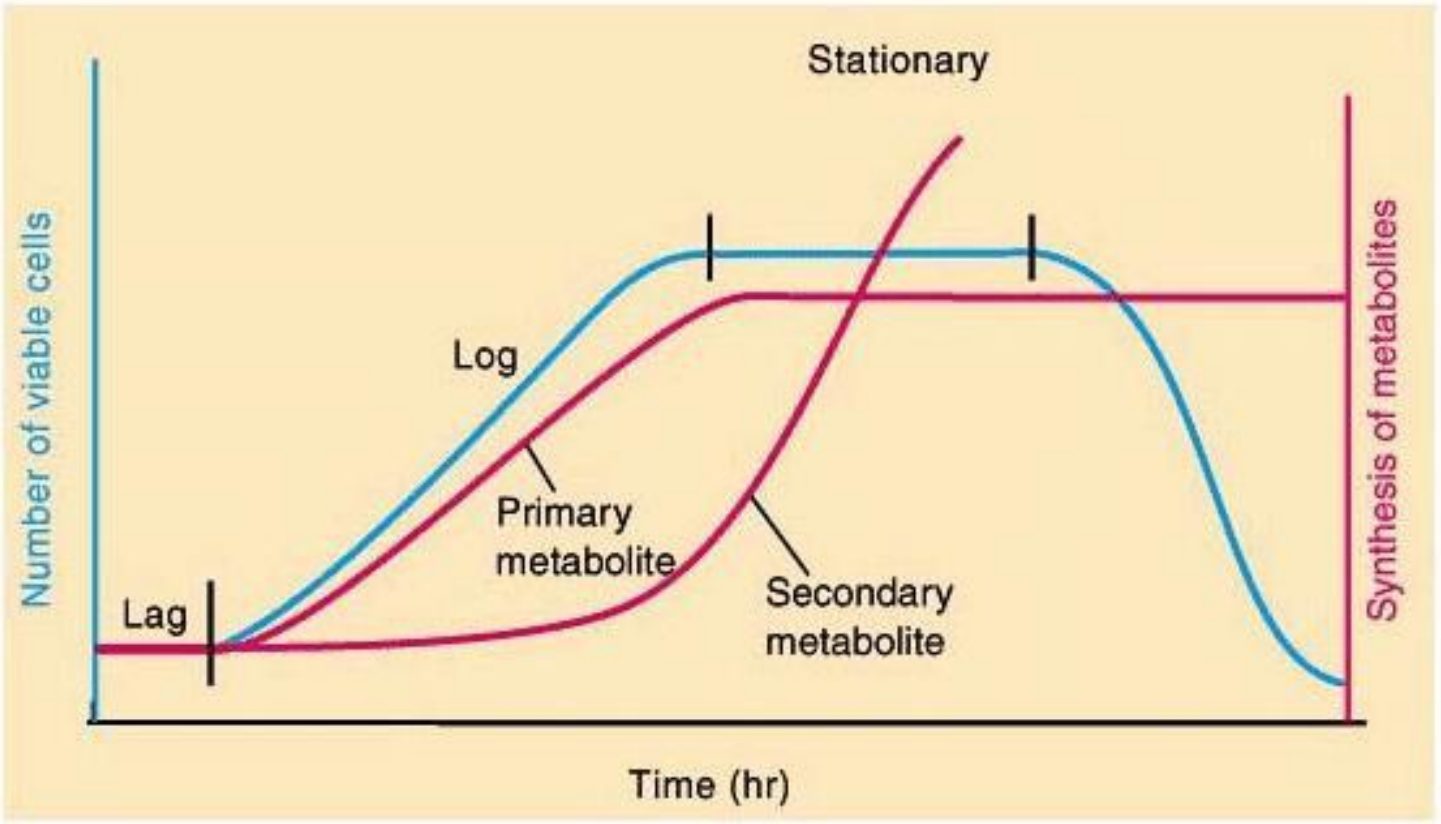
（3）酶系活跃，代谢旺盛。

（4）细菌细胞的形态特征均匀一致，最代表种的特征。

（5）微生物的生化特性均匀一致，并且典型。



stationary phase（稳定期）

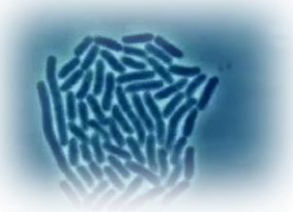


- Accumulation of **secondary metabolite** (次级代谢物)

微生物次级代谢及次级代谢产物

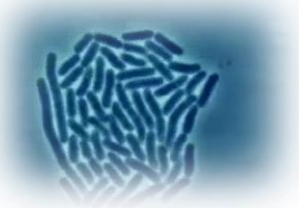
微生物从外界吸收营养，生成维持生命活动物质和能量的过程为初级代谢。

次级代谢以初级代谢产物为前体，合成一些对微生物生命活动无明确功能的一些物质的过程。有：抗生素、激素、生物碱、毒素、维生素等



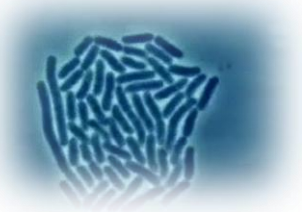
death phase（衰亡期）

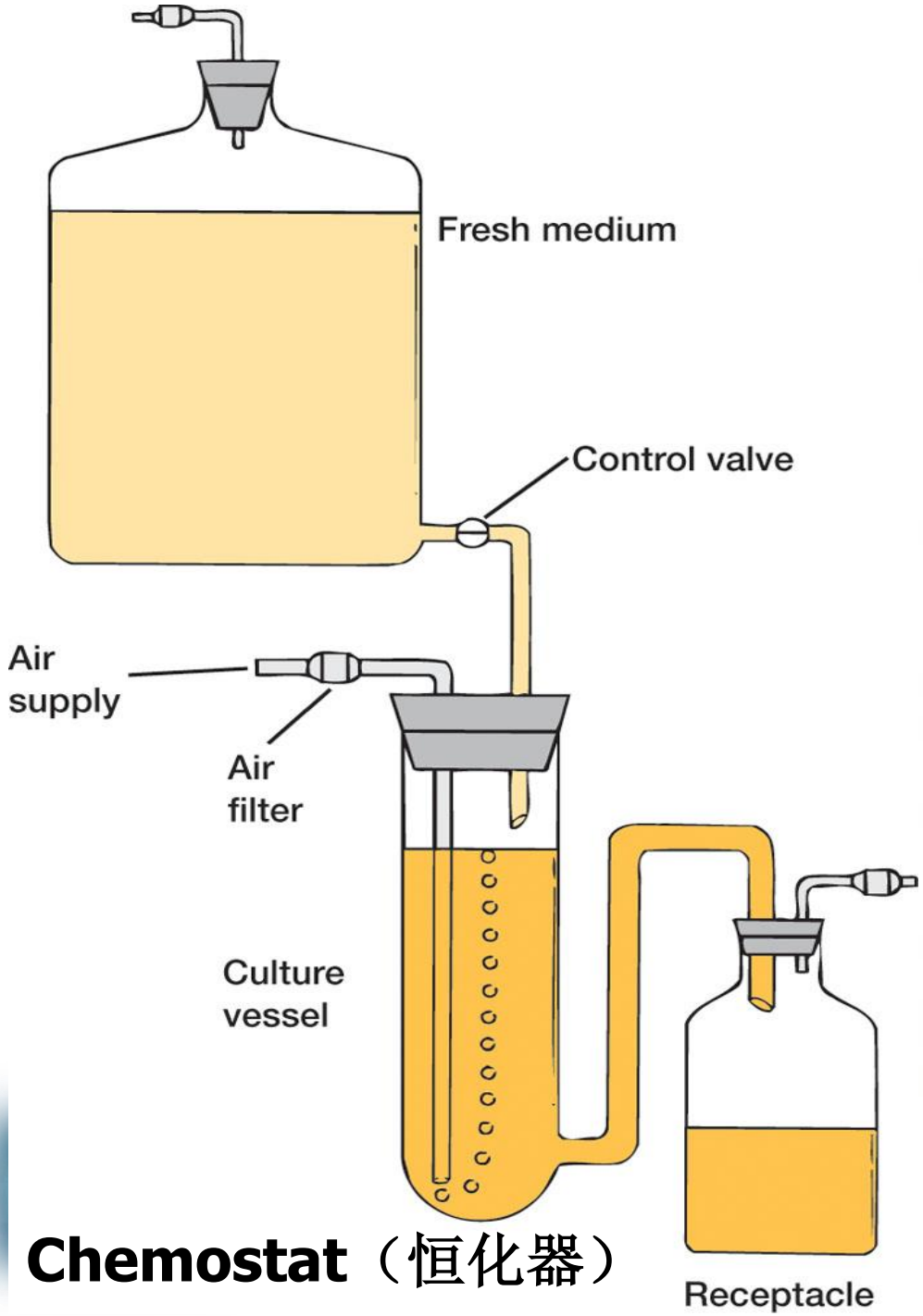
细胞形态出现不正常，呈多样性，细胞种的特征典型、生理生化出现异常现象。



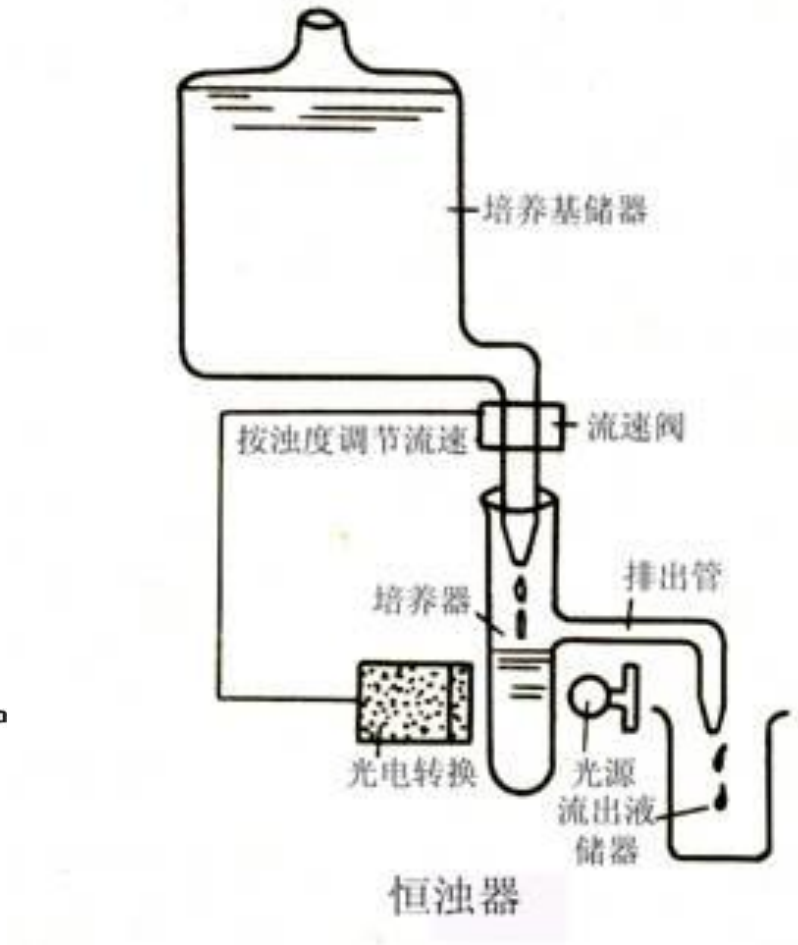
Batch and Continuous Growth

- **batch culture (批培养) -- a closed system without the addition of new material.**
- **continuous culture (连续培养) -- nutrients are supplied and end products continuously removed so that the exponential growth phase is maintained.**
  - ❖**Chemostat (恒化器)**
  - ❖**Turbidostat (恒浊器)**





Chemostat（恒化器）

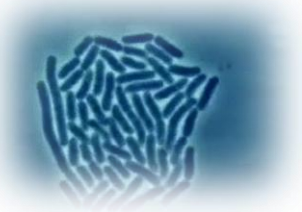


Turbidostat（恒浊器）

同步培养（synchronous culture）

同步培养法：所有微生物细胞处于相同的生长阶段的培养方法。

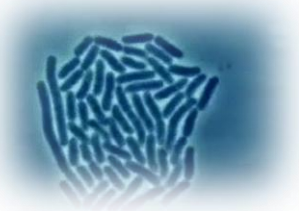
同步生长：培养物中所有的微生物细胞都处于同一生长阶段，并能同时分裂的生长方式。



如何获得同步培养?

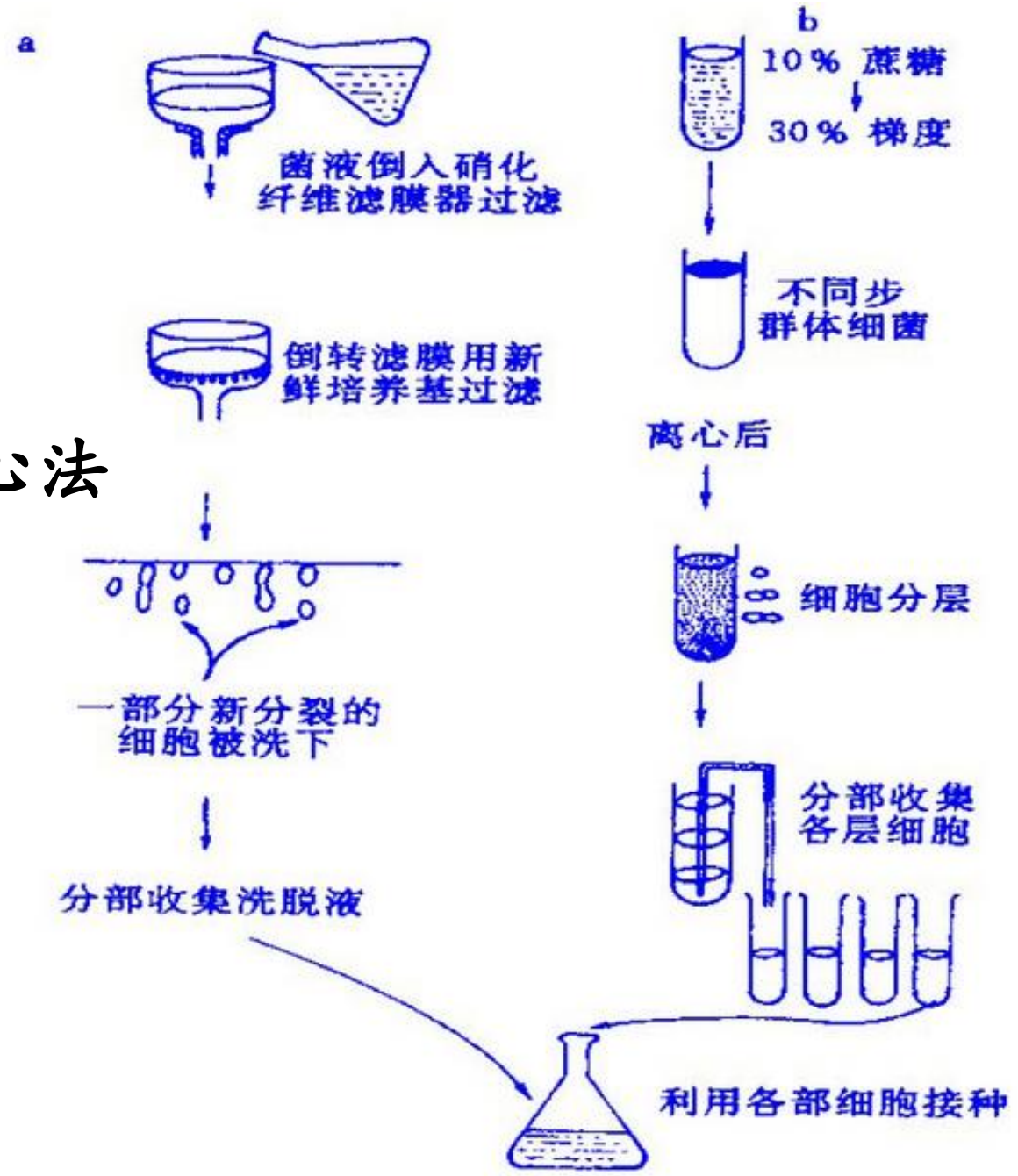
诱导法：采用理化因子使微生物在某生长阶段停下来，再去除该因子，以达到诱导微生物同步生长的目的。

选择法：膜过滤和密度梯度离心法。



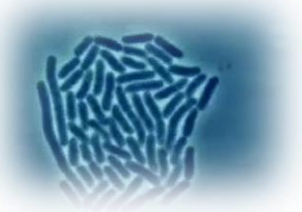
选择法：

- 膜过滤
- 密度梯度离心法

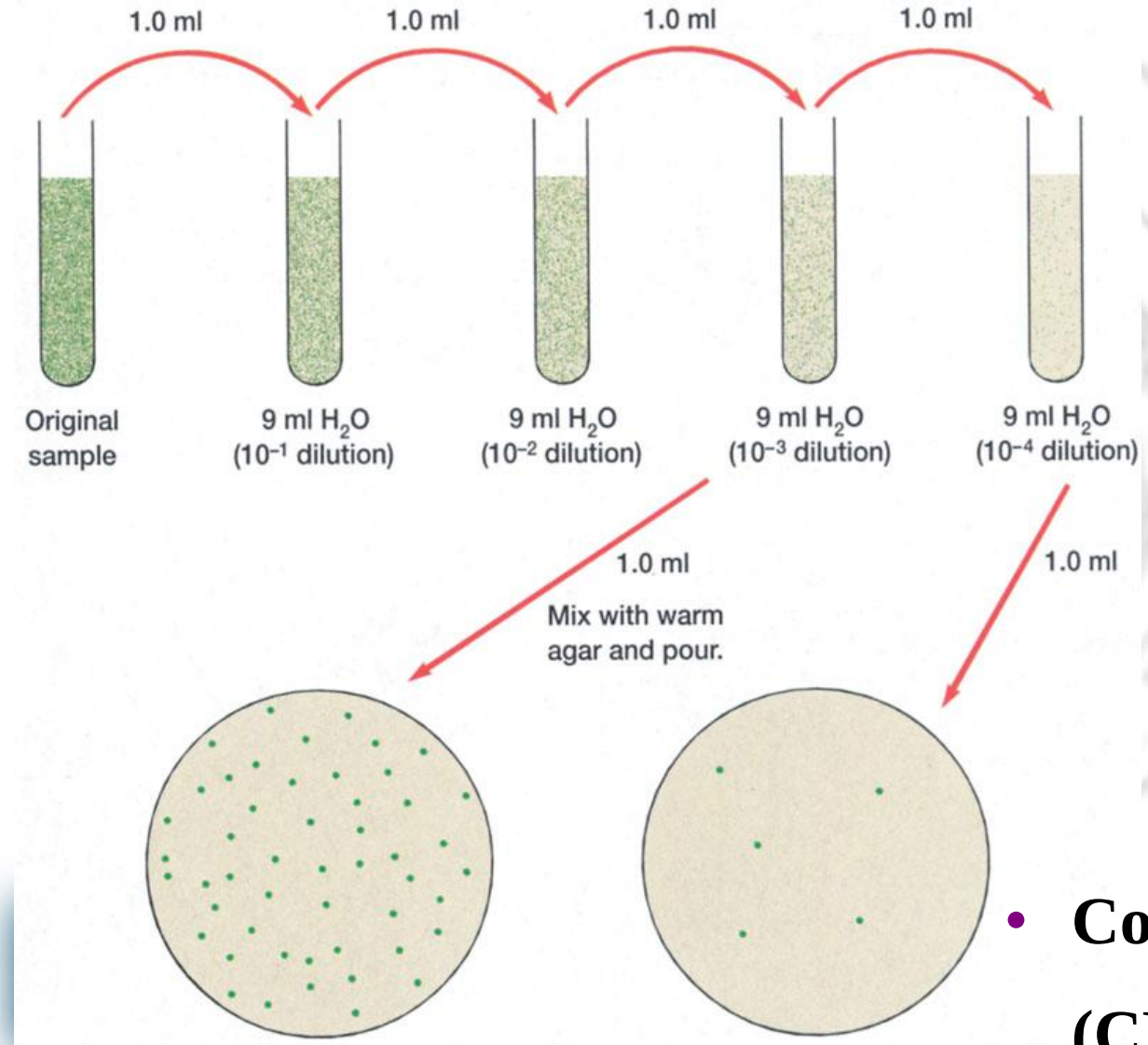


measurement of Bacteria

- viable count procedure
  - spread plate technique
  - pour plate technique
  - membrane filtration procedure
  - most probable number procedure
- directed count procedure
- other procedures



Viable Count Procedures

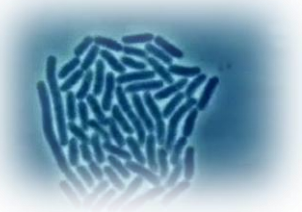


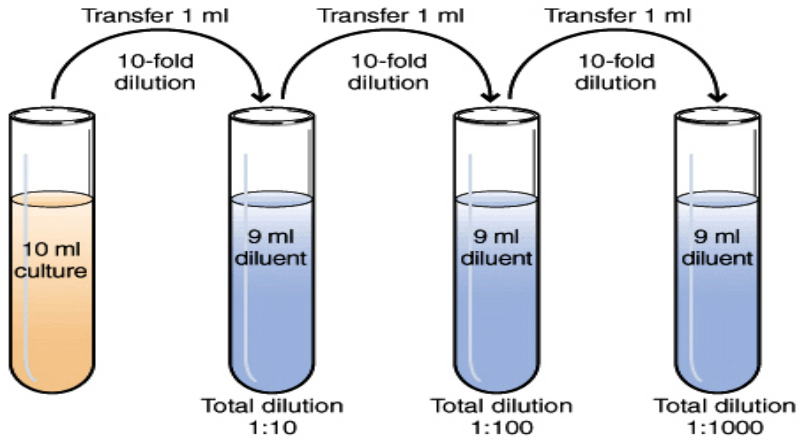
- **Colony forming unit (CFU, 菌落形成单位)**

Viable Count Procedures

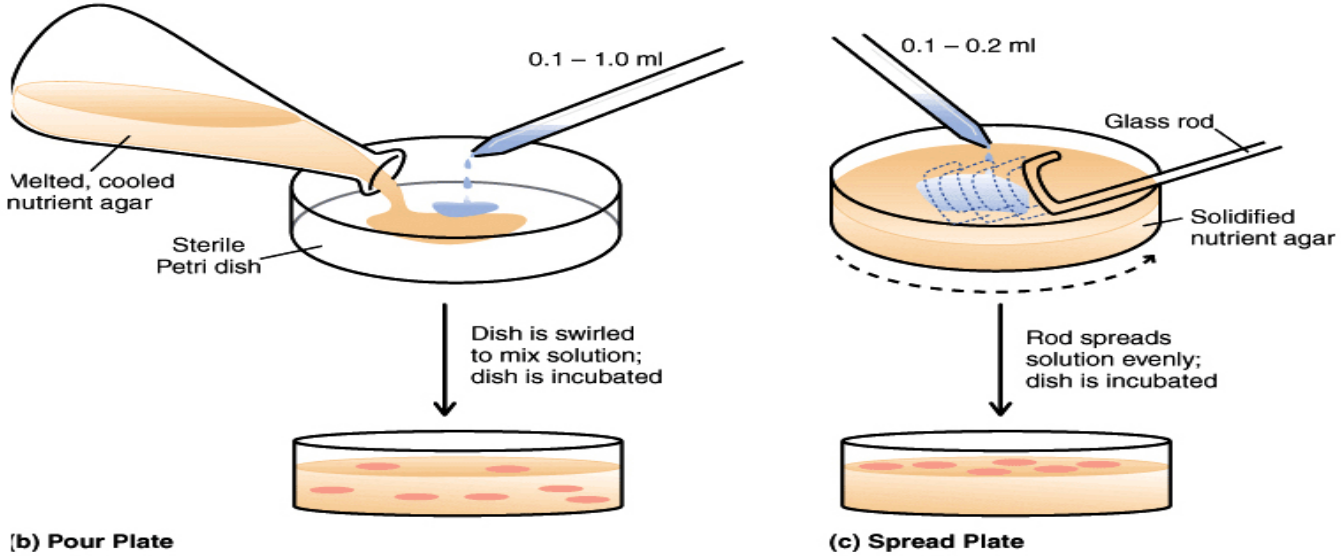
- **30 ~ 300 colonies/ plate**
- **A major limitation is that it is selective. Why?**

  -- Cells that are capable of growth on the given medium under the set of incubation conditions.
- **Unsuitable for that grow in chains or clumps that are hard to disperse.**





(a) Serial Dilutions

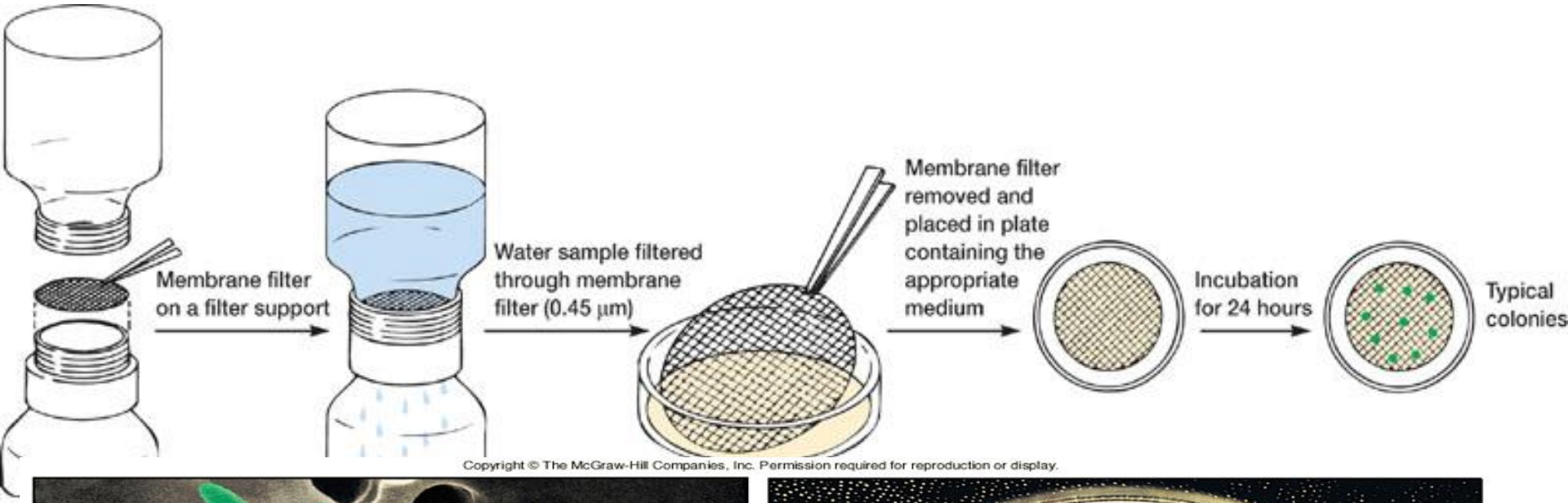


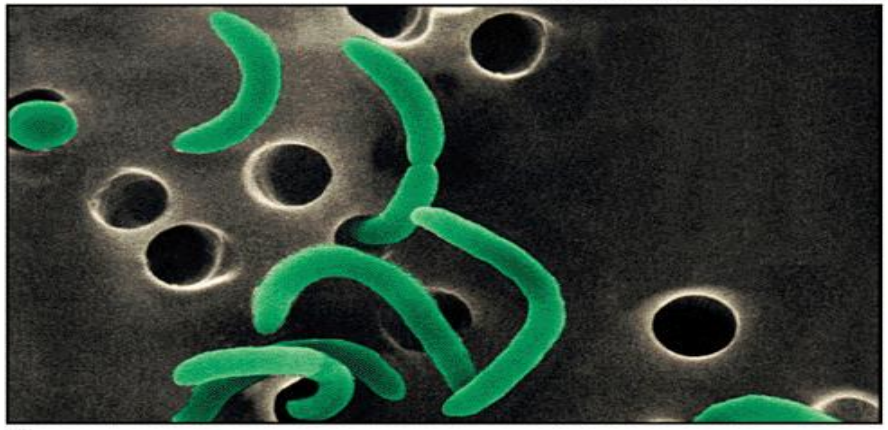
(b) Pour Plate

(c) Spread Plate

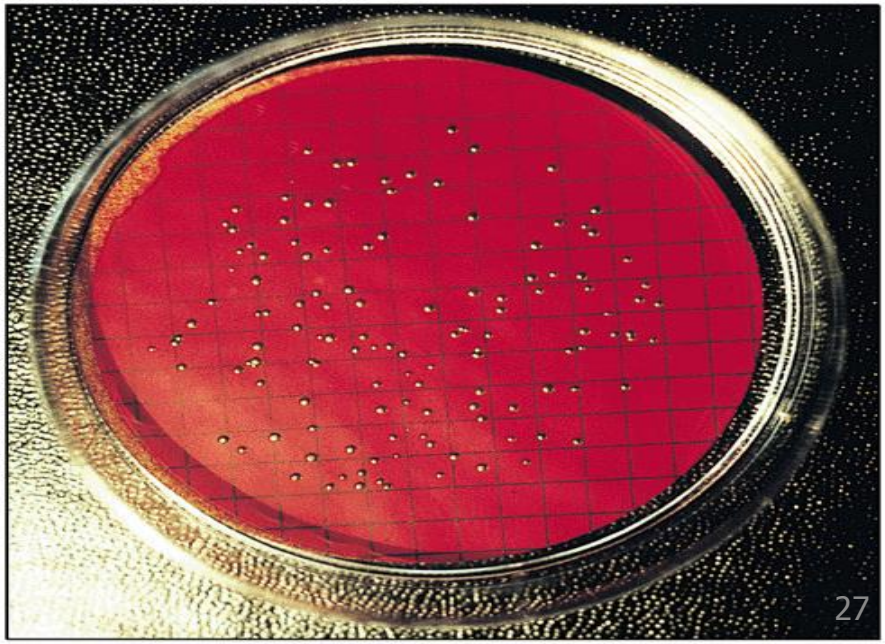
- **spread plate technique (平板涂布法)**
- **pour plate technique (平板倾注法)**

Membrane Filtration Procedure





(a)



(b)

Most probable number procedure

如某一细菌在稀释法中的生长情况如下：

稀释度	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
重复数	5	5	5	5	5	5
出现生长的管数	5	5	5	4	1	0

稀释度	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
重复数	4	4	4	4	4	4
出现生长的管数	4	4	3	2	1？	0

HJ

中华人民共

水质

多管发酵

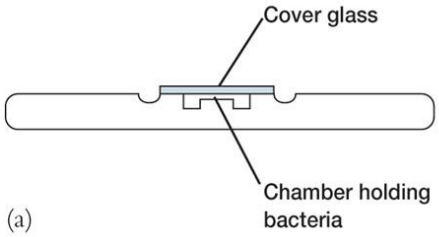
Water quality—Determinatio

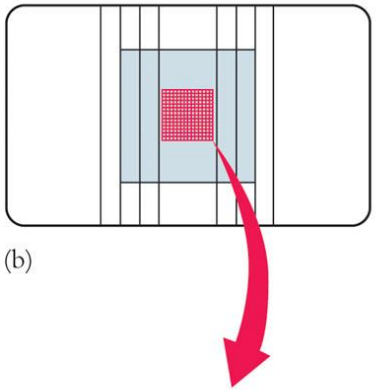
表 XIV-2　大肠菌群检数表

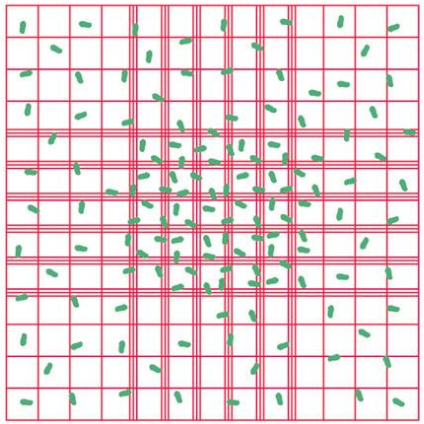
接种水样总量 300ml（ 100ml2 份， 10ml10 份）

10ml 水量的阳性管数 \ 100ml 水量的阳性管数	0	1	2
	每升水样中大肠菌群数	每升水样中大肠菌群数	每升水样中大肠菌群数
0	< 3	4	11
1	3	8	18
2	7	13	27
3	11	18	38
4	14	24	52
5	18	30	70
6	22	36	92
7	27	43	120
8	31	51	161
9	36	60	230
10	40	69	> 230

Direct Count Procedures



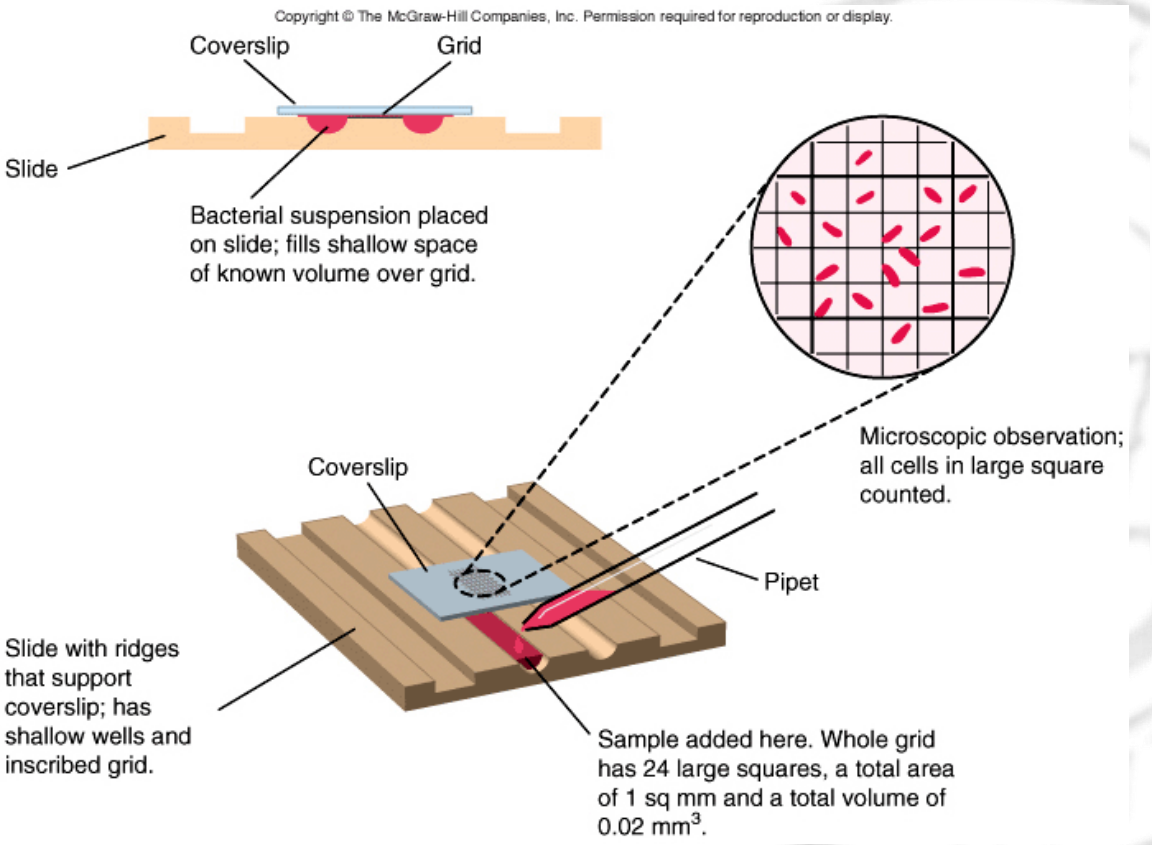


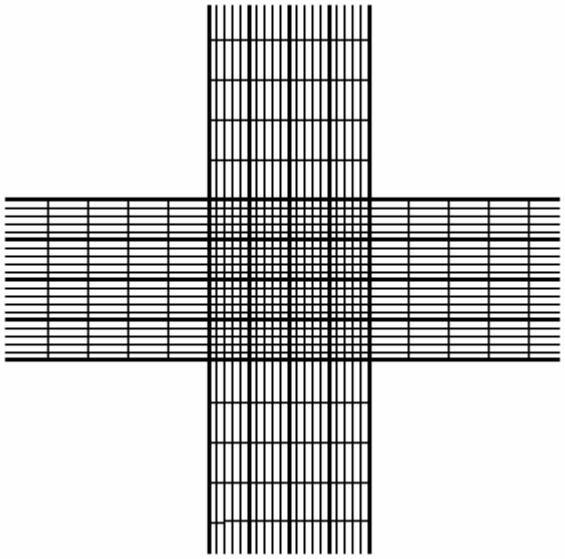


- do not need bacteria culture
- Hemocytometers (血细胞计数器)
- the limitation?

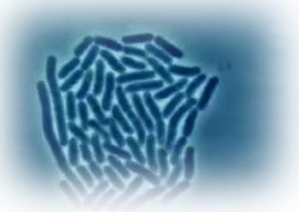
-- in establishing the metabolic status of the observed bacteria.

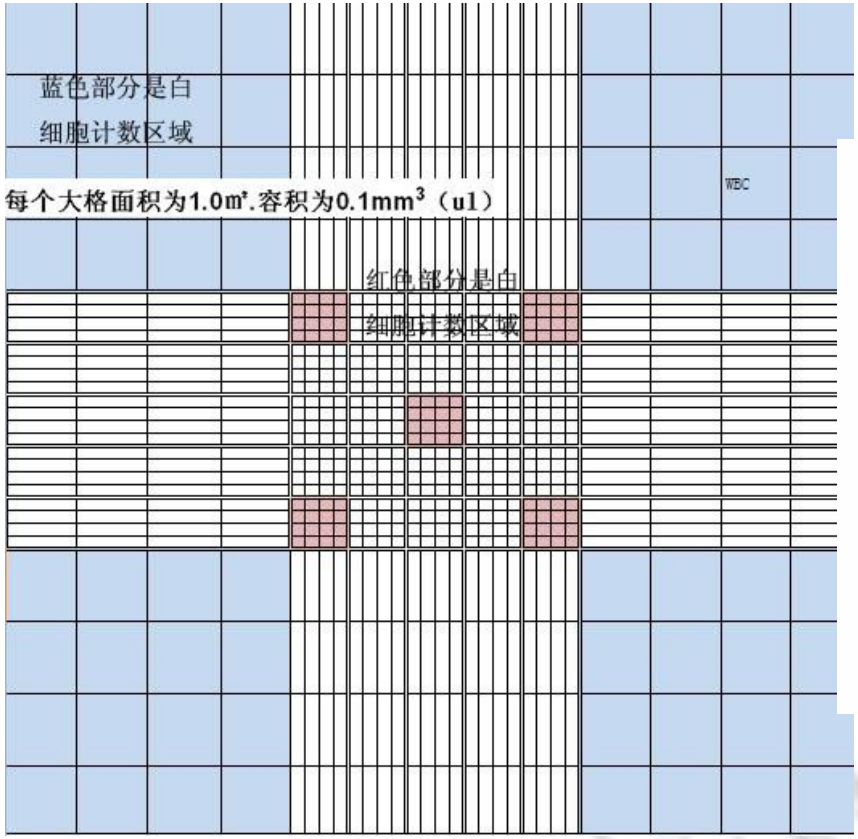
Direct Count Procedures



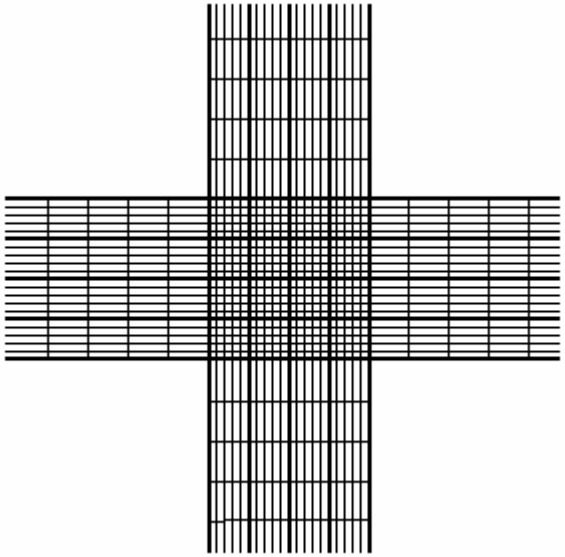


计数室0.1mm^3
占1ml体积的1/10000

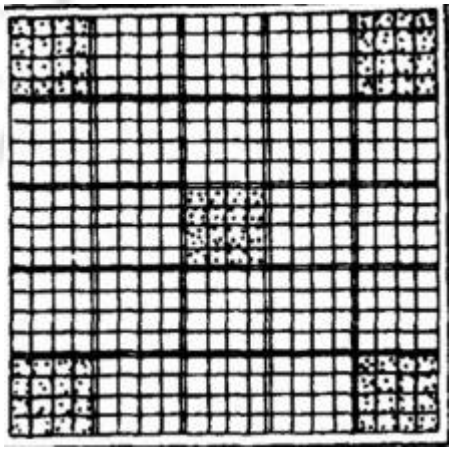




16×25型：麦氏血球计数板



25×16型：希里格式血球计数板

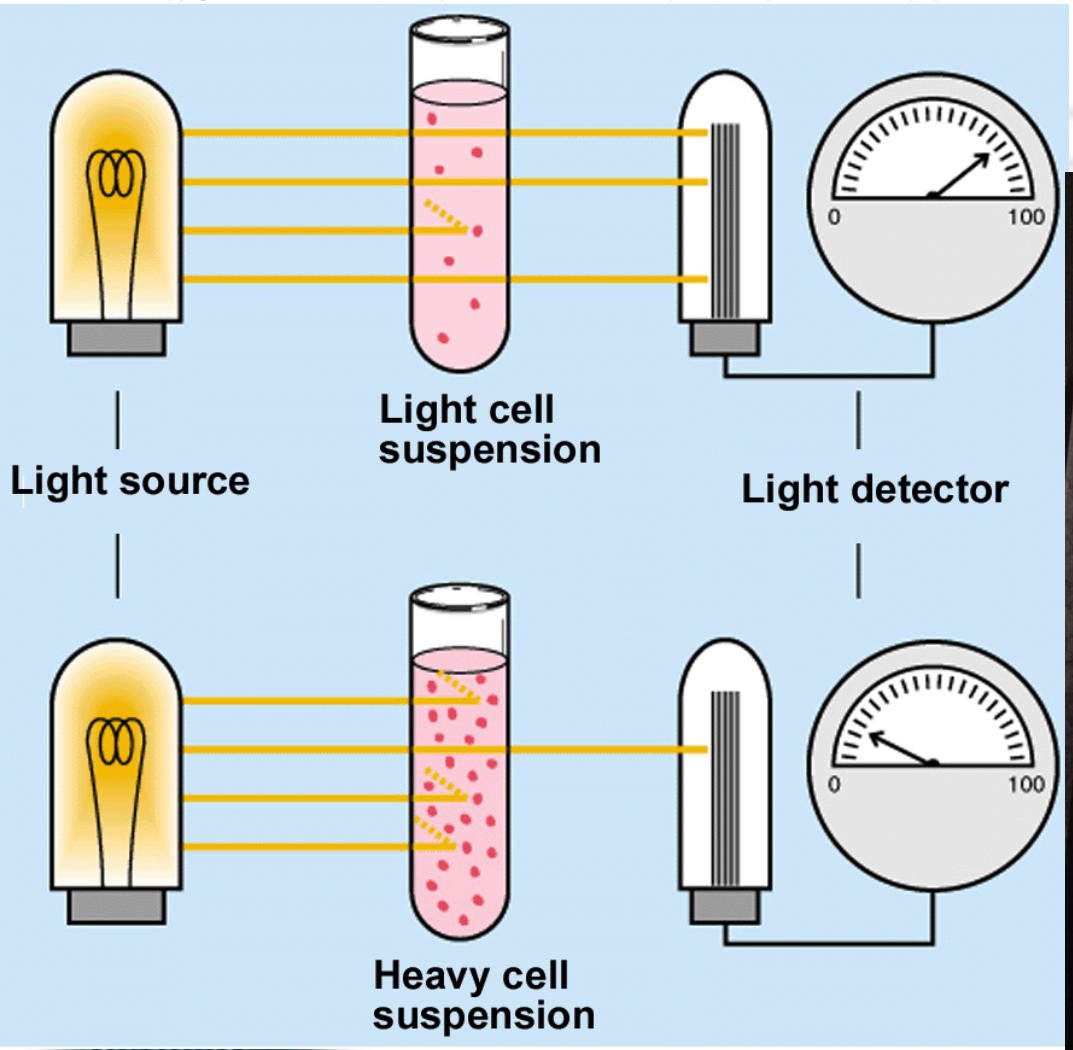


每ml细菌数=

每小格平均数×400 ×10000 ×稀释倍数

计数时数上不数下，数左不数右

Measuring Turbidity



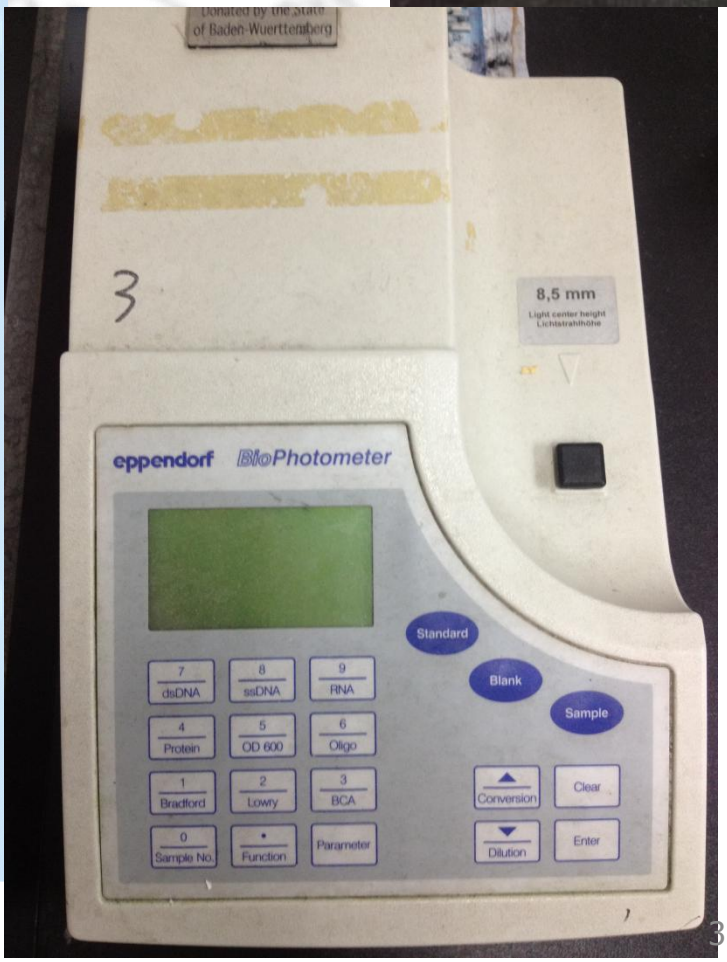
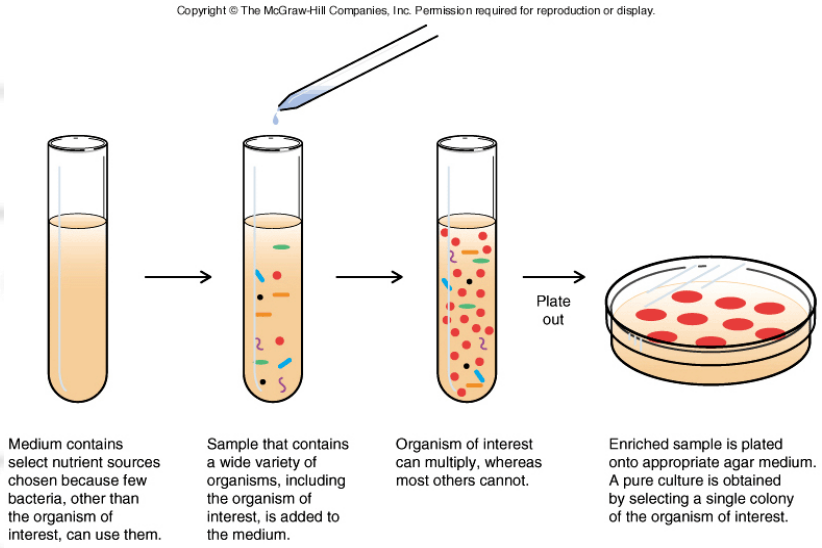


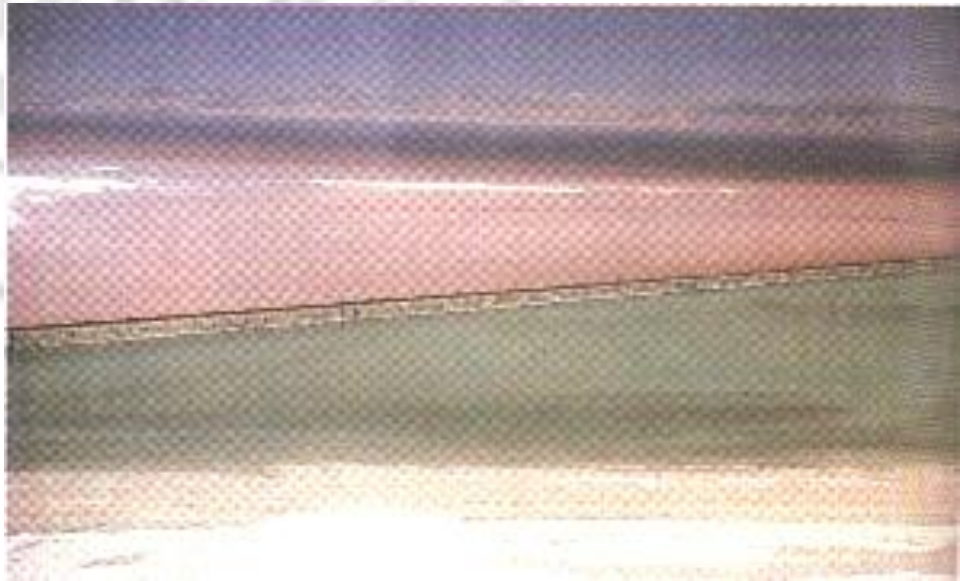
TABLE 4.1 Methods Used to Measure Bacterial Growth

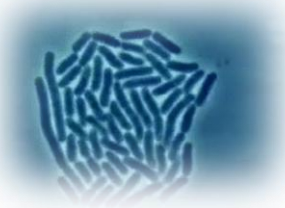
Method	Characteristics and Limitations
Direct Cell Counts	Used to determine total number of cells; can be used for those bacteria that cannot be cultured.
Direct microscopic count	Rapid, but at least 10^7 cells/ml must be present to be effectively counted. Counts include living and dead cells.
Cell-counting instruments	Coulter counters and flow cytometers count total cells in dilute solutions. Flow cytometers can also be used to count organisms to which fluorescent dyes or tags have been attached.
Viable Cell Counts	Used to determine the number of viable bacteria in a sample, but only includes those that can grow in given conditions. Requires an incubation period of approximately 24 hours or longer. Selective and differential media can be used to enumerate specific species of bacteria.
Plate count	Time-consuming but technically simple method that does not require sophisticated equipment. Generally used only if the sample has at least 10^2 cells/ml.
Membrane filtration	Concentrates bacteria by filtration before they are plated; thus can be used to count cells in dilute environments.
Most probable number	Statistical estimation of likely cell number; it is not a precise measurement. Can be used to estimate numbers of bacteria in relatively dilute solutions.
Measuring Biomass	Biomass can be correlated to cell number.
Turbidity	Very rapid method; used routinely. A one-time correlation with plate counts is required in order to use turbidity for determining cell number.
Total weight	Tedious and time-consuming; however, it is one of the best methods for measuring the growth of filamentous microorganisms.
Chemical constituents	Uses chemical means to determine the amount of a given element, usually nitrogen. Not routinely used.
Measuring Cell Products	Methods are rapid but must be correlated to cell number. Frequently used to detect growth, but not routinely used for quantitation.
Acid	Titration can be used to quantify acid production. A pH indicator is often used to detect growth.
Gases	Carbon dioxide can be detected by using a molecule that fluoresces when the medium becomes slightly more acidic. Gases can be trapped in an inverted Durham tube in a tube of broth.
Luminescence	Firefly luciferase catalyzes light-emitting reaction when ATP is present.

Factors Influencing Bacterial Growth

- 营养：加富培养基
- 温度：培养箱，嗜热菌
- 氧气：摇床或厌氧
- 盐度：嗜盐菌（3%-15%NaCl）
- pH：嗜酸菌
- 辐射：有色素菌耐辐射







How to control the growth of bacteria

- 1）Control by Physical Factors （物理因素）
  - ❖Physical Exclusion of Microorganisms（过滤）：气体和液体
  - ❖High Temperatures
    - Pasteurization (巴斯德消毒法)
    - Sterilization (灭菌)
  - ❖Low Temperatures
  - ❖Removal of Water
  - ❖Radiation : UV

Pasteurization (巴斯德消毒法)

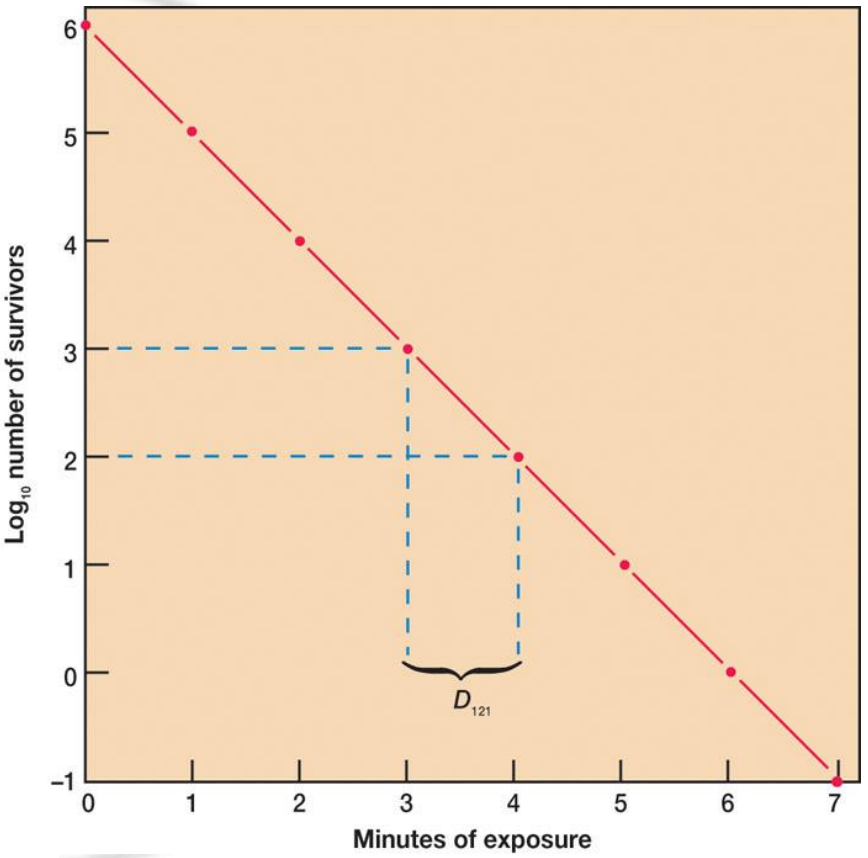
- **Brief exposures to moderately high temperatures to reduce viable microorganisms and eliminate human pathogens but does not eliminate all viable microorganisms.**
- **LTH (Low Temperature-Hold, 低温维持) process —— 62.8℃, 30 min.**
- **HTST (High Temperature-Short Time, 高温瞬间) process —— 71.7℃, 15 s.**

135-150℃，5-15 秒，工业上发酵培养基
135-150℃，2-6 秒，牛奶或其它液态食品

（超高温灭菌, UT）

High Temperatures

- **Thermal death point (TDP, 热力致死温度) is the lowest temperature required to kill all of the microorganisms in a liquid suspension in 10 minutes.**
- **Decimal reduction time (D value, 十倍致死时间) : the time required for a ten-fold reduction in the number of viable cells at a given temperature.**

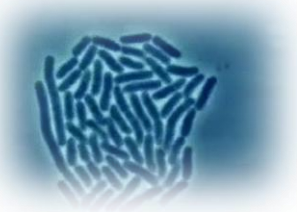


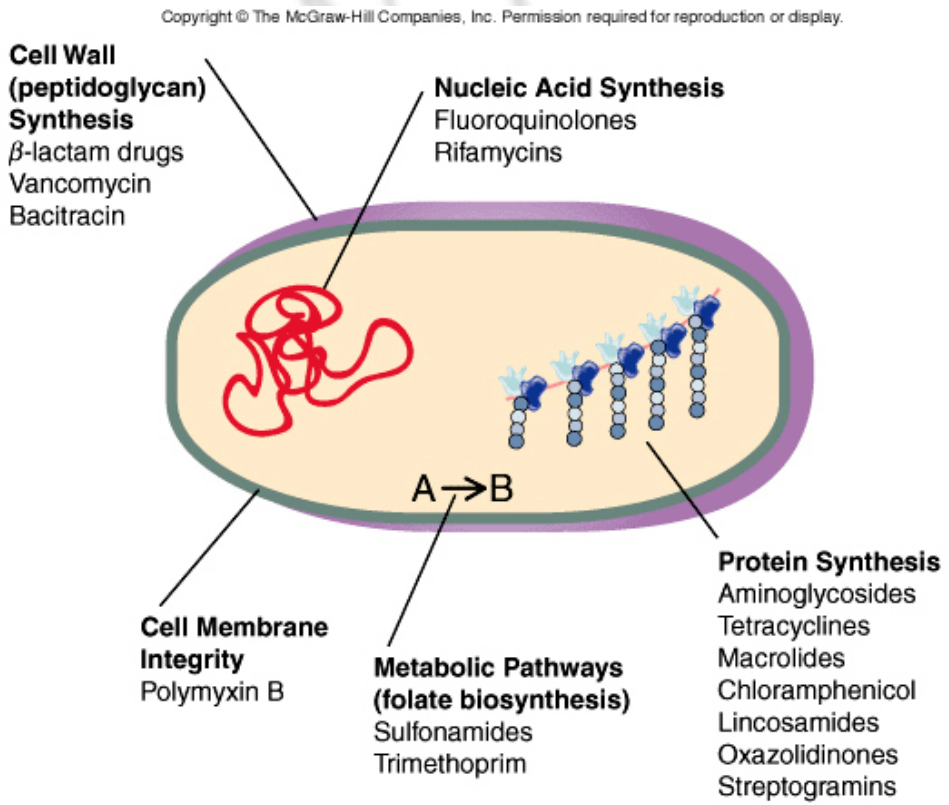
2）Control of Microbial Growth by Antimicrobial Agents

- **Antimicrobial agents:** chemicals that kill or prevent the growth of microorganisms.
- **Growing** microorganisms are more **sensitive** than dormant stages.
  - ❖**Disinfectant (消毒剂)：** 杀死或灭活所有病原微生物
  - ❖**Antiseptic (防腐剂)：** 抑制微生物生长
  - ❖**Chemotherapeutic agent**
    - ➢antibiotics

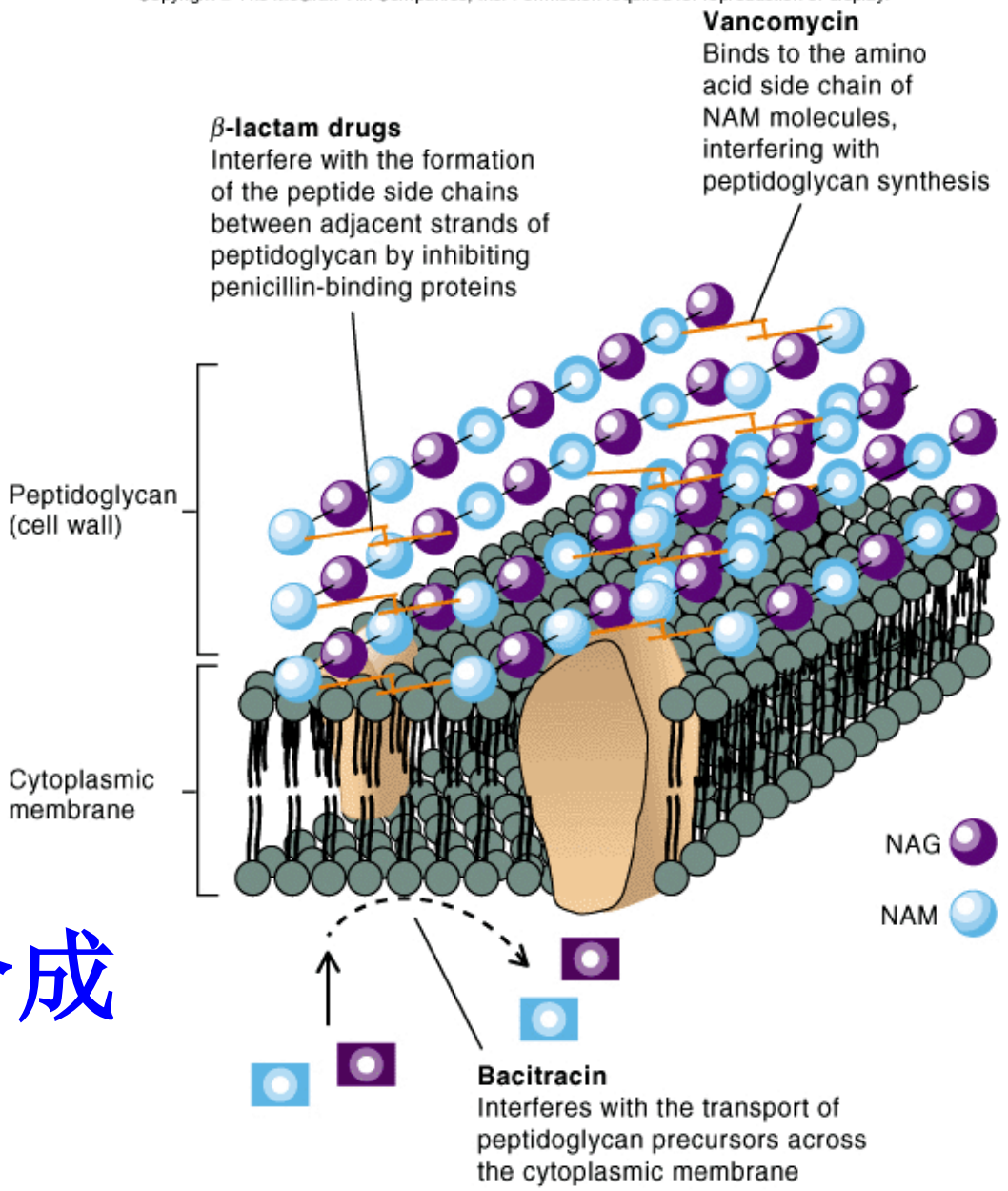
Antibiotics

- interfere with cell wall synthesis
- inhibit protein synthesis
- damage plasma membrane
- inhibit nucleic acid synthesis
- Metabolic pathway

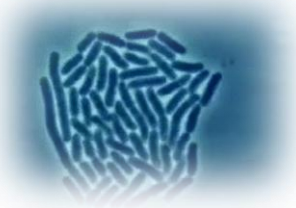




名称及类型	作用机制	作用后果
抑制细胞壁合成		
D-环丝氨酸	抑制 L-ala 变为 D-al	
万古霉素	抑制糖肽聚合物的	
瑞斯托菌素	抑制糖肽聚合物的	
杆菌肽	抑制糖肽聚合物的	
青霉素	抑制肽尾与肽桥间	
氨苄青霉素	抑制肽尾与肽桥间	
头孢菌素	抑制肽尾与肽桥间	
引起细胞壁降解		
溶葡球菌素	水解肽尾和分解胞	

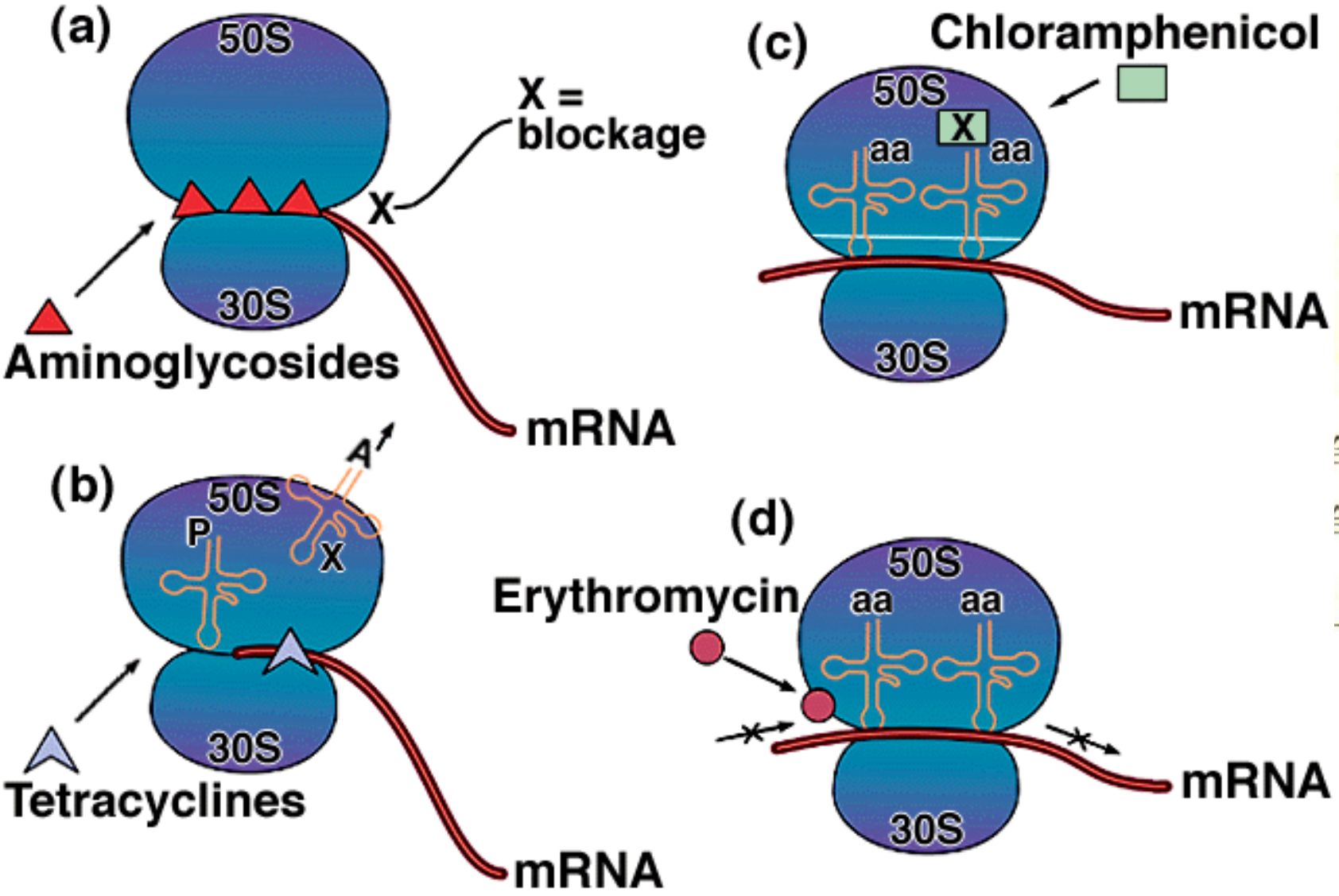


干扰CW合成



干扰细胞膜
短杆菌酪
短杆菌肽
多粘菌素
抑制蛋白质
链霉素
新霉素
卡那霉素
四环素
伊短菌素
噁吟霉素
氯霉素
红霉素
林可霉

Effects of drugs on ribosomes



干扰蛋白合成

磺胺药物最早发现，最常见的化学疗剂，抗菌谱广，能治疗多种传染性疾病。

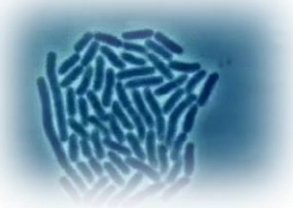
- 大多数革兰氏阳性细菌（如肺炎球菌、溶血性链球菌等）
- 某些革兰氏阴性细菌（如痢疾杆菌、脑膜炎球菌、流感杆菌等）

对放线菌也有一定的作用。

1934 年，德国 I. G. Farben 染料厂的 G. Domagk

一种红色染料（prontosil，百浪多息），白鼠静脉注射，可治疗因链球菌引起的感染，但在体外却无作用。

1935 年，进一步证明 prontosil 对人的链球菌病也有效。



Glu

+

PABA

+

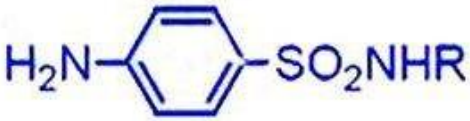
dihydropterin

FH_2 synthetase

FH_2

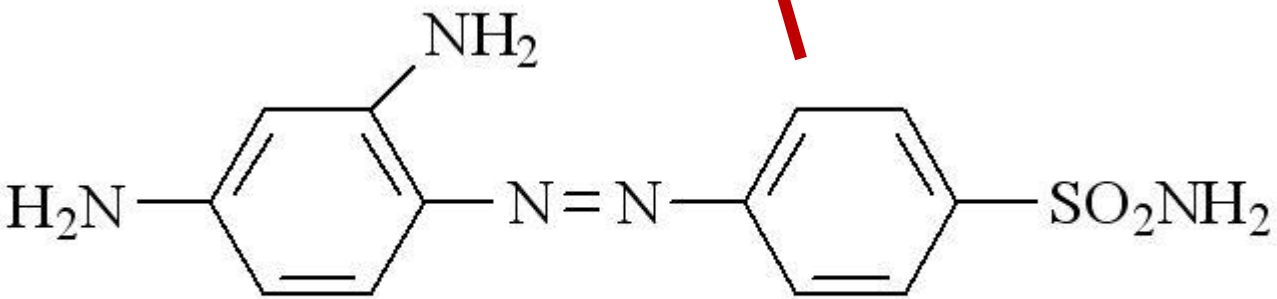
FH_2 reductase

FH_4



Sulfanilamide

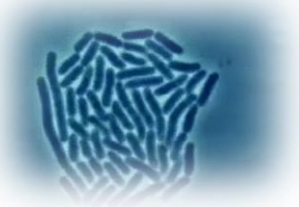
Methotrexate



百浪多息

本章小结

- 细菌群体生长（生长曲线、代时、批培养、同步培养和连续培养）
- 细菌生长测定
- 细菌生长繁殖影响因素



1.杆菌和球菌分裂时肽聚糖分布特点

2.细菌生长曲线，各时期都有何特点?

3.什么叫连续培养? 连续培养有哪两类? 什么是同步培养? 同步培养有哪些方法?

4.细菌计数方法有哪些?

5.CFU

6.影响微生物生长的因素有哪些?

7.如何控制微生物生长?

8.热力致死温度， D value

9.抗生素杀菌机理

10.抗生素生产菌为何不会杀死自己?