CHAPTER 3

Growth

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GROWTH CYCLE

Bacteria reproduce by **binary fission,** a process by which one parent cell divides to form two progeny cells. Because one cell gives rise to two progeny cells, bacteria are said to undergo exponential growth (logarithmic growth). The concept of exponential growth can be illustrated by the following relationship:

Number of cells	1	2	4	8	16
Exponential	2^0	2^1	2^2	2^3	2^4

Thus, 1 bacterium will produce 16 bacteria after 4 generations.

The doubling (generation) time of bacteria ranges from as little as 20 minutes for *Escherichia coli* to as long as 18 hours for *Mycobacterium tuberculosis.* The exponential growth and the short doubling time of some organisms result in rapid production of very large numbers of bacteria. For example, 1 *E. coli* organism will produce over 1000 progeny in about 3 hours and over 1 million in about 7 hours. The doubling time varies not only with the species, but also with the amount of nutrients, the temperature, the pH, and other environmental factors.

The growth cycle of bacteria has four major phases. If a small number of bacteria are inoculated into a liquid nutrient medium and the bacteria are counted at frequent intervals, the typical phases of a standard growth curve can be demonstrated (Figure 3–1).

(1) The first is the **lag** phase, during which vigorous metabolic activity occurs but cells do not divide. This can last for a few minutes up to many hours.

(2) The **log** (logarithmic) phase is when rapid cell division occurs. β-Lactam drugs, such as penicillin, act during this phase because the drugs are effective when cells are making peptidoglycan (i.e., when they are dividing). The log phase is also known as the **exponential** phase.

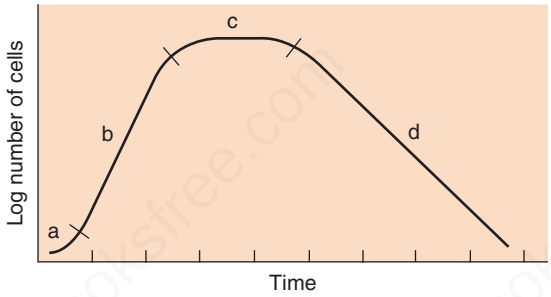

FIGURE 3–1 Growth curve of bacteria: a, lag phase; b, log phase; c, stationary phase; d, death phase. (Reproduced with permission from Joklik WK et al. *Zinsser Microbiology.* 20th ed. Originally published by Appleton & Lange. Copyright 1992, McGraw-Hill.)

(3) The **stationary** phase occurs when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die, resulting in a steady state. Cells grown in a special apparatus called a "chemostat," into which fresh nutrients are added and from which waste products are removed continuously, can remain in the log phase and do not enter the stationary phase.

(4) The final phase is the **death** phase, which is marked by a decline in the number of viable bacteria.

OBLIGATE INTRACELLULAR GROWTH

Most bacterial pathogens of humans are capable of growing on artificial media in the clinical laboratory. The term, artificial, means that the medium is composed of purified

chemicals such as sugars, amino acids, and salts, such as sodium chloride. Often blood is added in the form of sheep's blood but that is for nutritional purposes rather than for the need of the bacteria to grow within the red blood cells.

However, certain bacterial pathogens of humans, notably *Chlamydia* and *Rickettsia* (see Chapters 25 and 26, respectively), and *Ehrlichia* and *Anaplasma* (see Chapter 26) can *only* grow within living cells. They are **obligate intracellular parasites**, meaning that it is obligatory that they grow within cells. The main reason for this is that they lack the ability to produce sufficient ATP and must use ATP produced by the host cells.

AEROBIC & ANAEROBIC GROWTH

For most organisms, an adequate supply of oxygen enhances metabolism and growth. The oxygen acts as the hydrogen acceptor in the final steps of energy production catalyzed by the flavoproteins and cytochromes. Because the use of oxygen generates two toxic molecules, hydrogen peroxide (H_2O_2) and the free radical superoxide (O_2^-) bacteria require two enzymes to detoxify these molecules when oxygen is utilized. The first is **superoxide dismutase,** which catalyzes the reaction

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

and the second is **catalase,** which catalyzes the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The response to oxygen is an important criterion for classifying bacteria and has great practical significance because specimens from patients must be incubated in a proper atmosphere for the bacteria to grow.

(1) Some bacteria, such as *M. tuberculosis,* are **obligate aerobes;** that is, they require oxygen to grow because their ATP-generating system is dependent on oxygen as the hydrogen acceptor.

(2) Other bacteria, such as *E. coli,* are **facultative anaerobes;** they utilize oxygen, if it is present, to generate energy by respiration, but they can use the fermentation pathway to synthesize ATP in the absence of sufficient oxygen.

(3) The third group of bacteria consists of the **obligate anaerobes,** such as *Clostridium tetani,* which cannot grow in the presence of oxygen because they lack either superoxide dismutase or catalase, or both. Obligate anaerobes vary in their response to oxygen exposure; some can survive but are not able to grow, whereas others are killed rapidly.

FERMENTATION OF SUGARS

In the clinical laboratory, identification of several important human pathogens is based on the fermentation of certain sugars. For example, *Neisseria gonorrhoeae* and *Neisseria meningitidis* can be distinguished from each other on the basis of fermentation of either glucose or maltose (see page 131), and *E. coli* can be differentiated from *Salmonella* and *Shigella* on the basis of fermentation of lactose (see page 151).

The term **fermentation** refers to the breakdown of a sugar (such as glucose or maltose) to pyruvic acid and then, usually, to lactic acid. (More specifically, it is the breakdown of a monosaccharide such as glucose, maltose, or galactose. Note that lactose is a disaccharide composed of glucose and galactose and therefore must be cleaved by β-galactosidase in *E. coli* before fermentation can occur.) Fermentation is also called the glycolytic (glyco = sugar, lytic = breakdown) cycle, and this is the process by which facultative bacteria generate ATP in the absence of oxygen.

If oxygen is present, the pyruvate produced by fermentation enters the Krebs cycle (oxidation cycle, tricarboxylic acid cycle) and is metabolized to two final products, CO_2 and H_2O. The Krebs cycle generates much more ATP than the glycolytic cycle; therefore, facultative bacteria grow faster in the presence of oxygen. Facultative and anaerobic bacteria ferment, but aerobes, which can grow only in the presence of oxygen, do not. Aerobes, such as *Pseudomonas aeruginosa,* produce metabolites that enter the Krebs cycle by processes other than fermentation, such as the deamination of amino acids.

In fermentation tests performed in the clinical laboratory, the production of pyruvate and lactate turns the medium acid, which can be detected by a pH indicator that changes color upon changes in pH. For example, if a sugar is fermented in the presence of phenol red (an indicator), the pH becomes acidic and the medium turns yellow. If, however, the sugar is not fermented, no acid is produced and the phenol red remains red.

IRON METABOLISM

Iron, in the form of ferric ion, is required for the growth of bacteria because it is an essential component of cytochromes and other enzymes. The amount of iron available for pathogenic bacteria in the human body is very low because the iron is sequestered in iron-binding proteins such as transferrin. To obtain iron for their growth, bacteria produce iron-binding compounds called **siderophores**. Siderophores, such as enterobactin produced by *E. coli*, are secreted by the bacteria, capture iron by chelating it, then attach to specific receptors on the bacterial surface, and are actively transported into the cell where the iron becomes available for use. The fact that bacteria have such a complex and specific mechanism for obtaining iron testifies to its importance in the growth and metabolism of bacteria.

PEARLS

- Bacteria reproduce by **binary fission,** whereas eukaryotic cells reproduce by mitosis.
- The bacterial growth cycle consists of four phases: the **lag** phase, during which nutrients are incorporated; the **log** phase, during which rapid cell division occurs; the **stationary** phase, during which as many cells are dying as are being formed; and the **death** phase, during which most of the cells are dying because nutrients have been exhausted.
- Some bacteria can grow in the presence of oxygen (**aerobes** and **facultatives**), but others die in the presence of oxygen (**anaerobes**). The use of oxygen by bacteria generates toxic products such as **superoxide** and **hydrogen peroxide.** Aerobes and facultatives have enzymes, such as **superoxide dismutase** and **catalase,** that detoxify these products, but anaerobes do not and are killed in the presence of oxygen.
- The fermentation of certain sugars is the basis of the laboratory identification of some important pathogens. Fermentation of sugars, such as glucose, results in the production of ATP and pyruvic acid or lactic acid. These acids lower the pH, and this can be detected by the change in color of indicator dyes.

SELF-ASSESSMENT QUESTIONS

1. Figure 3–1 depicts a bacterial growth curve divided into phases a, b, c, and d. In which one of the phases are antibiotics such as penicillin most likely to kill bacteria?

(A) Phase a
(B) Phase b
(C) Phase c
(D) Phase d

2. Some bacteria are obligate anaerobes. Which of the following statements best explains this phenomenon?

(A) They can produce energy both by fermentation (i.e., glycolysis) and by respiration using the Krebs cycle and cytochromes.
(B) They cannot produce their own ATP.
(C) They do not form spores.
(D) They lack superoxide dismutase and catalase.
(E) They do not have a capsule.

ANSWERS

1. **(B)**
2. **(D)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 709. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.