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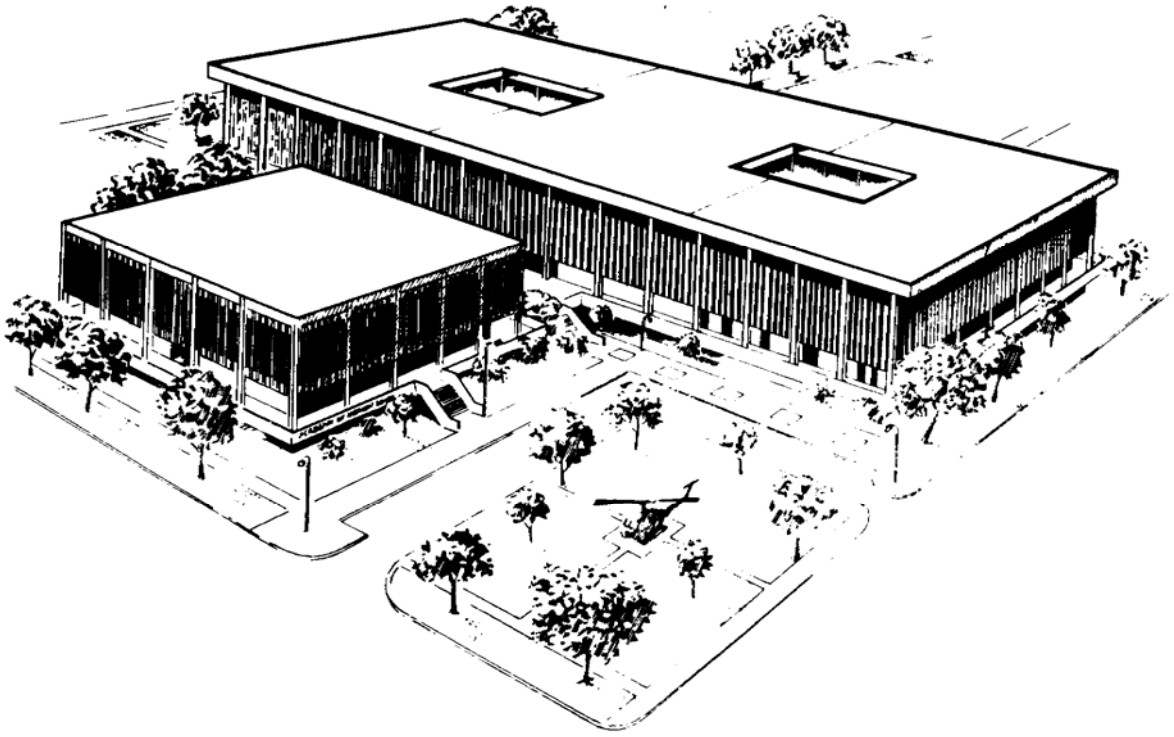

MICROBIOLOGY FOR THE VETERINARY SPECIALIST

SUBCOURSE MD0722 EDITION 100

DEVELOPMENT

This subcourse is approved for resident and correspondence course instruction. It reflects the current thought of the Academy of Health Sciences and conforms to printed Department of the Army doctrine as closely as currently possible. Development and progress render such doctrine continuously subject to change.

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CORRESPONDENCE COURSE OF
THE U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL

SUBCOURSE MD0722

MICROBIOLOGY FOR THE VETERINARY SPECIALIST

INTRODUCTION

What is the streak plate method? What foods are involved in an outbreak of staphylococcus food poisoning? What type of disease is caused by Bacillus cereus? If you cannot answer these questions now, you will be able to when you have completed this subcourse. For those who already know this material, let it serve as a review.

Why are we interested in microbiology? Microorganisms are one of the major causes of food spoilage and food deterioration in the Armed Forces. Millions of dollars worth of subsistence is lost due to microbial spoilage each year. Additionally, many man-hours are lost due to outbreaks of foodborne illness. Therefore, in order to protect the health of the troops, the veterinary specialist must have a basic knowledge of microbiology.

Subcourse Components:

The subcourse instructional material consists of three lessons as follows:

Lesson 1, Introduction to Microbiology.
Lesson 2, Microorganisms Causing Foodborne Illness and Other Diseases of Public Health Significance.
Lesson 3, Food Spoilage Due to Microorganisms.

Here are some suggestions that may be helpful to you in completing this subcourse:

--Read and study each lesson carefully.

--Complete the subcourse lesson by lesson. After completing each lesson, work the exercises at the end of the lesson, marking your answers in this booklet.

--After completing each set of lesson exercises, compare your answers with those on the solution sheet that follows the exercises. If you have answered an exercise incorrectly, check the reference cited after the answer on the solution sheet to determine why your response was not the correct one.

Credit Awarded:

Upon successful completion of the examination for this subcourse, you will be awarded 10 credit hours.

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Branch at Fort Sam Houston, Texas.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

A listing of correspondence courses and subcourses available through the Nonresident Instruction Section is found in Chapter 4 of DA Pamphlet 350-59, Army Correspondence Course Program Catalog. The DA PAM is available at the following website: http://www.usapa.army.mil/pdffiles/p350-59.pdf.

LESSON ASSIGNMENT

LESSON 1	Introduction to Microbiology
LESSON ASSIGNMENT	Paragraphs 1-1 through 1-43.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

1-1. Given a list of microbiological terms and their definitions, match the microbiological term with the correct definition.

1-2. Given a list of bacteria, select those of public health importance.

1-3. Identify the four reagents used in the gram stain and the purpose of each reagent.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 1

INTRODUCTION TO MICROBIOLOGY

Section I. INTRODUCTION

1-1. GENERAL

As the population of the world increases, it is essential that food production keep pace. It will be necessary not only to produce more food, but also to maintain this food so that it is safe and wholesome for human consumption. In this endeavor, the veterinary specialist will play an important role.

1-2. SIGNIFICANCE

a. The significance of microorganisms in food depends upon the following factors:

(1) The number of microorganisms found.

(2) The types of microorganisms.

(3) The type of food.

(4) The treatments to which the food has been exposed.

(5) The processing or storage treatments the food will receive.

(6) Whether the food is to be eaten as is, or heated and/or cooked.

(7) The individuals that might consume the food.

b. We also know that microorganisms may have one or more of four functions in a food. They may have a useful function, cause spoilage, be a health hazard, or just be present without a role in the food item. As a veterinary specialist, you are very concerned with those microorganisms that cause spoilage and those that may be a health hazard. These organisms will be discussed in detail in this subcourse.

Section II. MICROBIOLOGICAL RELATIONSHIPS

1-3. CAUSATION OF MICROBIAL DISEASE

Microbial disease must be understood in terms of the interrelationship among parasites, the host, and the environment. For example, the general health of the host may determine whether a disease occurs and how severe it may be. Even though a microorganism may be part of the ordinary flora, it can cause disease if the host's natural defenses are not fully effective. Some common symbiotic relationships between two organisms, such as man and a microorganism, are:

a. **Mutualism.** In this case, both organisms benefit.

b. **Commensalism.** Commensalism is a relationship where one organism benefits without producing harmful effects for the other.

c. **Parasitism.** In parasitism, one organism benefits at the expense of the other. The parasite may be facultative or obligatory. If the parasite is able to live independently of the host, it is considered a facultative parasite. When the parasite has a absolute requirement for a host and cannot live outside the host, it is an obligatory parasite. In the microbial association of parasitism, disease results if the host is injured by the parasite. In this regard, the parasite has certain characteristics favoring its establishment in the host. On the other hand, the host has certain characteristics which oppose the establishment of the parasite. The outcome of the host-parasite relationship depends on the active interplay between factors.

1-4. TERMINOLOGY

a. Parasites that usually cause disease are called pathogens, and pathogenicity is the term used to denote disease-producing ability. Virulence denotes the degree of pathogenicity, it is used to describe a particular strain or variety of a species. Pathogenicity or virulence may be due to the bacteria's invasiveness or toxigenicity. By invasiveness is meant the ability of the bacteria to enter, spread, and multiply in host tissue. Toxigenicity refers to the ability of the bacteria to produce toxic substances.

b. Virulence is usually expressed by the LD_{50}, that is, that concentration of microorganisms representing a lethal dose for 50 percent of the experimental animals under standardized conditions. The LD_{50} is specific to the laboratory animal employed.

c. The process whereby a pathogenic microbe enters into a relationship with the host is known as infection. The infection may or may not result in overt disease. In this regard, the host may overcome the infection, the host may develop latent, that is, inapparent infection, or the host may become a healthy carrier of the pathogen.

d. Bacteria reproduce asexually by splitting at right angles to their long axis, a process called binary fission.

1-5. THE INFECTIOUS PROCESS

Various steps are required for the infectious process.

a. First, an appropriate portal of entry is required. The respiratory tract, the gastrointestinal tract, direct contact, and insect bites may each represent the portal of entry. The portal of entry is usually specific for any particular pathogen.

b. Second, the pathogen must establish itself in and reproduce within the host. This involves its spread through the host's tissues via the lymphatics and the blood stream.

c. Third, to complete the transmission of the pathogen to a new host, a portal of exit is required. The portal of exit may be the respiratory tract, the gastrointestinal tract, direct contact, or an insect bite.

d. Finally, to ensure perpetuation of the parasite, the pathogen must be introduced to a new host. This may occur by the ingestion of contaminated food or drink, by direct contact with infected persons, by contact with contaminated objects (fomites), or by insect vectors.

Section III. ASPECTS OF PATHOGENICITY

1-6. CAPSULATION

Of primary consideration is the presence of a capsule on most pathogenic bacteria. Encapsulated bacteria are able to resist phagocytosis by leukocytes better than nonencapsulated bacteria. The loss of the capsule in certain bacteria (pneumococci) results in the loss of virulence. The capsule is generally regarded as a factor of invasiveness.

1-7. BACTERIAL TOXINS

Bacterial toxins are known to play a role in the ability of bacteria to cause disease. Toxins are divided into endotoxins and exotoxins.

a. Exotoxins are secreted by bacteria into their environment and are potent and specific in their action upon host tissue. The ability to form exotoxins is usually attributed to certain gram-positive rods such as Clostridia and Corynebacteria. The exotoxin has been found to be a highly antigenic, heat labile, proteinaceous substance that is usually destroyed by proteolytic enzymes. However, the very potent and toxic exotoxin of Clostridium botulinum is the exception; it is not destroyed by proteolytic enzymes. The toxic properties, but not the antigenic properties, of exotoxins are destroyed by formalin, heat, or prolonged storage. Such treated toxins are called toxoids and find great practical use in immunization programs.

b. Endotoxins are found intracellularly, and are considered to be part of the bacterial cell wall. They are released upon destruction of the bacterial cell. Endotoxins are usually associated with gram-negative bacteria, and considered to be weakly antigenic. Among their other properties, endotoxins are heat-stable polysaccharides that are not digested by proteolytic enzymes. Endotoxins are weakly toxic and induce a generalized reaction in the host which manifests itself in the form of a febrile response. Endotoxins may also cause shock.

1-8. EXTRACELLULAR ENZYMES

Certain microorganisms produce extracellular enzymes which contribute to their pathogenicity.

a. Production of the enzyme coagulase has been correlated to the pathogenicity of the staphylococci. Coagulase acts upon plasma causing it to coagulate. This activity results in the walling off of the site of infection and causes a layer of fibrin to form on the cell wall of the bacteria which enables the staphylococci to resist phagocytosis.

b. Collagenase is an enzyme that is produced by some of the Clostridium species. It acts upon collagen, a constituent of connective tissue. The breakdown of collagen promotes the spread of bacteria in tissue.

c. Hyaluronidase is an enzyme that is known as the spreading factor. It acts upon hyaluronic acid, a constituent of connective tissue. The enzyme is produced by staphylococci, clostridia, streptococci, and pneumococci. The activity of hyaluronidase contributes to the spread of the pathogens through tissue.

d. Streptokinase, also known as fibrinolysin, is an enzyme produced by streptococci, staphylococci, and Clostridium perfringens. Streptokinase activates a proteolytic enzyme of plasma known as plasmin. Plasmin is able to dissolve coagulated plasma, and this activity may aid in the spread of the bacteria.

e. Hemolysins are a group of soluble substances produced by staphylococci, clostridia, and groups A and C of the streptococci. These hemolysins destroy red blood cells and probably tissue cells.

f. Leukocidins are substances that destroy leukocytes, they are produced by streptococci and staphylococci.

Section IV. HOST RESISTANCE

1-9. NONSPECIFIC FACTORS

a. **Skin and Mucous Membrane Barriers.**

(1) Physical barriers.

(a) Intact skin.

(b) Mucous-sticky lining.

(c) Nasal hair.

(d) Cilia.

(e) Peristaltic action.

(f) Normal flora occupying attachment sites.

(2) Chemical barriers.

(a) Acid pH (stomach skin).

(b) Bile salts (intestine).

(c) Lysozyme (eyes).

(3) Microbial antagonism.

(a) Bacteriocins from normal flora.

(b) Antimicrobial factors from serum.

(c) Competition for nutrients.

b. **Phagocytosis.**

(1) Monocytes and macrophages ingest foreign particles in the host.

(2) Numerous enzymes act to degrade ingested particles.

(3) Local tissue damage and inflammation may occur.

c. **Emotional and Nutritional States.** Emotional and nutritional states (hormones, vitamins, and so forth) play an undefined role in resistance.

1-10. SPECIFIC FACTORS-IMMUNOLOGICAL RESPONSE

a. **Antibodies.** Antibodies may:

(1) Neutralize the antigen.

(2) Opsonize the antigen. (Prepare the antigen for phagocytosis).

b. **Cell-Mediated Immunity.**

(1) Antigen stimulates the release of biologically active substances called lymphokines.

(2) Lymphokines enhance phagocytosis.

c. **Complement System.** The complement system is a complex system of serum proteins which assist antibodies in:

(1) Neutralizing the antigen.

(2) Lysing bacteria.

d. **Interferon.** Interferon is a cell protein produced by a cell after stimulation by viruses and other microorganisms. It provides a local defense against an antigen.

Section V. THE BACTERIAL CELL

1-11. AUTOTROPHIC BACTERIA

Autotrophic bacteria obtain energy and grow on inorganic media, employing carbon dioxide ($C0_2$) as their sole source of carbon. From $C0_2$ and ammonia (NH_3), autotrophs build an entire protoplasmic structure of protein, fat, and carbohydrates. Autotrophs use the oxidation of ammonia to obtain energy for their other processes.

1-12. HETEROTROPHIC BACTERIA

Heterotrophic bacteria obtain energy from organic carbon sources. Heterotrophs require the addition of sugars, amino acids, purines, pyrimidines, and vitamins to their culture media. The fermentation of sugar is their primary source of energy.

a. Parasitic bacteria are heterotrophs. They have become adapted to an environment in which many kinds of organic materials are normally available. In many cases, such organisms have lost their ability to synthesize certain complex organic substances needed for their growth. Bacterial parasites require nutrients from living organisms and may cause harm to the host.

b. Saprophytic bacteria are heterotrophs which utilize decaying organic matter for nutrients and usually do not harm the host.

1-13. MORPHOLOGY

The size of bacteria is measured in thousandths of a millimeter (millionths of a meter). This measure is the micron (μ). See figure 1-1. Generally, the following dimensions apply. The coccus has a diameter approximately 1 micron (μ). The bacillus appears as a rod with a width of 0.5 μ and a length of 2 μ. The spirochete appears as a corkscrew with a width of 0.2 μ and a length of 10 μ. Bacteria can occur in a number of arrangements and a predominant arrangement is usually specified for a bacterium. In this regard, prefixes are added to the word indicating the shape of the bacterium.

a. **Coccus (Plural, Cocci).** Spherical bacteria occurring in any of the following arrangements:

(1) Singly (cocus) (figure 1-2A).

(2) In pairs (diplococcus) (figure 1-2B).

(3) In chains (streptococcus) (figure 1-2C).

(4) In clusters (staphylococcus) (figure 1-2D).

(5) In clusters of 4 (tetrad) or 8 (cube) (figure 1-2E).

b. **Bacillus (Plural, Bacilli).** Rod-shaped bacteria occurring in any of the following arrangements:

(1) Singly (bacillus) (figure 1-2F).

(2) In pairs (diplobacillus) (figure 1-2G).

(3) In chains (streptobacillus) (figure 1-2H).

(4) In palisades (palisade) (figure 1-2I).

c. **Spirillum (Plural, Spirilla).** Spiral, corkscrew-shaped organisms whose long axes remain rigid while in motion. A spirochete is a spiral microorganism whose long axis flexes when it is in motion (figure 1-2J).

d. **Coccobacillus.** A short, plump bacillus with rounded ends, resembling a coccus in shape and arrangement.

e. **Vibrio.** A comma-shaped bacillus resembling the spirillum because of its motility.

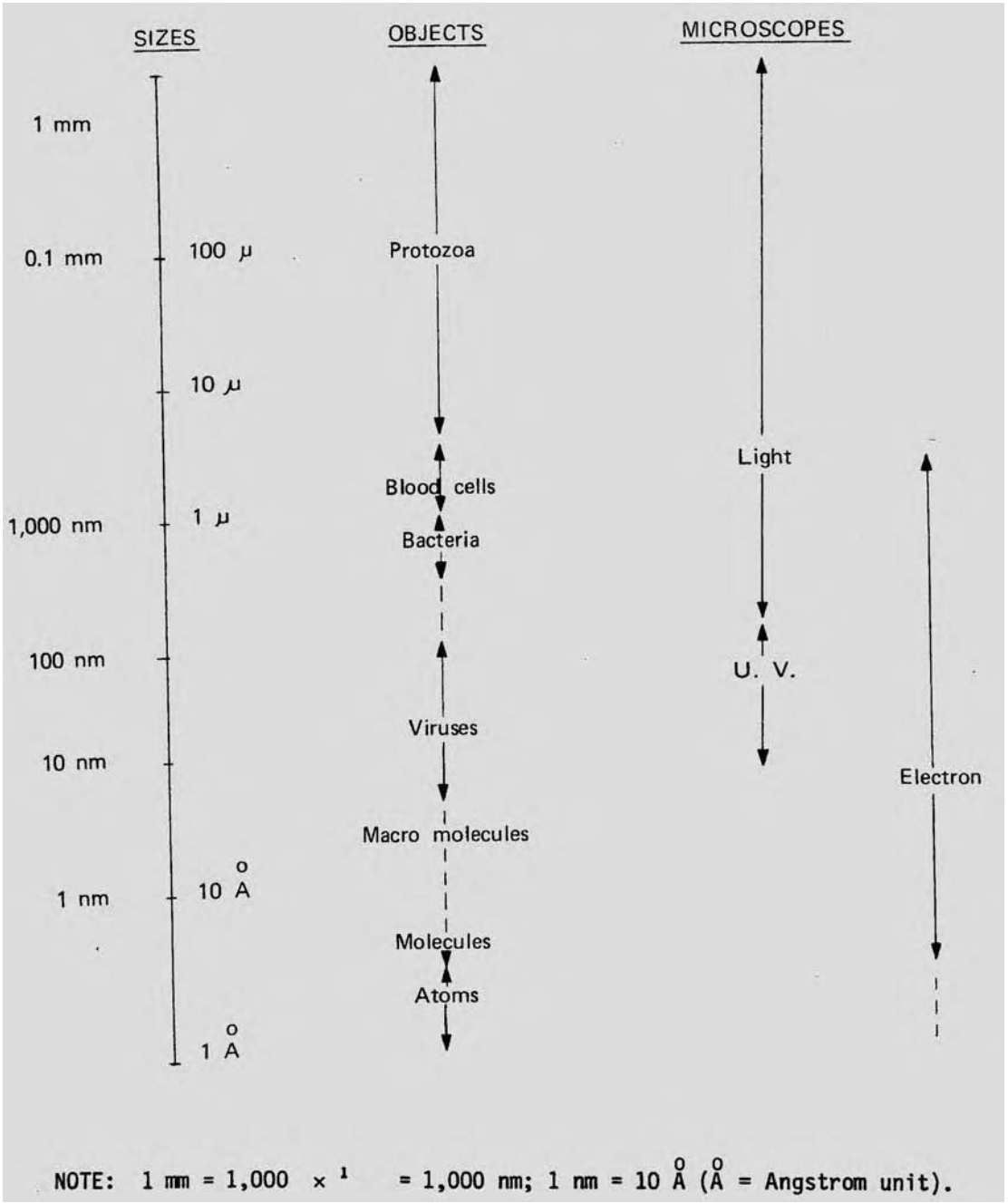
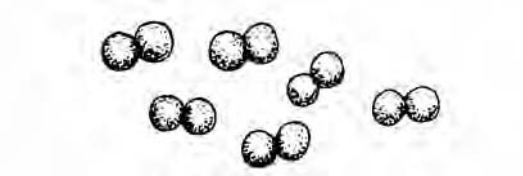
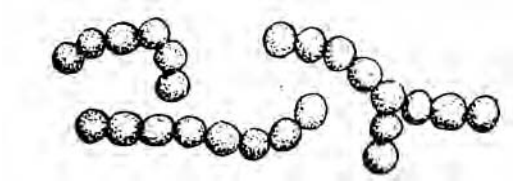


Figure 1-1. Size range of objects with different types of microscopes, (Reproduced for instructional purposes from Textbook of Virology, dated 1968, 5th ed., by A.J. Rhodes and C.E. Van Rooyen, Figure 1/2/1, adapted from Endeavor, Volume 15, page 153, written consent of the copyright owner has been obtained.)

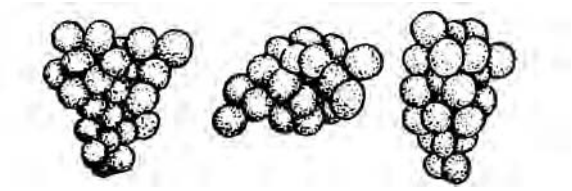
A Cocci (sing., coccus) occurring singly.



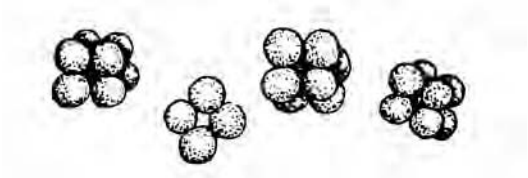
B Diplococci, in pairs.



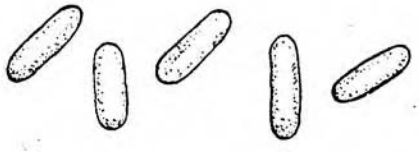
C Streptococci, in chains.



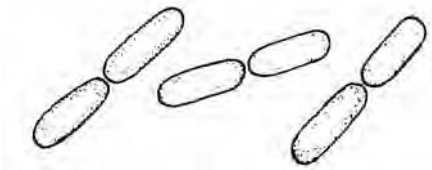
D Staphylococci, in clusters.



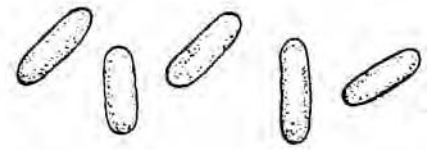
E Cocci in tetrad
(Group of four; Gaffkya) and cubes
(Groups of eight; Sarcina).



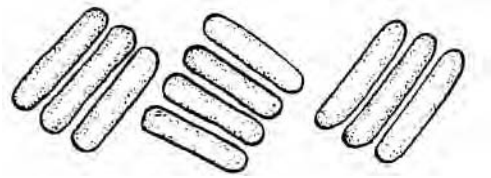
F Bacilli (sing., bacillus),
occurring singly.



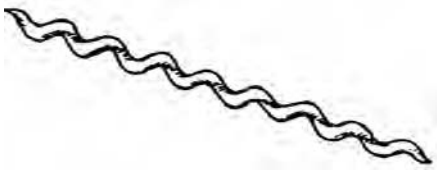
G Diplobacilli, in pairs.



H Streptobacilli, in chains.



I Bacilli in palisade arrangement.



Spirillum or spirochete, always

J

occurs singly.

Figure 1-2. Shapes and arrangements of bacteria.

1-14. STRUCTURE OF BACTERIA

The typical structure of a bacterial cell is shown in figure 1-3. It consists of general structures found in all bacterial cells and in specific types of bacterial cells.

a. **General Structures.**

(1) Cell wall. A thin, rigid cellulose covering that encloses the protoplasm of the cell and gives rigidity to the bacterial shape.

(2) Cytoplasmic membrane. A semipermeable membrane which is located directly beneath the cell wall, and which governs osmotic activity.

(3) Cytoplasm. The protoplasmic or vital colloidal material of a cell exclusive of the nucleus.

(4) Nucleus. Diffused chromatin material responsible for replication of the cell. The bacterial cell does not have a nuclear membrane nor a well-defined nucleus.

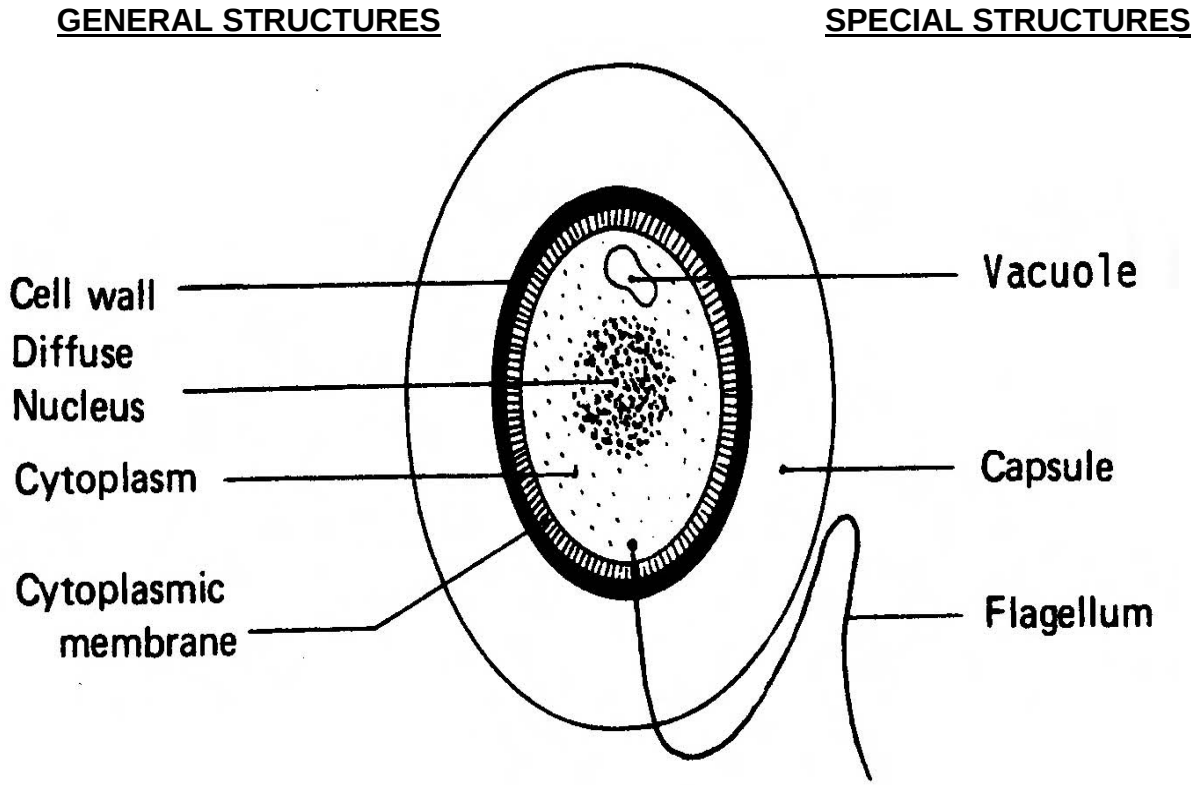


Figure 1-3 Bacterial cell structure.

b. **Special Structures.**

(1) Capsule. An accumulation of high-molecular-weight, excretory substances (slime layer) around a bacterium or bacteria. A capsule serves as a defense mechanism against phagocytosis by white blood cells and penetration by viruses.

(2) Flagellum. A protoplasmic strand of elastic protein originating in the cytoplasm and extending from the body of the cell. A flagellum serves as an organ of locomotion. The arrangement of flagella (plural) is peculiar to the species.

(3) Spore. Metabolically resistant body formed by a vegetative bacterium to withstand an unfavorable environment. Only bacilli form spores. The position and size of a spore within a bacillus is peculiar to the species.

(4) Inclusion bodies. Vacuoles of reserve or waste materials contained within the cytoplasm.

1-15. IDENTIFICATION OF BACTERIA

Since there are several thousand species of bacteria, it would be impossible to identify them on the basis of appearance alone. Therefore, the bacteriologist employs a wide variety of techniques, based upon known characteristics of specific bacteria, to arrive at the identity of a given specimen. The following characteristics, which are used frequently as terms of reference, assist the microbiologist in the positive identification of bacteria as well as in eliminating them from consideration.

a. **Food Requirements.**

(1) Natural media.

(a) Saprophytes grow on dead organic matter.

(b) Parasites grow on living tissue.

(2) Artificial media.

(a) Grow on any culture medium.

(b) Grow only on special culture media.

(c) Will not grow on any artificial culture medium.

b. **Oxygen Requirements.**

(1) Aerobes grow in the presence of free oxygen.

(2) Anaerobes grow without free oxygen.

(3) Obligate aerobes must have free oxygen for growth.

(4) Obligate anerobes must not have free oxygen for growth.

(5) Facultative aerobes are able to adjust to an aerobic environment.

(6) Facultative anaerobes are able to adjust to an anaerobic environment.

(7) Microaerophiles require small amounts of free oxygen for growth.

c. **Colony Morphology.** See figure 1-4.

d. **Microscopic Examination.**

(1) Size.

(2) Shape.

(3) Spore formation-sporeformers or nonsporeformers.

(4) Capsule formation-encapsulated or nonencapsulated.

(5) Motility-motile or nonmotile.

(6) Staining characteristics. Specimens are normally stained prior to microscopic examination. Various species react differently to the stains.

(a) Gram-positive-bacteria which, when stained by the gram stain method, retain the crystal violet stain (purple or blue).

(b) Gram-negative-bacteria, which, when stained by the gram stain method, do not retain the crystal violet stain, but retain the color of the counterstain (red).

(c) Acid-fast-bacteria, which, when stained with certain dyes and then treated with an acid, followed by a counterstain, retain the color of the dye.

(d) Nonacid-fast-bacteria, which, when treated as in (c) above, retain the counterstain rather than the dye.

e. **Pathogenicity.**

(1) Hemolytic or nonhemolytic.

(a) Beta hemolytic-can cause complete hemolysis (dissolution) of red blood cells.

(b) Alpha hemolytic-cause partial hemolysis of red blood cells.

(c) Gamma forms-do not cause hemolysis.

(2) Production of toxins.

(a) Exotoxins are extremely potent poisons which are produced in bacterial cells, which diffuse freely into the cells of host tissues, causing severe systemic poisoning.

(b) Endotoxins are less potent than exotoxins, produced in bacterial cells, and diffuse into the host cells only after the bacterial cell disintegrates.

FORM

Punctiform

Irregular

Circular

Rhizoid

Filamentous

Spindle

ELEVATION

Flat

Pulvinate

Raised

Umbonate

Convex

Umbilicate

MARGIN

Entire

Erose

Undulate

Filamentous

Lobate

Curled

Figure 1-4. Colony characteristics.

1-16. PATHOGENIC BACTERIA

Figure 1-5 presents a list of the principal pathogenic bacteria of public health importance, organized to illustrate the aids in identification discussed above. The organisms producing foodborne illness will be discussed in the section on various organisms.

IDENTIFICATION GROUP	ORGANISM	CAUSATIVE AGENT OF	REMARKS
GRAM-POSITIVE COCCCI			
Alpha hemolytic	Diplococcus pneumonia	Lobar pneumonia	
Beta hemolytic	Streptococcus pyogenes	Impetigo, septic sore throat, scarlet fever, foodborne disease	Produces exotoxin causing skin rash.
	Staphylococcus aureus	Upper respiratory infections, boils, surgical infections, food poisoning	Produces exotoxin causing food poisoning.
GRAM-NEGATIVE COCCCI	Neisseria gonorrhoeae	Gonorrhea, gonorrheal conjunctivitis	Kidney-shaped diplococci
	Neisseria meningitidis	Epidemic cerebrospinal meningitis	Kidney-shaped diplococci

Figure 1-5. Pathogenic bacteria of public health importance (continued).

IDENTIFICATION GROUP	ORGANISM	CAUSATIVE AGENT OF	REMARKS
GRAM-POSITIVE BACILLI			
Aerobic	Corynebacterium diphtheriae	Diphtheria	Produces powerful exotoxin causing inflammation of mucosa and impairment of vital organs.
Aerobic, spore-forming	Bacillus anthracis	Anthrax(Chiefly in herbivorous animals, but also in man)	Forms a capsule.
	B. cerus	Gastroenteritis	
Aerobic, acid-fast	Mycobacterium tuberculosis	Tuberculosis (man)	
	M. bovis	Tuberculosis (cattle and man)	
	M. leprae	Leprosy	
Anaerobic, spore-forming	Clostridium botulinum	Food poisoning	Produces powerful lethal exotoxin.
	C. tetani	Tetanus (lockjaw)	Produces powerful lethal exotoxin.
	Clostridium perfringens	Gas gangrene, food poisoning	

Figure 1-5. Pathogenic bacteria of public health importance (continued).

IDENTIFICATION GROUP GRAM-NEGATIVE BACILLI	ORGANISM	CAUSATIVE AGENT OF	REMARKS
Saprophytic enterics		Part of the normal flora of the adult intestinal tract, but pathogenic to infants or when introduced into other parts of the body	All nonspore-forming
	Escherichia coli[1]		[1]Causes enteric disease syndrome
	Aerobacter aerogenes		
	Proteus vulgaris		
Pathogenic enterics	Salmonella typhi	Typhoid fever	
	S. paratyphi	Paratyphoid fever	
	S. typhimurium	Acute gastroenteritis (Salmonellosis-"food poisoning")	Produces a somatic endotoxin.
	Shigella dysenteriae[2]	Bacillary dysentery	[2]Also produces a paralytic exotoxin.
	Sh. flexnerei		
	Sh. sonnei[3]	[3]Infant diarrhea	
	Vibro cholerae	Cholera (gastrointestinal illness)	Motile, comma-shaped.
	V. parahaemolyticus	Gastroenteritis	

Figure 1-5. Pathogenic bacteria of public health importance (continued).

IDENTIFICATION GROUP SMALL GRAM-NEGATIVE BACILLI	ORGANISM	CAUSATIVE AGENT OF	REMARKS
Non-motile nonspore-forming	Brucella abortus[1]	Contagious abortion in animals; brucellosis (undulant fever) in man	[1]Occurs in cattle.
	Br. suis[2]		[2]Occurs in swine.
	Br. melitensis[3]		[3]Occurs in sheep.
	Haemophilus infuenzae	Pharyngitis, otitis, sinusitis, pneumonitis, or (rarely) meningitis	Encapsulated.
	H. pertussis (Bordetella)	Whooping cough	
	H. ducreyi	Chancroid	
	Pasteurella (Yersina) pestis	Plague	
	P. (Francisella) tularensis	Tularemia	

Figure 1-5. Pathogenic bacteria of public health importance (continued).

IDENTIFICATION GROUP	ORGANISM	CAUSATIVE AGENT OF	REMARKS
SMALL GRAM-NEGATIVE BACILLI			
SPIROCHETES	Treponema pallidum	Syphilis	Does not stain with ordinary stains or grow on artificial media.
	Borrelia recurrentis	Relapsing fever	May be stained and cultured (chick embryo).
	Leptospirosa ichterohemorrhagiae (also Leptospirosa canicola, Leptospirosa autumnalis, Leptospirosa pomona)	Leptospirosis (Weil's disease, infectious jaundice)	

Figure 1-5. Pathogenic bacteria of public health importance (concluded).

Section VI. COLLECTING AND PROCESSING OF BACTERIOLOGICAL SPECIMENS

1-17. INTRODUCTION

The improper collecting and processing of bacteriological specimens is often responsible for the failure to isolate and identify the bacterial agent of disease. Certain basic principles, therefore, should be followed at all times.

1-18. BASIC PRINCIPLES

a. First, instruments, containers, and other equipment in direct contact with specimens must be sterile. The cartons for the collection of stool specimens are an exception to this rule.

b. Second, material for culture must not come in contact with chemicals, disinfectants, or germicidal agents.

c. Third, material for culture should be obtained before the patient receives antibiotic therapy. If this is not possible, the type of therapy should be indicated on the bacteriology request form. Such substances as penicillinase should then be added to the media if penicillin is indicated, or para-aminobenzoic acid if sulfonamides are indicated.

d. Fourth, specimens should be properly labeled and dated. Specimens should be inoculated to media immediately after delivery to laboratory.

e. Fifth, if anaerobic cultures are requested, the specimen should be inoculated to fluid thioglycollate medium at the time the specimen is collected, or as soon thereafter as possible. Exposure to atmospheric oxygen should be avoided completely if possible.

f. Sixth, to avoid contamination, always culture the specimen before making smears or performing special tests.

Section VII. MICROSCOPIC EXAMINATION OF BACTERIA

1-19. GENERAL COMMENTS

The direct observation of microorganisms in stained or unstained slide mounts of cultures or specimens is an essential part of clinical microbiology. Characteristics, such as the shape and grouping of the cells, the presence or absence of special structures, and the reaction to stains, are of value in identification.

1-20. GRAM STAIN

The most widely used staining procedure in the bacteriological laboratory is the gram stain. The purpose of the gram stain is to differentiate bacteria on the basis of their gram-staining reaction.

a. **Principle.** Gram-positive bacteria, following initial staining with crystal violet, will retain the purple dye upon subsequent treatment with a mordant (iodine) and the application of alcohol or acetone-alcohol decolorizing agents. Gram-negative bacteria, which lack specific cellular substances responsible for binding crystal violet, fail to retain the dye upon similar treatment. The latter forms are, therefore stained red upon application of safranin counterstain.

b. **Reagents.**

(1) Crystal violet stain.

(a) Make SOLUTION A by dissolving 4 g of crystal violet in 20 ml of 95 percent ethyl alcohol.

(b) Make SOLUTION B by dissolving 0.8 g of ammonium oxalate ($(NH_4)_2C_20_4\,H_20$) in 80 ml of distilled water.

(c) Mix solutions A and B ordinarily in equal parts.

(d) This procedure may sometimes cause organisms to retain the basic dye and resist decolorization. To avoid this, solution A may be diluted as much as ten times. Then the diluted solution A is added in equal parts to solution B.

(2) Iodine solution.

(a) Grind 1 gram of iodine (I_2) and 2g of potassium iodide (KI) in a mortar. Dissolve the ground reagents in 5 ml of distilled water and add sufficient distilled water to make 240 ml. Add 60 ml of 5 percent sodium bicarbonate (made by adding enough distilled water to 5 g of sodium bicarbonate ($NaHC0_3$) to make 100 ml).

(b) When a color loss of the iodine solution is noted, prepare a fresh solution. The iodine solution will remain stable longer if stored in a dark bottle.

(3) Decolorizer. Mix equal volumes of 95 percent ethyl alcohol and acetone.

(4) Safranin counterstain. Dissolve 0.5 g of safranin in a small amount of distilled water. Add sufficient distilled water to make 100 ml.

c. **Technique.**

(1) Cover the heat-fixed smear with crystal violet solution, allowing the stain to remain for 1 minute.

(2) Remove all stains with a gentle flow of water.

(3) Cover the smear with iodine solution (mordant) for at least 1 minute.

(4) Wash with water.

(5) Treat with decolorizer solution by flowing the reagent dropwise over the smear while the slide is held at an angle. Decolorization should be stopped as soon as the drippings from the slide become clear.

(6) Remove excess decolorizer immediately with a gentle flow of water.

(7) Apply the safranin counterstain for 30 seconds.

(8) Remove counterstain with gentle flow of water and gently blot dry.

(9) Examine the smear microscopically using the oil immersion objective.

d. **Interpretation.**

(1) Most bacteria may be placed in one of two groups by their reaction to the gram stain. If an organism retains crystal violet (cells purple or blue), it is referred to as gram-positive. Organisms, which lose crystal violet stain under treatment with the decolorizing agent and are stained red upon applying the safranin counterstain, are termed gram-negative.

(2) Gram-positive organisms may become gram-negative as a result of autolysis, aging, acidity of culture medium, improper temperature of incubation, or the presence of toxic substances (drugs, metabolic wastes, and so forth). For best results, gram stains should be prepared on cultures 18-24 hours old. A known gram-positive organism may be used as a control.

(3) If films are prepared unevenly, or excessively thick, dense clumps of growth will be present which retain crystal violet upon decolorization, regardless of the gram reaction. Under these conditions, gram-positive appearing clumps may be present in an otherwise gram-negative smear.

(4) Smears should be completely dry before being heat-fixed; otherwise, any protein material carried over into the smear from culture media or specimens will be precipitated. As a result, the background of the smear will be difficult to decolorize and may be filled with debris and misleading artifacts.

(5) False decoloration of gram-positive cells may result from the use of an iodine (mordant) solution which has deteriorated. Gram's iodine solution will remain stable for longer periods of time when protected from light by storage in a dark colored bottle. When iodine (mordant) solution fades from a dark brown to light amber or yellow, it is no longer suitable for use.

(6) Too drastic a treatment with the decolorizing solution usually results in a false gram-negative reaction. Immediately after the drippings appear clear, when treating the smear with acetone-alcohol mixture, the slide must be washed with water to prevent overdecolorization.

(7) Although most bacteria are either gram-positive or gram-negative, certain species exhibit a definite tendency to display both gram-positive and gram-negative forms ("gram-variable"). Usually whether positive, negative, or variable, the gram reaction is species specific.

e. **Discussion.**

(1) The gram stain is extremely valuable as a screening technique for differentiating bacterial types. By correlating the gram reaction with a gram-stained smear, tentative generic identification is often accomplished. This enables intelligent selection of suitable culture media for isolation and confirmation of the organism involved. Furthermore, the gram reaction is often concomitant with other properties of a given group or species of bacteria, for example, habitat, resistance or susceptibility to therapeutic agents, disease manifestations, and so forth.

(2) The gram stain is routinely employed as the first step in the examination of specimens and cultures.

Section VIII. CULTIVATION OF BACTERIA

1-21. INTRODUCTION

a. In order to identify bacteria in a specimen, it is usually necessary to inoculate artificial culture media with samples of the specimen and perform studies on bacterial colonies found in the cultures after incubation. This necessitates an understanding of the growth requirements for cultivating bacteria.

b. The nutritional spectrum of bacteria varies from the self-reliant autotrophs (that is, those bacteria that are able to synthesize cellular materials from inorganic nutrients) to the more nutritionally demanding heterotrophs (which require a carbon source in the form of organic materials). It is in the latter group, the heterotrophs, that we find the parasites, both saprophytes and the pathogens.

c. Most media are available commercially in the dehydrated form. Since dehydrated products have been economically used with considerable success in most laboratories, it is recommended that commercial media be prepared according to the manufacturer's directions.

d. For additional information about specific culture media, consult printed information provided by Difco Laboratories, Detroit, and BioQuest (Division of Becton, Dickinson and Company), Cockeysville, Maryland.

1-22. COMPOSITION OF MEDIA

a. Agar, a complex carbohydrate obtained from seaweed, is used as a solidifying agent in many media. It is inert and is not a source of nutrients. A broth (liquid) medium usually contains no agar.

b. Peptones are nitrogenous compounds derived from specific proteins or protein mixtures by hydrolysis to provide a more available form of nitrogen for bacteria.

c. Meat extracts, meat infusions, and other natural products are sometimes used to enrich media.

d. There is a trend to the use of media which are better defined chemically. Modern media tend to consist of well-defined components, such as the peptones described in the US Pharmacopoeia and pure chemicals.

e. Culture media without glucose generally give more reliable and consistent results than media with glucose. Glucose fermentation may result in a pH which is harmful to acid-sensitive organism. In a blood agar base medium, the presence of glucose can make it harder to differentiate between alpha and beta hemolysis. However, glucose is useful in several specific media.

1-23. HYDROGEN ION CONCENTRATION (pH)

The growth of microorganisms is very markedly affected by the pH of the medium. A proper hydrogen ion concentration must be established and maintained by use of buffering systems to ensure maximum growth of a bacterial population. The pH must be adjusted to the proper value before sterilization. Generally, a narrow pH range of 6.8 to 7.4 is considered optimal for pathogenic bacteria since most bacteria, especially pathogenic forms, grow best near the neutral point.

Section IX. ENVIRONMENTAL FACTORS

1-24. TEMPERATURE

The correct temperature is very important for the proper growth of bacteria since bacteria vary considerably with respect to temperature requirements. Some bacteria grow best in a temperature range of 10º to 20ºC; these bacteria are called psychrophilic (psychrotrophic) bacteria. Other bacteria have an optimum temperature in the 30º to 40ºC range; they are referred to as mesophilic bacteria. The thermophilic bacteria have an optimum temperature range from 50º to 60ºC. The majority of human pathogens are mesophilic, growing best at 37ºC and, for this reason, a constant temperature incubator adjusted to 37ºC (normal body temperature) and containing sufficient moisture satisfies the temperature requirement in a clinical bacteriology section. However, some authorities suggest that most incubators should be set at 35ºC, so that a temperature higher than 37ºC will be unlikely at any time.

1-25. OXYGEN REQUIREMENTS

It is essential that the proper gaseous environment be furnished when attempting to cultivate bacteria. The specific needs apply to oxygen and carbon dioxide. Often, the failure to isolate pathogenic microorganisms from clinical materials is due to inadequate provisions with respect to aeration. Bacteria are divisible into two broad groups on the basis of their oxygen requirements: (1) aerobic forms which require free oxygen for growth, and (2) anaerobic forms which require the absence of free oxygen for growth. Aerobic and anaerobic bacteria are further divided into several categories (figure 1-6).

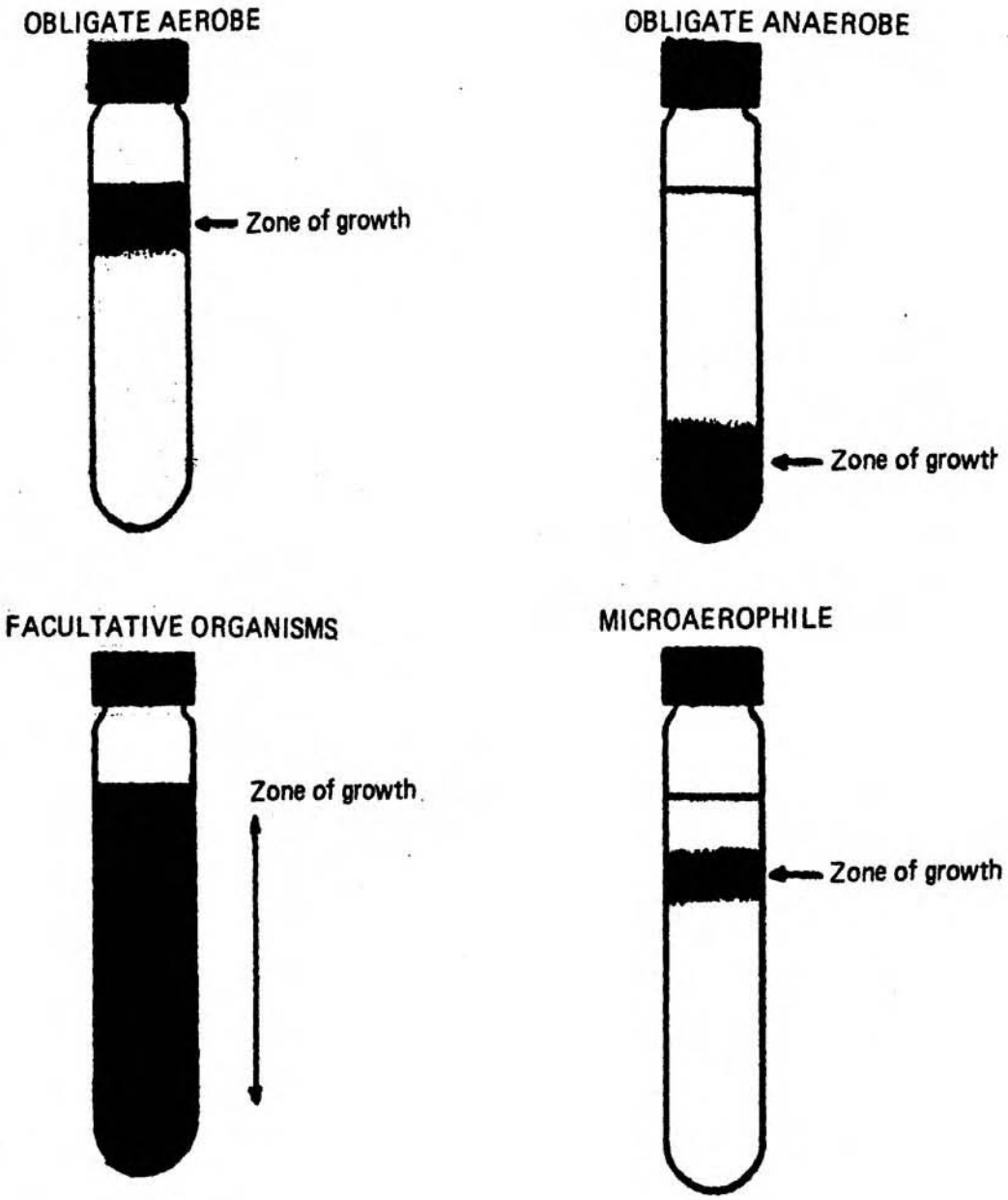


Figure 1-6. Aerobic and anaerobic bacteria divided into categories.

a. **Obligate Aerobes.** An obligate aerobe must have free atmospheric oxygen in order to grow. These forms grow best on agar media in a normal atmosphere. They will also grow in the upper portion of a broth medium. Some members of the genus Bacillus are strict aerobes.

b. **Microaerophiles.** For optimal growth these forms require a greatly reduced atmosphere of oxygen such as that supplied in the lower portion of a broth medium. These organisms will also grow well on an agar plate, when incubated in an atmosphere of increased carbon dioxide (candle jar). Haemophilus species and many streptococci are microaerophiles.

c. **Facultative Organisms.** Those organisms capable of adapting to either presence or absence of atmospheric oxygen fall into this group.

d. **Obligate Anaerobes.** These forms require the strict absence of atmospheric oxygen for growth. Free oxygen is toxic to obligate anaerobes because of resultant enzyme destruction or inactivation. Special measures must be taken to remove and exclude oxygen from these cultures during incubation. Some pathogenic bacteria are obligate anaerobes on primary isolation yet will adapt to aerobic conditions upon subculture. Examples of true obligate anaerobes are represented in the genus Clostridium and some streptococci.

1-26. CARBON DIOXIDE REQUIREMENTS

Many microorganisms, aerobic and anaerobic, require a carbon dioxide concentration above normal atmospheric levels. Examples of aerobes requiring increased CO_2 are Brucella and Mycobacterium.

Section X. ANAEROBIC METHODS

1-27. INTRODUCTION

The anaerobic bacteria can be isolated and studied quite readily provided certain cardinal principles of anaerobic bacteriology are rigidly applied.

1-28. BASIC PRINCIPLES

Four of the most important considerations in the cultivation of anaerobic bacteria are:

a. Proper collection and transport of the material to be examined.

b. Culture of the material as soon as possible after collection.

c. Use of freshly prepared and properly reduced media.

d. Proper anaerobic conditions.

Section XI. ANTIBACTERIAL AGENTS, STERILIZATION, AND ASEPTIC TECHNIQUE

1-29. ANTIBACTERIAL AGENTS

Sterility may be defined as freedom from all living organisms, and sterilization is the process of killing or removing microorganisms from the environment. An agent exerts a bactericidal effect if it kills bacteria, while a bacteriostatic agent inhibits bacterial reproduction. On removal of the bacteriostatic agent, bacterial reproduction resumes. A disinfectant is a bactericidal agent that acts as a general protoplasmic poison. Because disinfectants are generally very toxic, their use is restricted to fomites (nonliving objects such as glassware, bench tops, and floors). An antiseptic is a bactericidal agent that is less potent than a disinfectant. Antiseptics are used topically, externally, on the skin. An understanding of these terms is necessary for consideration of the effects of physical and chemical agents upon bacteria.

a. **pHisoHex.** pHisoHex is the brand name of a detergent that is used as an antiseptic. This detergent contains the phenolic derivative, hexachlorophene, which acts as a bactericidal agent. Small amounts of this detergent should be used to wash the hands whenever bacterial cultures or clinical specimens have been handled.

b. **Wescodyne.** Wescodyne is the brand name given to a solution of active iodine. It acts as an oxidizing agent and is used as a general disinfectant. It is further characterized as nonirritating to the skin.

c. **Phenol.** Phenol is an organic compound whose germicidal activity is taken as the standard for all disinfectants; that is, the activity of other disinfectants is compared to that of phenol. A 5 percent solution of phenol readily destroys spores and vegetative cells. It is used on inanimate or nonliving objects such as glassware, bench tops, and floors.

d. **Alcohol.** Alcohol is used extensively as an antiseptic. It is relatively ineffective as a bactericidal agent, but exerts its maximum effect at a concentration of 70 percent.

1-30. GENERAL COMMENTS ON STERILIZATION

a. Bacteriological identification requires that pure cultures of microorganisms be studied. Since microorganisms are ubiquitous, all materials used in the cultivation of bacteria must be subject to preliminary sterilization.

b. The fundamental principle of media sterilization is to remove or destroy all living material on or within a medium without rendering it ineffective for cultivating the desired microorganisms. The methods most commonly used involve moist or dry heat and filtration. The particular sterilization method employed is governed by the items to be sterilized and their intended use.

c. The usual items of glassware must be scrupulously clean. Flasks and tubes are plugged with nonabsorbent cotton to prevent entry of bacteria after sterilization. Small portions of cotton are inserted in the mouthpiece of pipets which are subsequently wrapped in paper or placed in metal canisters. All materials are wrapped in paper or placed in canisters. All materials are wrapped to preclude contamination after sterilization. It is a good practice to cap cotton plugs of flasks with paper or foil. Glassware prepared in this manner will remain sterile up to 30 days if properly stored.

1-31. MEDIA PREPARATION

Just as it is important to observe aseptic technique in the processing and culturing of specimens, it is equally important to prepare the media used in bacteriology with great care. The results obtained depend directly on the quality of the media used. Before autoclaving, the flask containing the medium should be covered. This cover is kept in place except when the medium is actually being poured after autoclaving. Before the medium is poured from the flask, and occasionally while pouring, the mouth of the flask should be flamed to kill any air contaminants which may have landed on the mouth. Plates are poured by lifting the lip of the Petri plate only enough to pour in the agar. The flask must not be allowed to touch the plate while pouring, and as soon as a plate has been poured, the lid must be put back on. Liquid media are prepared either by pouring or dispensing into tubes and then autoclaving, or by pipetting sterile media into sterile tubes.

1-32. HEAT

In any discussion of the effect of heat upon bacteria, it must be realized that a time-temperature relationship exists in all cases. In this regard, if vegetative bacterial cells are exposed to a temperature of 55º to 58ºC for 30 minutes, all psychrophiles and mesophiles will be destroyed, while some thermophiles will survive. The process of pasteurization is based upon this time-temperature relationship. Heat may be applied in a dry or wet form.

a. **Dry Heat.** The use of dry heat to control bacteria is frequently employed in the bacteriology laboratory. The flame of the Bunsen burner is used to sterilize bacteriological loops and needles. Contaminated materials which will burn are destroyed by burning. The hot air oven is used to dry glassware and to sterilize glassware and metalware. Sterilization is brought about by employing a temperature of 170º to 180ºC for 1/2 to 2 hours. Heatlabile substances such as culture media, paper, rubber, plastic items, and non-heat-resistant glassware cannot be sterilized by using the hot air oven.

b. **Moist Heat.** Control systems based upon the use of moist heat are used extensively. Moist heat rather than dry heat is used in the sterilization of culture media.

(1) Boiling water. The most basic system using moist heat to control bacteria is seen in the practice of placing surgical instruments in boiling water. This procedure does not always provide sterile conditions. Ideally, boiling water provides a temperature of 100ºC, and although vegetative bacterial cells are destroyed by such treatment, spores of bacteria are resistant to boiling water. Accordingly, boiling is not a recommended procedure, and it is not used in the bacteriology laboratory to establish sterile conditions.

(2) Free-flowing steam. Free-flowing steam finds limited use in the bacteriology laboratory as a means of sterilizing media. It must be remembered that in terms of temperature, steam is formed at 100ºC. Any sterilization procedure based upon the utilization of free-flowing steam will be limited to the destruction of vegetative cells. For this reason, media to be sterilized by tyndallization, the name given to the procedure of sterilization by the use of free-flowing steam, must be treated on three successive days. Most spores present in the media would germinate, and the resultant vegetative cells would be susceptible to destructive action of free-flowing steam. The Arnold sterilizer is based upon the free-flowing steam principle.

(3) Steam under pressure. At this point it should be apparent that if spores are to be destroyed along with vegetative cells, a temperature higher than 100ºC is required. Temperatures higher than 100ºC are made possible by placing steam under pressure; the autoclave is the name of the instrument whose operation is based upon steam under pressure. It is the increased temperature which destroys microorganisms; the pressure acts to increase the temperature of the steam. Usually the items to be sterilized are exposed to a temperature of 120ºC for 15 minutes at a setting of 15 pounds pressure. (Exposure time starts after the desired temperature level and pressure are reached.) It must be remembered, however, that the increased temperature of the autoclave may result in the breakdown of thermolabile substances such as urea and carbohydrates. When media containing these substances are to be sterilized, adjustments in the sterilization procedure are required. Autoclaving is the most extensively used method for sterilizing culture media.

1-33. FILTRATION

Sterilization by filtration represents a mechanical means of removing bacteria from liquids. When the relative size of bacteria and spores is recalled, it should be apparent that the porosity of the filter that is used must be extremely small. The Seitz filter and the membrane filter are examples of very fine filters that are used in the bacteriology laboratory. The use of these filters is recommended for the sterilization of liquids containing thermolabile substances such as carbohydrates, urea, and sera.

1-34. RADIATION

Bacteria, like all living systems, are susceptible to the lethal effects of radiation. In the laboratory, ultraviolet (UV) light sources are sometimes built into isolation hoods. Special UV lighting devices are sometimes installed in rooms where highly infectious specimens, such as those from tuberculosis patients, are handled. Exposure of specimens or equipment must be direct and sufficiently prolonged. (Severe damage to the eyes can result from even a short exposure to ultraviolet rays. Highly penetrating rays such as x-rays and gamma rays are not routinely used in the medical bacteriology laboratory.)

1-35. GENERAL COMMENTS ON ASEPTIC TECHNIQUE

In the bacteriology section of a laboratory, the specialist is constantly exposed to microorganisms that can and do cause disease in man. Aseptic technique is a manipulative skill that prevents self-infection when working with pathogenic microorganisms and also prevents the introduction of extraneous microorganisms into a system. This skill is applied when dealing with bacteria or with anything that can come into contact with bacteria. The purpose of aseptic technique is to protect the specialist and his co-workers, and to prevent contamination of the specimen with which he is working.

1-36. WORKING AREA

a. The working area must be decontaminated before starting the day's work and again at the completion of the day. In addition, if a specimen should be spilled onto the working area, the affected area should be immediately decontaminated. One way to accomplish decontamination of working space is to soak a paper towel with the decontaminant and then wipe the working area with this solution and allow to air-dry. Ultraviolet light can also be effectively used, but its use is ordinarily restricted to a specific area, such as an inoculating hood, or to nighttime use.

b. Unauthorized personnel should be forbidden access to the bacteriological section. Doors should remain closed.

1-37. DISPOSAL OF CONTAMINATED MATERIALS

The media, specimens, and equipment which are used in the processing of specimens must be correctly disposed of to avoid the possibility of infecting yourself or your co-workers, and to avoid the possibility of contamination of other specimens. This material may be safely decontaminated by incineration, autoclaving, or by immersion in disinfectant solutions.

a. Loops and needles are sterilized by incineration. Swabs and disposable equipment may also be incinerated, but should not be incinerated by a Bunsen burner.

b. Autoclaving can be used on all contaminated materials such as swabs, Petri dishes, and test tubes. Swabs and other disposable materials should then be discharged. Glass Petri dishes, tubes, and closures should be cleaned and sterilized for reuse.

c. After use, glassware is in a disinfectant solution. Next it is autoclaved, washed, plugged with cotton (as applicable), and sterilized in pipet canisters or individual packets for reuse.

d. The method used by any laboratory depends on the local procedures of that laboratory, but strict attention must be paid to aseptic technique, regardless of the procedure used.

1-38. PERSONAL CLEANLINESS

A veterinary specialist working in a food microbiology laboratory must take measures to protect himself. To adequately accomplish this, it is frequently necessary to wash your hands in disinfectant solution or with surgical soap. It is always necessary to practice aseptic technique. You should never put objects such as fingers or pencils into your mouth. Avoid mouth pipetting. If you should accidentally spill a specimen on yourself, you must decontaminate yourself with a noncaustic disinfectant solution such as Wescodyne. To ignore any of these precautions is to invite trouble in the form of an infection or illness.

Section XII. ISOLATION OF BACTERIA

1-39. INTRODUCTION

a. Disease-producing bacteria usually occur in specimens in association with other bacteria, rather than in the pure state. In laboratory identification of microorganisms it is necessary that pure cultures (cultures containing only a single species) be studied.

b. To secure a pure culture of a given organism from a specimen or sample containing mixed flora, it is necessary to isolate a single cell from all other cells present. The cell is cultivated in such a manner that its collective progeny remain isolated.

c. Two common methods used to inoculate specimens or broth cultures to agar media, are the streak plate method and the pour plate method.

1-40. INOCULATING LOOPS AND NEEDLES

The tools used in these techniques are the loop and the needle.

a. The inoculating loop and the inoculating needle are composed of platinum or nichrome wire affixed to a handle. The needle is used to transfer colonies from one broth medium to another or to an agar medium.

b. During use, the loop or needle must never be allowed to touch the outside or lip of a container since contamination may result. Contamination gives a distorted picture of the contents of the specimen which is being cultured. When using a needle to pick a colony from an agar medium, touch the needle to the top center of the colony, avoid visible contaminants, and do not dig into the agar.

c. Before a loop or needle is used, it must be sterilized. It is held downward at a 45º angle within the blue part of the flame of a Bunsen burner and is heated to red hot, starting at the base of the wire next to the handle and moving slowly toward the tip. If the loop or needle is not clean and dry, it should be slowly heated at first to avoid spattering and contamination of the surroundings. After being sterilized, the loop or needle is cooled for approximately 1 to 20 seconds to prevent heat destruction of the microorganisms in the specimen.

d. When not in use, the loop or needle is kept in a rack or a special stand. It should never be placed on a working surface.

1-41. STREAK PLATE

a. The plate is kept in an inverted position during processing and incubation. Only the part of the Petri dish containing the medium is picked up when working with the dish in order to prevent contamination of the surface of the plating medium and therefore contamination of the specimen. This is done whether the streaking is for isolation or for sensitivity studies. The Petri dish should be labeled with the specimen number or patient's name, and the date and time of inoculating. This is done prior to inoculation to prevent any possibility of the interchange of plates and the reporting of incorrect results.

b. A loopful of inoculum is collected on a flame-sterilized wire loop and streaked over approximately one-quarter of the agar surface. After flaming the loop again and without collecting more inoculum, the plate is rotated slightly and another quadrant of the agar surface is streaked, overlapping the original quadrant as shown in figure 1-7. This process of diluting and spreading the inoculum over the medium is continued until the entire agar surface is covered. As the streaking continues, fewer and fewer cells remain on the loop, and finally single cells are deposited on the agar. Each isolated cell will give rise to a visible colony under suitable environmental conditions. If clinical materials on cotton swabs are to be cultured, the swab is rolled over a small area of the agar surface at the edge of the plate. With a wire loop the inoculum is spread over the four quadrants of the agar surface in the previously described manner (figure 1-7).

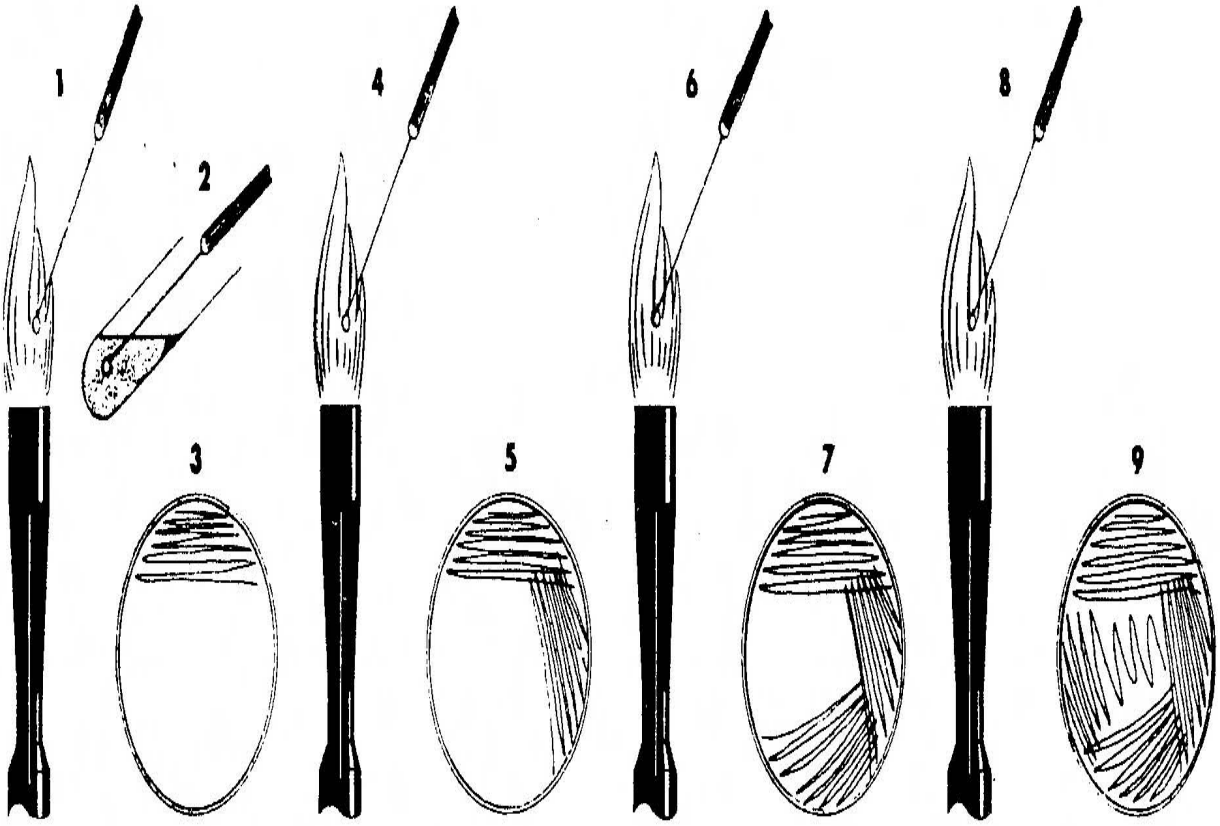


Figure 1-7. Streak plate method.

c. The streak plate method is the most common means of securing isolated colonies, and when properly done is just as reliable and much more rapid than the pour-plate method. Although any agar base medium may be used, blood agar streak plates are usually employed for primary isolation of pathogenic bacteria. After inoculation, the plates are ordinarily incubated at 37ºC for 18-24 hours in the inverted position. Growth of isolated colonies is examined grossly and microscopically for characteristics of various genera and species. Using a wire needle, pure cultures are obtained by picking growth from the center of the colony and subculturing to suitable broth or agar media.

1-42. POUR PLATE METHOD

a. At times it may be necessary to utilize a pour plate technique for securing isolated colonies. To accomplish this, culture tubes containing approximately 12 ml of sterile infusion agar or other suitable medium are placed in boiling water to melt the agar. After cooling the medium to about 48ºC in a water bath, approximately 0.7 to 1.0 ml of sterile, defibrinated blood is aseptically added to each tube of melted agar. Each blood medium mixture is then inoculated with a sample of the appropriately diluted specimen, mixed well by twirling the tube, and dispensed to sterile Petri dishes. It is important that the inoculum be diluted properly in preparing pour plates when many organisms are observed in the gram smears of the specimen. This dilution may be accomplished by inoculating a loopful of the original specimen to 5 to 6 ml of sterile broth or normal saline. After mixing thoroughly, one loopful of this dilution is inoculated to the melted blood agar.

b. Upon media solidification, individual cells in the inoculum are immobilized in various areas of the agar. During incubation each cell will multiply to form a visible colony. Blood agar pour plates are primarily used for determining the type of hemolysis produced by strains of streptococci. When subcultures or stains are to be prepared from pour plates cultures, it is necessary to secure growth from individual colonies within the agar using a sterile needle.

1-43. TEST TUBE CULTURES

After isolated colonies are obtained by the streak plate or pour plate technique, it is often necessary to subculture growth to tubed media to permit further study. Tubed media are prepared by dispensing broth or agar media into appropriate test tubes. The tubes are plugged with small sections of rolled cotton which are bent in the middle and inserted in the tube 2.5 cm and should project at least 2 cm outside the lip of the tube. Cotton plugs should fit snugly, but not so tight that difficulty will be experienced in removing and replacing the plug during bacteriological manipulations. After sterilization, broth and agar stab tubes are allowed to cool in the upright position. Agar slants are prepared by inclining the tubes of melted agar medium on a table top until solidification occurs. To prepare pure cultures, each type of medium is inoculated as follows:

a. **Liquid Cultures (Broth).** Using a wire loop, emulsify a small amount of growth on the moist wall of the tube just above the liquid and wash down by tilting the tube. If the inoculum is liquid, a loopful is simply placed in the broth and dispersed by gentle agitation.

b. **Slant Cultures.** Slant cultures are prepared by streaking the inoculum over the slant surface from bottom to top. If the slant contains some water of condensation at its base, do not spread the water over the surface of the slant since the resulting growth will not yield characteristic colony appearance.

c. **Stab Cultures.** Stab cultures are made with a straight needle into tubes of unslanted solid or semisolid medium. The stab line, centered without lateral movement, should extend approximately one-half to two-thirds the depth of the medium.

Continue with Exercises

EXERCISES, LESSON 1

INSTRUCTIONS. Answer the following exercises by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. The significance of microorganisms in food depends upon which of the following factors:

   a. Type of food.

   b. Processing treatment the food will receive.

   c. Individual consuming the food.

   d. Type of microorganisms.

   e. Number of microorganisms.

   f. All of the above.

   g. None of the above.

2. The term invasiveness may be defined as:

   a. Ability of the bacteria to produce toxic substances.

   b. Degree of pathogenicity.

   c. Ability to cause disease.

   d. Ability of the bacteria to enter, spread, and multiply in the host tissue.

3. The term virulence denotes the degree of invasiveness.

   a. True.

   b. False.

4. The term infection may be defined as:

    a. Bacteria reproducing asexually by binary fission.

    b. Ability to cause disease.

    c. Process whereby a pathogen enters into a relationship with the host.

    d. Degree of pathogenicity.

5. The two types of toxins are:

    a. Antigenic and labile.

    b. Endotoxins and exotoxins.

    c. Virulent and invasive.

    d. Pathogenic and antigenic.

6. All of the following are extracellular enzymes except:

    a. Coagulase.

    b. Hyaluronidase.

    c. Lysozyme.

    d. Streptokinase.

7. One would expect to find spores with:

    a. Cocci.

    b. Bacilli.

    c. Spirilla.

    d. All of the above.

8. The formation of spores by bacteria is generally considered as a means of:

    a. Reproduction.

    b. Toxin formation.

    c. Survival.

    d. Resistance to phagocytosis.

9. A toxin that is found in the cell wall of a living organism is:

    a. An endotoxin.

    b. An enterotoxin.

    c. An exotoxin.

    d. A pseudotoxin.

10. Heterotrophic bacteria require _____ media.

    a. Organic.

    b. Inorganic.

11. The outer layer that gives rigidity to a bacterial cell is known as the:

    a. Cell wall.

    b. Cytoplasmic membrane.

    c. Nuclear membrane.

    d. Cytoplasm.

12. Most pathogenic bacteria grow best at a pH near:

    a. 3.0.

    b. 4.5.

    c. 7.0.

    d. 9.0.

13. Alcohol is a rather ineffective bactericidal agent but it exerts its maximum effect at a concentration of:

    a. 70 percent.

    b. 80 percent.

    c. 90 percent.

    d. 100 percent.

14. Prior to sterilization, materials are packaged or wrapped to preclude:

    a. Breakage.

    b. Heat damage.

    c. Chemical breakdown.

    d. Contamination after sterilization.

15. Which of the following is the most extensively used method for sterilizing culture media?

    a. Dry heat.

    b. Autoclaving.

    c. Filtration.

    d. Tyndallization.

16. What is the disease of public health importance for bacteria Clostridium tetani?

    a. Typhoid fever.

    b. Paratyphoid fever.

    c. Anthrax.

    d. Plague.

    e. Lockjaw.

    f. Tuberculosis.

    g. Syphilis.

17. What is the disease of public health importance for bacteria Pasteurella pestis?

    a. Typhoid fever.

    b. Paratyphoid fever.

    c. Anthrax.

    d. Plague.

    e. Lockjaw.

    f. Tuberculosis.

    g. Syphilis.

18. What is the disease of public health importance for bacteria Mycobacterium bovis?

    a. Typhoid fever.

    b. Paratyphoid fever.

    c. Anthrax.

    d. Plague.

    e. Lockjaw.

    f. Tuberculosis.

    g. Syphilis.

19. What is the disease of public health importance for bacteria Bacillus anthracis?

    a. Typhoid fever.

    b. Paratyphoid fever.

    c. Anthrax.

    d. Plague.

    e. Lockjaw.

    f. Tuberculosis.

    g. Syphilis.

20. What is the disease of public health importance for bacteria Salmonella typhi?

a. Typhoid fever.

b. Paratyphoid fever.

c. Anthrax.

d. Plague.

e. Lockjaw.

f. Tuberculosis.

g. Syphilis.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 1

1. f (para 1-2)
2. d (para 1-4a)
3. b (para 1-4a)
4. c (para 1-4c)
5. b (para 1-7)
6. c (para 1-8)
7. b (para 1-14b(3))
8. c (para 1-14b(3))
9. a (para 1-7b)
10. a (para 1-12)
11. a (para 1-14a(1))
12. c (para 1-23)
13. a (para 1-29d)
14. d (para 1-30c)
15. b (para 1-32b(3))
16. e (figure 1-5)
17. d (figure 1-5)
18. f (figure 1-5)
19. c (figure 1-5)
20. a (figure 1-5)

End of Lesson 1

LESSON ASSIGNMENT

LESSON 2	Microorganisms Causing Foodborne Illness and Other Diseases of Public Health Significance.
LESSON ASSIGNMENT	Paragraphs 2-1 through 2-50.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

2-1. Identify the three principal causes of foodborne illness.

2-2. Given a foodborne illness, identify the illness as to causative agent, symptoms of the disease, foods involved in the outbreak, and the methods for prevention of the disease.

2-3. Given a list of diseases and the causative agent, match the disease with the correct causative agent.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 2

MICROORGANISMS CAUSING FOODBORNE ILLNESS AND OTHER DISEASES OF PUBLIC HEALTH SIGNIFICANCE

Section I. INTRODUCTION TO FOODBORNE ILLNESS

2-1. GENERAL

a. Toward the end of the last century, food poisoning was thought to be caused by ptomaines that had been formed in foods as a consequence of protein decomposition. This hypothesis was invalidated later when it was determined that many foodborne diseases were caused by microorganisms.

b. The greatest foodborne disease hazard now appears to be pathogenic bacteria. The great majority of foodborne disease incidents are attributable to errors in food service in the home, in public and private institutions, or in commercial eating establishments. Increased application of good sanitary and personal hygiene practices markedly reduces the incidence of foodborne illness.

c. Foodborne disease is an inclusive term for many syndromes. Typical is acute gastroenteritis with sudden onset of vomiting or diarrhea, or both, with accompanying abdominal pain. The incubation time (time between eating and onset of first symptom), the type of symptoms, and the duration of symptoms are variable and depend upon the etiologic agent.

2-2. CAUSES

The principal causes of foodborne diseases fall into three categories:

a. Metallic or poisonous chemicals.

b. Toxicants, occurring naturally in plants or animals.

c. Microbiological agents (bacteria, viruses, protozoa, helminths, molds) or their toxins.

2-3. CHEMICALS

Food poisoning outbreaks caused by chemicals are relatively uncommon. Metallic poisonings can occur when high-acid foods (such as fruit juices and carbonated beverages) are stored in, or allowed to flow through, metal-containing (copper or cadmium) or metal-coated (zinc, antimony, or lead) vessels or pipelines. Other chemical poisonings occur because workers inadvertently mistake poisons (pesticides) for food stuffs, or add excessive amounts of flavor intensifiers, curing agents, or preservatives to foods. Such contamination may occur at any point in the food chain.

2-4. TOXICANTS

a. Illnesses from inherently poisonous foods occur because uninformed persons mistake toxic fish, shellfish, crustacea, mushrooms, or uncultivated plants for edible varieties. Fortunately, such foods are normally excluded from the commercial food chain.

b. Paralytic shellfish poisoning is contracted by eating contaminated oysters, mussels, or clams. The dinoflagellates from which the oysters, mussels, or clams obtain the toxin have a wide distribution in marine waters. Masses of toxic dinoflagellates give rise to "redtide" of the sea. Prevention of this type of food hazard consists of avoiding seafood from waters laden with toxic dinoflagellates.

2-5. MICROBIOLOGICAL AGENTS OR THEIR TOXINS

a. The following food poisoning bacteria will be discussed in the following sections on the various genera of bacteria:

(1) Staphylococcus aureus.

(2) Streptococcus species.

(3) Bacillus cereus.

(4) Clostridium botulinum.

(5) Clostridium perfringens.

(6) Escherichia coli.

(7) Salmonella species.

(8) Shigella species.

(9) Vibrio parahaemolyticus.

b. Occasionally outbreaks of viral hepatitis have been traced to food, but there is little evidence of foodborne transmission of other viral agents. The proof that viruses are important agents of foodborne diseases awaits better methods of recovering viruses from foods. Therefore, food is known to serve as a vehicle for only two viral diseases of man, poliomyelitis and infectious hepatitis.

c. Some molds have been found to produce mycotoxins in foods. These toxins have caused illness in individuals who ate large quantities of moldy foods. Aspergillus flavus is one of the storage fungi that develop on a variety of stored grains such as wheat, peanuts, soybeans, and corn.

d. The only protozoan disease of any known importance in food microbiology is toxoplasmosis. Infected meat serves as a source of human toxoplasmosis. The encysted parasites are scattered and too small to be seen with the naked eye, thus making their detection by visual inspection all but impossible. In adults, symptoms consist of fever with a rash, headache, muscle aches and pain, and swelling of lymph nodes.

e. Of the flatworms that are parasitic for man, three are obtained by eating the flesh of pork, beef, and fish.

(1) The beef tapeworm lives as an adult in the human intestine and in larval form in the muscles of bovines. The form of the organism found in the flesh is referred to as a cysticerus.

(2) The pork tapeworm has a life cycle similar to that of the beef tapeworm but man can serve as the intermediate host as well. Both the beef and pork tapeworms are distributed worldwide, with the incidence being highest where raw or improperly cooked pork is eaten.

(3) The third tapeworm which man acquires from food is the broadfish tapeworm. Man obtains this organism by ingesting fish such as pike or trout which become infested upon by the ingestion of copepods.

f. The nematode of greatest importance from the standpoint of food is Trichinella spiralis. Trichinosis is caused by migration through the human body of larvae of Trichinella spiralis and by their encystment in muscles after ingestion of raw or insufficiently cooked, infected meat. Clinical disease in man is highly variable. Usually a mild febrile disease, but can range from inapparent infection to a fulminating, fatal disease.

(1) Mode of transmission. Trichinosis is transmitted by eating the raw or insufficiently cooked flesh of animals containing viable encysted trichinae. This chiefly involves pork and pork products. Beef products such as hamburger, adulterated with pork, may also be involved.

(2) Methods of control.

(a) Regulations help to assure adequate processing of pork products.

(b) Pork must be ground in a separate grinder, or the grinder must be thoroughly cleaned before processing other meats.

(c) Garbage should be cooked before feeding to swine.

(d) All fresh pork and pork products should be cooked at a temperature and time sufficient to allow all parts to reach at least 65.6ºC (150ºF) or until meat changes from pink to grey.

(e) Bear meat should be thoroughly cooked.

(f) Low temperature(s) maintained throughout infected meat are effective in killing trichinae, that is, holding at -27ºC for 36 hours or at higher temperatures for longer periods of time. At least 20 days of storage at -18ºC (0ºF), the temperature of the home freezer, is required.

Section II. STAPHYLOCOCCI

2-6. GENERAL COMMENTS ABOUT GRAM-POSITIVE COCCI

The organisms comprising the gram-positive cocci are chiefly the staphylococci, streptococci, and pneumococci. Collectively, these organisms are responsible for a variety of human infections. Such infections range from relatively simple involvements of the skin and mucous membranes to more serious diseases which may be manifested in food poisoning, pneumonia, septicemia, rheumatic fever, acute glomerulonephritis, or deep tissue abscesses.

2-7. PATHOGENICITY OF STAPHYLOCOCCI

a. **Common Manifestations.** The staphylococci (figure 2-1) are ubiquitous in nature. They occur as normal inhabitants on the skin and in the respiratory and gastrointestinal tracts of man. The majority of such forms are the comparatively avirulent organisms. Staphylococcus epidermidis and the related forms of Micrococcus and Sarcina are saprophytes frequently isolated from the skin and mucous membranes.

(1) Staphylococcus aureus strains are usually responsible for the staphylococcal diseases of man. These forms occur especially in the upper respiratory tract of asymptomatic individuals. The asymptomatic carrier is of considerable importance in transmitting these organisms.

(2) Staphylococcal diseases are most commonly manifested in localized suppurations which may be in the form of simple pustules, hair follicle infections, boils, or extensive carbuncular conditions that may progress to form metastatic abscesses in any tissue. The latter results from the spread of the organism via the bloodstream.

(3) A majority of cases of osteomyelitis, enterocolitis, otitis media, and sinusitis are of staphylococcal etiology. Pneumonia, meningitis, and endocarditis are relatively infrequent manifestations of the staphylococci.

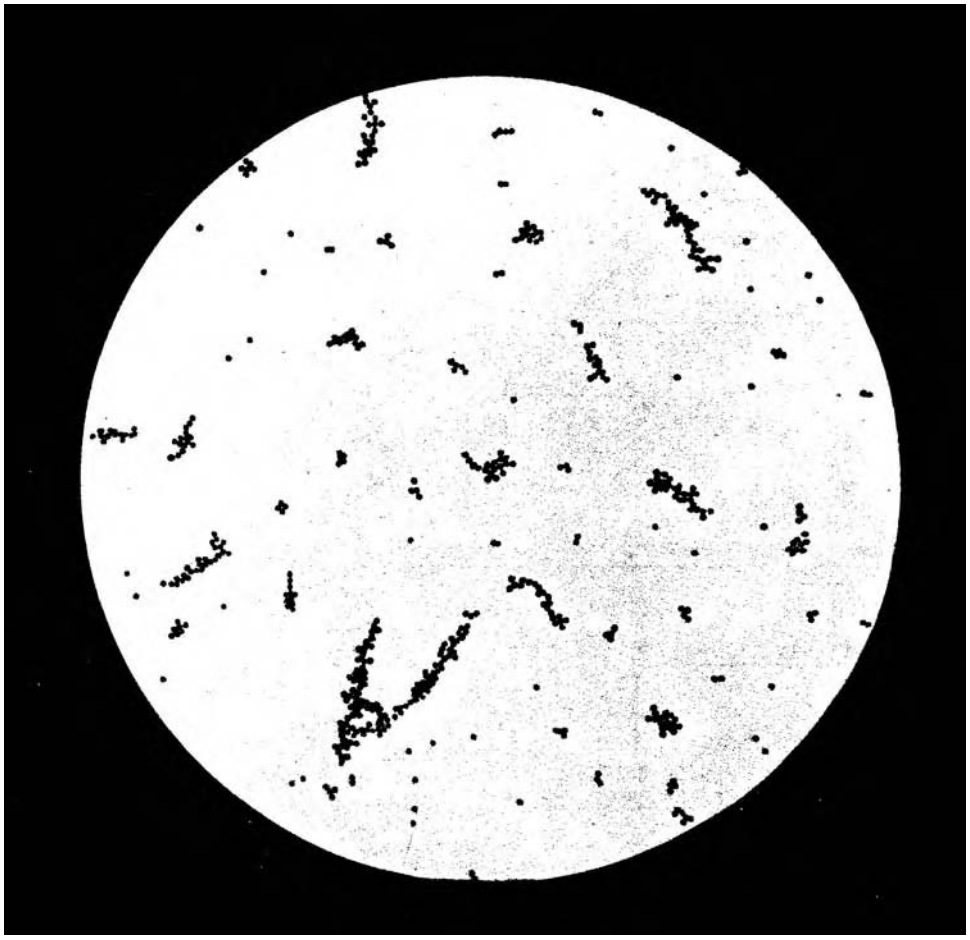


Figure 2-1. Gram-stained smear of Staphyloccocus species from broth culture.

b. **Enzyme Secretions.** The virulent staphylococci excrete a variety of substances which account for their ability to invade tissue and cause disease in man. Coagulase is an enzyme produced by pathogenic staphylococci. Coagulase causes a clotting of plasma which results in the formation of a layer of fibrin around a given staphylococcal lesion. Although this fibrin wall may confine the infection to a localized process, it also serves as a protective barrier for the organism against phagocytic activity and the action of antimicrobial drugs. This, then, is one of the very valuable clinical laboratory tests used to identify Staphlyococcus aureus.

c. **Toxin Production.** A variety to toxins may appear in cultures one to three days old when grown in a semisolid infusion agar of veal or beef. These cultures must be incubated in an atmosphere of 20 to 40 percent carbon dioxide. The exotoxins may be destroyed by heating to 55º to 60ºC. Any of the following may be present in such a filtrate: lethal toxin, hemolysin, leukocidin, and dermonecrotic toxin.

d. **Hospital Staphylococci.** Staphylococci have eventually become resistant to practically every antibiotic introduced to combat its presence. Each succeeding generation seems to be more resistant to drugs than the previous parental strain. This has been primarily due to the promiscuous use of antibiotics to treat everything from a cold to a sore toe.

(1) Since the advent of penicillin, when there were almost no drug-resistant strains, certain Staphylococcus species have been particularly adept at developing drug resistance. Since antibiotics were originally administered in low doses for such widely divergent ailments, those strains of organisms which were not eliminated by use of drugs, have been able to develop into strains which are particularly pathogenic and resistant to almost every antibiotic that has been developed.

(2) This has led to the necessity of determining a series of tests to discover which antibiotic is effective for each patient. The antibiotic that is more effective against one strain of cocci for one person may not be best for another person with a different strain. Some larger laboratories perform phage typing of hospital staphylococci for epidemiologic purposes.

e. **Foodborne Illness Due to S. aureus.** The initial symptoms of staphylococcal intoxication are excessive salivation and nausea. These are followed by a sudden onset of vomiting which is the predominant and the most severe symptom. Vomiting usually occurs 2 to 4 hours after a food that contains staphylococcal enterotoxin is ingested; however, it may occur in as short a time as 1 hour or as long as 8 hours. Abdominal cramps and diarrhea are often very marked. Other symptoms observed are sweating, cold and clammy skin, tetanic muscular contractions, weakness, depression, anorexia, shock, and dehydration. Mortality is low. Most of the deaths have occurred in children or the aged. On the other end of the spectrum of intoxication, mild attacks of just nausea or belching without vomiting or diarrhea also occur. The foods that are usually involved in staphylococcal intoxications are protein-containing products which are contaminated after cooking.

(1) Raw milk from individual cows has been incriminated as a vehicle in numerous outbreaks. For raw milk to cause outbreaks cows must have staphylococcal udder infections, and the milk must be ingested without heat processing such as pasteurization.

(2) Staphylococcal intoxication has resulted from the ingestion of cheese. Four contributing factors to the large number of staphylococci in the cheese were:

(a) Substantial contamination with staphylococci originating from cow's udder.

(b) Inadequate cooling of raw milk.

(c) Presence of antibiotic residues.

(d) Contamination with strains of staphylococci resistant to the antibiotics present in milk.

(3) In the United States (US), ham has been responsible for most reported outbreaks.

(4) Other foods responsible for outbreaks have been cream-filled pastry, chicken, turkey, beef, potato salad, meat mixtures, and egg products.

(5) In man, the main reservoir of S. aureus is the nose. From this source, these organisms find their way to the skin and into wounds. The two most important sources of this organism to foods are nasal carriers and individuals whose hands and arms are inflicted with boils and carbuncles and who are permitted to handle foods.

(6) When susceptible foods are produced with low numbers of staphylococci, they will remain free of enterotoxin and other food poisoning hazards if kept either below 4ºC or above 60ºC until eaten.

(7) The factors that have contributed the greatest to foodborne illness disease outbreaks have been inadequate refrigeration, preparing foods far in advance of planned service, infected persons practicing poor personal hygiene, inadequate cooking or heat processing, and holding food in warming devices at bacterial growth temperatures.

2-8. CULTURAL CHARACTERISTICS OF STAPHYLOCOCCI

a. Staphylococci are nonmotile, nonsporogenous, and usually do not form capsules. The exception to capsule formation is in very young broth cultures after a few hours incubation. These bacteria always stain gram-positive. Those cocci not staining gram-positive are due to old and dying cultures, organisms phagocytized by white cells, and those in the center of clusters.

b. The staphylococci grow readily on ordinary nutrient media without the presence of special enrichments. The more commonly occurring species are facultative organisms. A few staphylococci, which may be weakly pathogenic, are strict anaerobes. The staphylococci isolated from human disease grow well at 37ºC. Abundant growth usually takes place in 18 to 24 hours of incubation.

c. The colony morphology of Staphylococcus species is usually characteristic. After 18 hours incubation at 37ºC on an agar media, staphylococci form rather large colonies ranging from 2 to 4 mm in diameter. The colonies are opaque, round, smooth, raised, glistening, and with an entire (even) margin. The colonies may develop a characteristic golden, porcelain-white, or lemon-yellow pigment. The colony is soft or "butterlike" in consistency.

d. Pigmentation is more evident after plates of the cultivated organism have been exposed to room temperature overnight. Young colonies of staphylococci are not pigmented and appear colorless. As growth continues, a pigmentation results that will not diffuse into the surrounding medium. This is what gives pus and sputum a faint golden yellow color and usually indicates possible infection with staphylococci. All of the characteristics given up to this point relate to the genus Staphylococcus.

e. Colonies of Staphylococcus aureus typically have a golden pigmentation on initial isolation while colonies of Staphylococcus epidermidis are usually white when cultivated on blood agar. When grown on blood agar, Staphylococcus aureus usually causes beta hemolysis (complete lysis of the RBCs), resulting in a clear zone around the colony, while Staphylococcus epidermidis generally does not hemolyze the RBCs.

Section III. STREPTOCOCCI

2-9. INTRODUCTION TO THE STREPTOCOCCI

a. The streptococci (figure 2-2) are spherical to ovoid cocci ranging between 0.8 and 1.0 micron in diameter. The cocci predominantly occur in chains; however, paired or single cells may also be observed. Characteristic chains are more typical in smears from broth cultures. The streptococci are nonmotile and nonsporeforming. They are typically stained gram-positive, although gram-negative forms are occasionally observed in specimens of old cultures.

b. Certain Streptococcus species produce polysaccharide capsules, while others produce a capular substance composed of hyaluronic acid. The presence or absence of capsules is not a distinct feature for use in routine identification of streptococcal forms.

c. Several schemes for classifying the streptococci have been devised. The Lancefield classification has as its basis the antigenic structure of the organisms. The structure of a carbohydrate antigen ("C" substance) is different for each group in this series. The groups are designated by the letters A through O. According to this classification, group A strains are the most common human pathogens.

d. A second classification, devised by Sherman, has as its basis both the physiologic and immunologic characteristics of the streptococci. The Sherman classification is composed of the pyogenic streptococci, the viridans group, the enterococci, and the lactic streptococci. The human pathogens are in the pyogenic group.

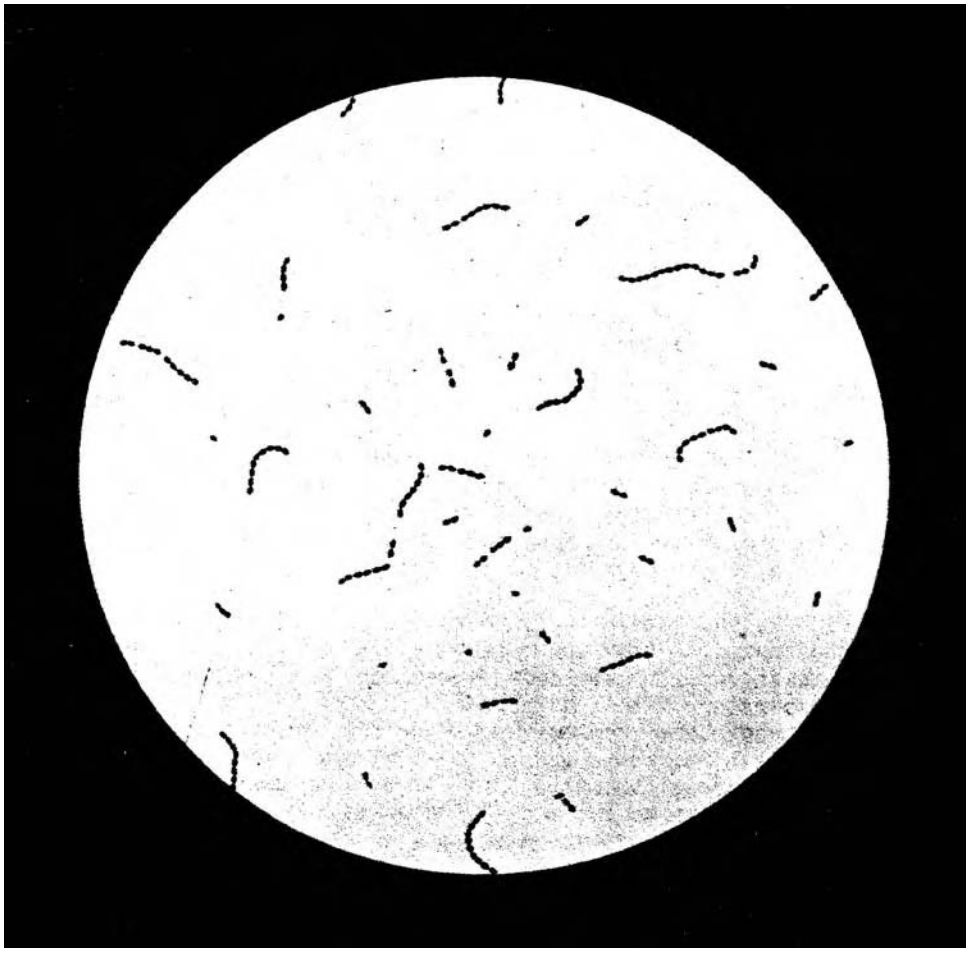


Figure 2-2. Gram-stained smear of Streptococcus species from broth culture.

2-10. HEMOLYTIC PATTERNS

The most useful method for preliminary differentiation of streptococci is the pattern of hemolysis on blood agar.

a. The hemolytic action of Streptococcus species is influenced by the type of blood used in blood agar. The blood of choice for study of hemolysis is defibrinated sheep blood in a concentration of 5 percent. Glucose should be excluded since it may obscure hemolytic reactions. If sheep blood is not available, use rabbit or horse blood.

b. The pour plate method is by far the best method for studying hemolytic activity; it provides subsurface colonies of streptococci whose hemolytic activity is not greatly affected by oxygen. For convenience, a general practice is to study the hemolytic patterns of surface colonies. It must be remembered, however, that the hemolytic activity of such surface colonies may not be completely typical.

c. The four types of hemolytic patterns, which are best observed under low-power (100X) magnification, are defined as follows:

(1) Alpha (α). An indistinct zone around a colony in which red cells are partially destroyed. There is often a greenish or brownish discoloration of the medium near the colony.

(2) Beta (β). A clear, colorless zone around a colony that indicates complete lysis of the red cells. This is best seen in deep colonies in a pour plate since oxygen affects the activity of hemolysins and may make surface colonies appear to be alpha or nonhemolytic.

(3) Gamma (γ). No apparent hemolytic activity or discoloration around the colony.

(4) Alpha prime (α^1) or wide zone alpha (Wfa). A small halo of intact or partially lysed cells immediately surrounding the colony, with a zone of complete hemolysis extending further into the medium. Without a microscope, this can be confused with beta hemolysis.

2-11. PATHOGENICITY OF THE STREPTOCOCCI

a. The majority of streptococcal infections of man are caused by beta hemolytic streptococci. A variety of diseases are manifested such as: puerperal fever, erysipelas, septic sore throat, scarlet fever, impetigo, and acute bacterial endocarditis. Of these infections, septic sore throat is, by far, the most common clinical entity. Approximately 2 to 3 weeks following recovery from a beta streptococcal pharyngitis, acute glomerulonephritis or rheumatic fever may develop, not as a direct effect of disseminated bacteria, but due to a tissue hypersensitivity.

b. The alpha hemolytic streptococci, especially those which are normal inhabitants of the upper respiratory and intestinal tracts, can cause disease if normal resistance is reduced. Alpha hemolytic members from the respiratory tract may cause subacute bacterial endocarditis. Group D streptococci commonly cause urinary tract infections.

c. The anaerobic streptococci commonly encountered in the normal vaginal flora, in the mouth, and in the intestine are capable of giving rise to suppurative lesions. Infections of these organisms produce pus with a foul odor.

d. The nonhemolytic streptococci are practically all saprophytic forms that have been isolated from milk and various dairy products. A few strains have been implicated as causing subacute bacterial endocarditis (SBE).

e. The group of streptococci generally associated with food poisoning consists of fecal streptococci, especially S. faecalis. Other outbreaks have also been caused by S. viridans and S. pyogenes. The symptoms of this syndrome are abdominal pain, vomiting, and diarrhea. The incubation period ranged from 2-22 hours after ingestion of food with a median of 10 hours. Vehicle foods of streptococcal food poisoning include turkey dressing, cured ham, barbecued beef, cheese, evaporated milk and turkey a la king.

Section IV. CORYNEBACTERIA AND RELATED SPECIES

2-12. GENERAL COMMENTS ABOUT THE GRAM-POSITIVE BACILLI

Members of the genera Corynebacterium, Bacillus, Clostridium and Mycobacterium are gram-positive bacilli.

a. Corynebacterium diphtheriae biotypes gravis, intermedius, and mitis cause the disease diphtheria. The genus Corynebacterium also includes saprophytic species (called diphtheroids) which are normal inhabitants of the respiratory tract. These require differentiation from C. diphtheriae when possible diphtheria exists.

b. The majority of Bacillus species are saprophytes found in soil, water, air, and on vegetation. One pathogenic member is B. anthracis which causes anthrax. Another pathogen is B. cereus which may cause gastroenteritis in man.

c. Members of the genus Clostridium are strict anaerobes. The Clostridium species cause several diseases which include botulism, tetanus ("lockjaw"), gas gangrene, and food poisoning. The clostridia are commonly found in soil, especially manured soil, and are prevalent in the intestinal tracts of man and animals.

d. Acid-fast bacilli make up the genus Mycobacterium. Unlike most bacteria, these organisms stain with difficulty. They require either prolonged contact with the dye, or the accompanying application of heat or surface-wetting agents to facilitate dye penetration of the cells. Once stained, the mycobacteria resist decolorization with acid-alcohol; thus, they are designated "acid-fast bacilli."

2-13. GENERAL COMMENTS ABOUT THE GENUS CORYNEBACTERIUM

a. The corynebacteria are slender, gram-positive rods, usually aerobic, measuring from 1 to 6 microns in length and 0.3 to 0.8 microns in breadth.

(1) These bacilli usually exhibit considerable pleomorphism (various forms). In addition to occurring as straight or slightly curved rods, they are frequently observed to be swollen on one or both ends, resulting in club or dumbbell-shaped forms.

(2) The diversity of shapes is due to the irregular distribution of cytoplasmic granules (metachromatic) that build up during growth and distort the cell wall. In stained smears the metachromatic granules appear as deeply stained bodies against lighter, area of cytoplasm. This gives the cell a transverse-banded, barred, or beaded appearance. Metachromatic granulation is satisfactorily demonstrated using methylene blue stain.

(3) The corynebacteria are characteristically arranged in palisades. V or Y shaped branching forms may also occur. Microscopic arrangements have been compared to Chinese letters composed with matches.

b. It is very important to remember that the saprophytic diphtheroids may resemble Corynebacterium diphtheriae. However, diphtheroids are usually short, thick, uniformly stained rods in palisade arrangement. In most cases these forms exhibit little or no plemorphism.

c, The corynebacteria are nonmotile, nonsporogeneous, nonencapsulated, and stain gram-positive, indicating they have retained the primary stain, crystal violet.

2-14. PATHOGENICITY OF THE CORYNEBACTERIA

a. **Varieties.** Of the corynebacteria, only the three biotypes of Corynebacterium diphtheriae (gravis, mitis, and intermedius) are generally recognized as pathogens.

(1) The rare species C. ulcerans is also pathogenic. The diphtheroids which normally inhabit the mucous membranes of the respiratory tract and the conjunctiva, for example, C. hofmanii and C. xerosis are not usually associated with the disease of man.

(2) Corynebacterium diphtheriae is usually found in the respiratory tract of asymptomatic carriers or infected individuals. The organism is rarely isolated from the skin or wounds.

(3) Diphtheria bacilli are spread by nasal or oral droplets from infected persons or by direct contact. Susceptible individuals are primarily within the 5 to 14 year old age group.

b. **Toxic Effects.** The virulent bacilli enter by way of the mouth or nose, invade the mucous membranes of the upper respiratory tract, multiply rapidly, and begin to produce a powerful exotoxin. The toxin is absorbed by the mucous membrane, resulting in acute inflammatory response and destruction of the epithelium.

(1) The exudation of fibrin, red blood cells, and white blood cells into the affected area results in the formation of a gray, clotted film, or "pseudomembrane" often covering the tonsils, pharynx, or larynx.

(2) As the disease progresses, the toxin is extended to more distant tissues causing necrosis, functional impairment, and sometimes gross hemorrhage of the heart, liver, kidneys, and adrenals. Neurotoxic manifestations are also evidenced by paralysis of the soft palate, eye muscles, or extremities.

(3) Diphtheria bacilli remain localized in the upper respiratory tract. It is the exotoxin, disseminated to the blood and deeper tissues, which accounts for the symptoms of systemic involvement. The potency of toxin excreted by a given variety of Corynebacterium diphtheriae determines the severity of the disease.

(4) Individuals possessing sufficient levels of specific neutralizing antitoxin in their blood stream and tissues are resistant to diphtheria. Susceptible individuals lack antitoxin immunity. Since the toxins produced by all three types of diphtheria bacilli are antigenically identical, infections or toxoid inoculations with any one will impart immunity to all.

2-15. LISTERIA MONOCYTOGENES

Listeria monocytogenes is a small, nonencapsulated, nonsporogenous, gram-positive bacillus.

a. It is aerobic to facultatively anaerobic in its oxygen requirements.

b. This organism is motile at 22ºC and much less motile at 37ºC. The effect of temperature on the motility of this bacterium can be the key differentiating procedure in its identification.

c. The colony on blood agar is less than 1 mm in diameter and is characterized by a weak zone of beta hemolysis which may often be seen only when the colony is removed from the medium surface.

d. Gram-stained smears reveal typical palisade arrangements.

e. Infection with this organism may be transmitted to man from animals from unpasteurized milk, infected meat, and direct contact.

f. The disease in man is usually a type of meningitis but may also cause abortion or still-birth in women.

2-16. ERYSIPELOTHRIX RHUSIOPATHIAE

Erysipelothrix rhusiopathiae (also known as Erysipelothrix insidiosa) is a nonsporogenous, nonencapsulated, nonmotile, thick, gram-positive bacillus.

a. It is isolated from blood culture or from characteristic lesions, resulting from contact with infected fowl, fish, or cattle. The translucent, glistening 1 mm colonies may mislead the observer into suspecting an alpha streptococcus; however, the gram stain will reveal the characteristic square-shaped rods in long tangled chains.

b. This organism may cause a localized cutaneous infection seen primarily as an occupational disease of persons handling animals, meat, poultry, and fish.

Section V. BACILLUS SPECIES

2-17. GENERAL COMMENTS ABOUT THE GENUS BACILLUS

The aerobic spore-forming bacilli include one important pathogenic species, Bacillus anthracis. In size, this is one of the largest pathogenic bacteria. Identification is based on cell morphology, staining, and cultural characteristics.

a. Members of the genus Bacillus are large, gram-positive, spore-forming rods, usually occurring in chains. Individual cells range between 1 to 1.25 microns in width and 3 to 10 microns in length.

b. The only encapsulated and nonmotile species is B. anthracis; the many saprophytic forms, B. subtilis, B. cereus, B. megaterium, and so forth, are nonencapsulated and are usually actively motile. The encapsulated cells of B. anthracis are usually found in direct smears of clinical specimens, but are rarely observed in smears of cultural growth.

c. Most Bacillus species appear as long, straight-sided rods with curved ends; the cells of B. anthracis often possess swollen, square, or concave ends, which give the chains a bamboo-like appearance.

2-18. BACILLUS SPORE FORMATION

In an unfavorable environment most Bacillus species, including B. anthracis, produce spores. Spores of B. anthracis are not observed in specimens from living tissue. Although the spores of Bacillus species cannot be stained by ordinary methods, their presence in gram-stained smears is evidenced by unstained areas within the cytoplasm of vegetative cells. The Wirtz-Conklin technique, employing heat, will satisfactorily stain the vegetative bacillary cells and spores of Bacillus species.

CAUTION: Ordinary heat fixation in preparing slides for staining will not destroy all anthrax spore. Strict aseptic technique should be used; a bacteriological hood may be employed in work with anthrax and other highly dangerous organisms.

2-19. BACILLUS CULTURES

All Bacillus species, including Bacillus anthracis, grow rapidly on simple basic media. The addition of special enrichments such as blood or carbohydrates does not substantially improve growth. Certain strains are strictly aerobic; others are facultative.

a. Growth occurs over a wide range of temperature for most species, especially the saprophytic forms. The optimum incubation temperature for B. anthracis is 37ºC. Spores are abundantly formed at 32º to 35ºC.

b. On blood agar after 18 to 24 hours' incubation, typical colonies of B. anthracis are 2 to 3 mm in diameter, off-white to gray, opaque, dull, with irregular edges and a rough ground-glass appearance. Since B. subtilis, B. cereus, B. megaterium, and other saprophytic species may exhibit the same colony picture, they are often referred to as pseudoanthrax bacilli.

c. Hemolysis is an important basis for differentiation. The colonies of anthrax bacilli are nonhemolytic or weakly hemolytic on blood agar, while pseudoanthrax forms are usually surrounded by a definite zone of hemolysis. When anthrax is suspected and hemolysis is not present, an unknown Bacillus species must be further studied to prove or disprove its pathogenicity.

2-20. PATHOGENICITY OF BACILLUS

a. **Origin of Infections.** We are concerned pathologically only with Bacillus anthracis and B. cereus.

(1) Although this genus contains many saprophytes, B. subtilis and B. megaterium are most often encountered. From a medical standpoint, these organisms are only important in that their microscopic and colonial morphology is often indistinguishable from the anthrax bacillus. The saprophytic species frequently occur as laboratory contaminants. This necessitates distinctions of such from B. anthracis where possible anthrax exists.

(2) Anthrax is primarily a disease of herbivorous animals. Infections of sheep and cattle are most common. Horses, swine, and other animals are occasionally infected. The soil of grazing regions becomes contaminated with anthrax spores from carcasses of dead animals, and other animals become infected during grazing. Viable spores enter the intestinal tract or the buccal mucosa, where they germinate and multiply. The bacilli are disseminated via the lymphatics to the blood stream and deeper tissues, rapidly resulting in death of the animal.

(3) Infections of man are almost always of animal origin. The organisms may enter through the skin, through the respiratory tract, or through the intestinal mucosa. The incidence of anthrax is highest among butchers, herdsmen, woolhandlers, tanners, and other occupational groups dealing with infected animals or their products.

b. **Forms of Infection.**

(1) Cutaneous anthrax is the most common form of human anthrax infection and most often results from direct contact with infected tissue, hides, hairs, or bristles. Skin lesions begin as a papule which rapidly progresses in sequence to a vesicle, pustule, and ultimately to a hard necrotic ulcer. Such infections may spread to deeper tissue resulting in septicemia and widespread involvement of internal organs.

(2) Primary pulmonary anthrax originates from inhalation of spores disseminated into the air in the process of handling infected materials, especially animal fibers (wool or fleece). Infected individuals exhibit signs of pneumonia, which often progresses to fatal septicemia. Fortunately, pulmonary infections are not very common.

(3) Intestinal anthrax may result from ingestion of insufficiently cooked meat of infected animals or from ingestion of foods contaminated with spores. Infections of the intestinal tract are very rare in man, but are the most common form of the disease in animals.

c. **Virulence.** Bacillus anthracis produces no soluble exotoxin or endotoxin.

(1) Virulence is apparently associated with the ability to form a capsule. The capsule is composed of polypeptide (protein complex of d(-) glutamic acid) material instead of a polysaccharide substance common to capsules of most other bacteria.

(2) While the vegetative cells of Bacillus species are no more resistant to deleterious influences than other bacteria, the spores are highly resistant. Anthrax spores have been known to survive for decades in soil. The spores ordinarily require boiling for at least 10 minutes to effect their destruction. Treatment of the spores with disinfectants usually requires prolonged exposure. For example, 0.1 percent mercuric chloride may not destroy anthrax spores even after 72 hours. Standard sterilization temperatures and periods of exposure successfully destroy all pathogenic bacterial spores. Though the spores of saprophytic species exhibit comparable or even greater resistance, their medical importance is negligible although in the clinical laboratory they must be differentiated from the pathogenic species.

(3) Intestinal anthrax may result from ingestion of insufficiently cooked meat of infected animals or from ingestion of foods contaminated with spores. Infections of the intestinal tract are very rare in man, but are the most common form of the disease in animals.

d. **Foodborne illness due to B. cereus.** The incidence of Bacillus cereus gastroenteritis in the US is presumed to be quite low. The incidence in Europe is much higher.

(1) The incubation period usually ranges from 1 to 16 hours. The symptoms include mild to profuse diarrhea, stomach cramps or abdominal pains, moderate nausea, and rarely vomiting or fever.

(2) Since large numbers of cells are needed to cause foodborne illness, the food is abused some time during preparation. Plant products (cereals, flour, starch, bakery products, spices), animal products, and mixtures of ingredients (spaghetti sauce, pudding, soup mixes, gravy mixes) can contain a few or many cells or spores of B. cereus.

(3) There is a similarity between foods involved in illness due to B. cereus and Clostridium perfringens. In either case, the food is prepared ahead of time, in large batches, which are not properly cooled prior to reheating (if needed) and serving. The reheating is not sufficient to destroy the cells. The methods for control of this foodborne illness are:

(a) Limit or prevent contamination of the food.

(b) Prevent or inhibit growth of the organism.

(c) Destroy the organism.

Section VI. CLOSTRIDIA

2-21. GENERAL COMMENTS ABOUT THE GENUS CLOSTRIDIUM

The members of the genus Clostridium are large anaerobic gram-positive rods of variable length and breadth, ranging from long filamentous forms to short plump bacilli.

a. In an appropriate environment, individual bacilli of most species produce a single spherical or ovoid spore which may be located centrally, subterminally, or terminally within the vegetative cell. In most instances the spores appear as swollen bodies since they are generally wider than the diameter of the rods in which they develop. The shape and position of the spore, as well as the swelling of the vegetative cell and characteristics which may contribute to species identification, are shown in figure 2-3.

b. Spores of Clostridium species are not stained by ordinary methods: therefore, in gram-stained smears, spores are evidenced as unstained areas within the dark-stained cytoplasm of vegetative bacilli or as free hyaline bodies. The relatively impervious spore bodies may be effectively stained.

CAUTION: Ordinarily heat fixation in preparing a slide for staining may not destroy all spores. Strict aseptic technique should be used; a bacteriological hood may be useful in work with spore formers.

c. The majority of the clostridia are motile, but Clostridium perfringens, the species most frequently isolated form clinical materials, is nonmotile.

Species	Surface colonies anaerobic blood agar	Mobility	Spores
Cl. tetani	Small (1 mm) flat, rhizoid or feathery surface; raised centers; single hemolytic zone.	+	Round, terminal, and swollen (drumstick).
Cl. botulinum	Large (5-10 mm); glistening translucent edges with brown centers; single hemolytic zone.	+	Oval, subterminal, and swollen.
Cl. perfringens	Small (2-5 mm) round, smooth, opaque, entire; double hemolytic zone.	-	Oval, central, nonswollen (rarely seen).
Cl. novyi	Small, delicate, flattened, transparent; blue gray and irregularly contoured; single hemolytic zone.	+	Oval, eccentric, subterminal, and swollen.
Cl. septicum	Small, with branching or rhizoid margins and deep, opaque centers; single hemolytic zone.	+	Oval, subterminal, and swollen.
Cl. sporogenes	Small, transparent, with rhizoid or amoeboid margins and raised centers; single hemolytic zone.	+	Oval, eccentric, subterminal, and swollen.
Cl. chauvoei	Small, flat, round or leaf-like; single hemolytic zone.	+	Oval, eccentric, subterminal, and swollen.
Cl. histolyticum	Minute, round, translucent with "dew drop" appearance single hemolytic zone.	+	Oval, subterminal, and swollen.
Cl. bifermentans	Small, transparent, with rhizoid or amoeboid margins and raised centers; single hemolytic zone.	+	Oval, eccentric, subterminal, and swollen.

Figure 2-3. Morphological differentiation of Clostridium species.

2-22. GROWTH REQUIREMENTS OF CLOSTRIDIA

The clostridia are obligate anaerobes. Growth may be obtained over a wide range of temperatures; however, 37ºC is optimum for pathogenic species. Although variations in nutritive requirements throughout the clostridia do exist, they may be successfully isolated from specimens using blood agar and thioglycollate broth (the addition of 0.6 percent glucose is helpful as an added growth factor). Anaerobic condition may be provided by incubating the inoculated blood agar plates in a Brewer anaerobic jar, or any of several methods for providing strict anaerobic conditions.

2-23. APPEARANCE OF CLOSTRIDIA

Stained smears of growth usually reveal spores, except when Cl. perfringens is present. This species fails to sporulate on most media, especially those media containing carbohydrates. On blood agar, after 48 hours' anaerobic incubation at 37ºC, typical colonies of the various Clostridium species appear as described in figure 2-3. Most clostridia produce distinct beta hemolysis on blood agar. Clostridium perfringens, however, may exhibit a "target" appearance or double zone of hemolysis. This is shown by a definite narrow 1 to 2 mm zone, immediately around the colonies, which is surrounded by a wide 4 to 5 mm zone of partial hemolysis. Although the microscopic and colonial morphology of certain clostridia may appear quite distinctive, final identification rests with the performance and interpretation of biochemical tests.

2-24. PATHOGENICITY OF CLOSTRIDIA

Since the natural habitat of the clostridia is worldwide in soil and in the intestinal tract of man and animals, pathogenic species are always present and may cause disease when the opportunity arises. The pathogens are those organisms responsible for botulism, tetanus, and gas gangrene.

a. **Tetanus.** Clostridium tetani causes tetanus. This disease results from the introduction of spores (from soil or feces) of the organism into puncture wounds, burns, surgical sutures, or other traumatic injuries. Spores of the organism germinate to form the vegetative bacilli, which multiply and produce a powerful exotoxin at the expense of the necrotic tissue, created by vascular destruction. The exotoxin spreads through rapid absorption and acts particularly on the tissue of the spinal cord and peripheral motor nerve endings. Toxemia is first evidenced by muscle spasms near the site of infection with subsequent spasms of the jaw muscles (lockjaw). The intoxication progresses to the nerves of other voluntary muscles causing tonic spasms, convulsions and, ultimately, death. Prevention of disease rests with active immunization of the populace with toxoid, or passive immunization with antitoxin as soon as possible after injury.

b. **Botulism.** Clostridium botulinum is responsible for a fatal type of food poisoning, botulism.

(1) The disease is an intoxication rather than an infection in that outbreaks occur following ingestion of food in which Cl. botulinum has grown and produced a highly potent exotoxin. Within the anaerobic environment of the foodstuff, the spores germinate to form vegetative bacilli, which in turn produce toxin. Since the spores of Cl. botulinum will withstand a temperature of 100ºC for at least 3 to 5 hours, they present a definite hazard to home canning.

(2) Toxin-containing foods may appear spoiled and rancid, cans may be swollen due to gas formation by the organism. In some cases the foodstuff may appear entirely harmless. The toxin is destroyed by heating the food at 100ºC for 10 minutes.

(3) Outbreaks of botulism are rare in the US because of the rigid regulation of commercial canning and food preservation. Most cases since 1910 have been those associated with home preparation of foodstuffs. Canned vegetables have accounted for the majority of outbreaks in the US. Meat, poultry and dairy products rarely are involved since these foods are consumed primarily as fresh, rather than canned food.

(4) There are five antigenic types of Cl. botulinum toxin, designated A, B, C, D, and E. Type A toxin is one of the most poisonous substances known. After approximately 18 to 48 hours following the consumption of toxic food, neurotoxic manifestation are evidence in visual disturbances, inability to swallow, and speech difficulty. Progressive signs of bulbar paralysis are exhibited and these lead to fatal termination from respiratory failure or cardiac arrest. Since the various toxin types are highly antigenic, potent antitoxins may be obtained by injecting toxoids into animals. Polyvalent antitoxin (A through E) is administered intravenously to affected individuals.

c. **Gas Gangrene.**

(1) The Clostridium species most commonly associated with gas gangrene are Cl. perfringens, Cl. novyi, and Cl. septicum. Cl. perfringens is the most frequent cause and is found either alone or mixed with other anaerobes.

(2) Gas gangrene often develops as a complication of severe traumatic injuries such as dirty, lacerated wounds, especially those accompanying compound fractures. In these and other injuries, the circulation to a local tissue area is often impaired or destroyed. The resulting necrotic tissue, void of oxygen and rich in nutrients, affords an ideal anaerobic environment in which the spores of gangrene organisms may germinate and multiply. The organisms actively metabolize tissue carbohydrates to acid and gas. The gangrenous process extends to other tissues primarily as a result of exotoxins excreted by pathogenic clostridia. The exotoxins include hyaluronidase, lecithinase, and collagenase.

(3) In addition, other enzymes may be present which exhibit hemolytic, necrotizing, and lethal effects on tissues. Gas gangrene is usually a mixed infection composed of toxigenic and proteolytic clostridia and other aerobic and anaerobic, gram-positive or gram-negative organisms.

(4) The accessory organisms may contribute to the severity of infection. Without the prompt administration of antitoxin or amputation of necrotic tissue, patients die from toxemia. The antitoxin employed usually consists of pooled concentrated immune globulins against toxin of Cl. perfringens, Cl. novyi, and Cl. septicum.

d. **Foodborne Illness Due to Cl. perfringens.** This illness has been called a food poisoning, an intoxication, a foodborne illness, an infection, and an infective food poisoning. These different designations are undoubtedly due to the fact that the release of the toxin is different from that of S. aureus or Cl. botulinum. Large numbers of the organism are associated with the illness. This is true for infections. The majority of the reported outbreaks are due to food consumed in mass-feeding establishments.

(1) The etiologic agent has been termed "Clostridium perfringens enterotoxin." Unlike the enterotoxin of S. aureus or the neurotoxin of Cl. botulinum, which is present in toxic food, the enterotoxin of Cl. perfringens normally is not found in food. Instead, it is produced in the intestine by a sporulating culture of enterotoxigenic strains of Cl. perfringens.

(2) The illness is the result of a sequence of events. The food is contaminated by the organism. During cooking, the vegetative cells and the heat-sensitive spores may be killed, but heat-resistant spores will survive. The heat during cooking may activate the spores to germinate. If the food is held at a temperature allowing growth, the vegetative cells will multiply. The organism must pass through the low pH of the stomach to reach the intestines. The presence of proteins protects the organism from the acid in the stomach. When the organisms reach the small intestine, they find an environment acceptable for multiplication and sporulation. The organism may sporulate in some foods, but usually, by the time the food is toxic, it also is not palatable.

(3) The two prominent symptoms of the illness are diarrhea and abdominal cramps. The illness is usually of short duration, from less than 12 hours to up to 24 hours. Due to the rather mild symptoms and short duration, usually no therapy is needed.

(4) The foods involved in outbreaks are usually protein-type foods that have been boiled, stewed or lightly roasted, or meat and poultry stews, sauces, gravies, pies, casseroles, salads, and dressings. Usually the incriminated food is cooked a day or two in advance, refrigerated, and then reheated prior to serving.

(5) The methods for control of this illness are the same as for B. cereus illness.

(a) Limit or prevent contamination of the food.

(b) Prevent or inhibit growth of the organism.

(c) Destroy the organism.

Section VII. MYCOBACTERIA

2-25. GENERAL COMMENTS ABOUT THE GENUS MYCOBACTERIUM

The genus Mycobacterium, whose members are also called the "acid-fast bacilli," contains many species of saprophytic acid-fast bacilli, but M. tuberculosis, M. bovis, M. avium, and M. leprae are clearly definable pathogens. M. tuberculosis is the principle cause of human tuberculosis. M. bovis, is primarily responsible for tuberculosis of cattle, although infections of cattle are transmissible to man. M. avium is infectious for fowl. The leprosy bacillus M. leprae is the causative agent of human leprosy. In contrast to the other pathogenic mycobacteria, M. leprae has not been routinely grown in vitro. Human leprosy is generally not transmissible to animals.

2-26. PATHOGENICITY

a. **Human Infections.** Mycobacterium tuberculosis causes about 90 percent of all mycobacterial infections. It is the principal cause of tuberculosis in man. Among communicable diseases, tuberculosis is the leading killer in the world today, although it is no longer the leading cause of death in countries where the standard of living is high.

b. **Routes of Infection.** Tubercle bacilli may enter the body by way of the respiratory or alimentary tracts, as well as the conjunctiva. The respiratory tract is the most frequent and important route of infection for man. Infected individuals in the process of sneezing, coughing, or expectorating produce an infectious aerosol of droplets and contaminated dust particles, which may be inhaled by susceptible individuals. Infections are also acquired from fomites (towels, drinking cups, doorknobs, and so forth). The ingestion of unpasteurized milk or inadequately cooked meat of infected cattle is an important source of infection where bovine tuberculosis is not well controlled.

c. **Spread of Disease Within the Body.** Following initial infection, tubercle bacilli form primary and secondary lesions within the tissues. The organisms may then spread to various tissues via the lymphatic system, blood stream, or by direct extension. Blood stream invasion results in transport of the bacilli throughout the body and thus gives rise to acute miliary or chronic disseminated tuberculosis.

(1) Practically any tissue of the body is subject to invasion by tubercle bacilli. However, more than 90 percent of the deaths from tuberculosis are due to the pulmonary type. Infections of the bone and joints, lymph nodes, spleen, liver, kidney, meninges, and gastrointestinal tract do occur, but with much less frequency. Disseminated infections are somewhat more prevalent in children.

(2) The lesions formed by pathogenic acid-fast bacilli are referred to as tubercles. Tubercles may rupture and discharge their bacilli to produce further infection or they may heal and permanently wall-off the bacilli by fibrosis or calcification.

d. **Acquired Resistance.** If man or lower animals survive the first infection with tubercle bacilli, they acquire some resistance to tuberculosis. Upon subsequent infections, the defense mechanisms of these subjects have an increased capacity to localize tubercle bacilli.

(1) Although antibodies are formed by the host against a variety of cellular antigens within the tubercle bacilli, such antibodies appear to be of little value in increasing resistance. The increased resistance to infection is largely attributed to the mononuclear cells which acquire a greater ability to ingest tubercle bacilli. Mononuclear cells develop this property in the course of primary infections.

(2) Nevertheless, antibody production in response to tuberculous infections is of value in the diagnosis of tuberculosis. This forms the basis of the tuberculin skin test. Individuals who have had no contact with tubercle bacilli exhibit no reaction to the skin test; however, the majority of normal adults are tuberculin-positive. In children, however, positive reactions are more suggestive of active infections. The tuberculin test is valuable as a screening test in examining children for possible tuberculosis.

Section VIII. ENTEROBACTERIACEAE

2-27. GENERAL

The family Enterobacteriaceae consists of gram-negative, aerobic (facultatively anaerobic), nonsporogenous bacilli which grow well on artificial media. They may be motile or nonmotile, but motile forms must be peritrichous, that is, possess flagella distributed over the entire surface of the bacterial cell. Members of the family reduce nitrates to nitrites, ferment glucose with the production of acid or of acid and gas, do not produce indophenol-oxidase, and do not liquefy alginate. Pectobacterium is the only genus of the family which liquefies pectate. The genera in the family Enterobacteriaceae are Escherichia, Shigella, Edwardsiella, Salmonella, Arizona, Citrobacter, Klebsiella, Enterobacter, Serratia, Proteus, Erwinia, Pectobacterium, and Yersinia.

2-28. NORMAL FLORA AND GENERAL IDENTIFICATION OF SPECIE

Many of these bacteria are normally present in the intestinal tract as part of the normal flora. It becomes the task of the bacteriology laboratory to differentiate between gram-negative, glucose-fermenting bacilli that are normally present in the intestines, and those that are considered to be pathogens. It must be remembered that the reactions and classifications given in this study guide are broadly accepted, but due to the very nature of the subject itself, different textbooks and authors may vary on certain points and the specific reactions of a particular organism. To completely identify any one of these enteric bacteria, all characteristics of the bacterium must be established to include colony morphology on differential and selective media, numerous biochemical patterns, and often serological characteristics.

2-29. MORPHOLOGY AND CULTURE

The enteric gram-negative rods range from 1 to 4 microns in length and from 0.4 to 0.8 microns in breadth. A few longer, filamentous cells may be exhibited by any of the species. The organisms possess no typical cellular arrangement and may be observed singly, in pairs, in clumps, and occasionally in short chains. Microscopic morphology is, therefore, of little diagnostic value. The majority of the enteric gram-negative rods are actively motile. The enteric bacilli grow well on ordinary nutrient media. Most species are facultative anaerobes. Although the majority of these organisms usually yield good growth between 20º and 40ºC, 37ºC is optimum for most species, especially the pathogens. A medium of approximately neutral ph is most favorable for growth of all enteric bacilli. A great variety of culture media may be employed for isolation and identification of pathogenic enteric bacilli in fecal specimens. This includes the use of differential, selective and inhibitory plating media as well as selective and enrichment broths.

2-30. PATHOGENICITY OF THE GENUS ESCHERICHIA

a. **E. coli.** Eschericia coli is one of the most abundant species of bacteria represented in the normal intestinal tract. In this region, the organism contributes to normal function and nutrition. E. coli and other enteric saprophytes become pathogenic when introduced into tissues outside the intestinal tract, especially the urinary and biliary tracts, peritoneum, or meninges. E. coli more frequently invades the urinary tract and is the most common cause of cystitis. The organism has also been isolated from local infections such as conjunctivitis. E. coli may also be the cause of septicemia. A number of E. coli serotypes have been associated with infant diarrhea, and when E. coli is isolated from pediatric patients it should always be serotyped.

b. **Foodborne Illness Due to E. coli.** There are two types of illness due to E. coli. The invasive strains cause dysentery similar to shigellosis, while the enterotoxin-producing strains cause an illness similar to cholera. The incubation period for the invasive strains is 6-36 hours while the enterotoxin-producing strains have an incubation period of 36-48 hours. The main symptom is diarrhea with dehydration. These symptoms are essentially the same as those due to enterotoxins, except bloody diarrhea does not occur. The only well documented foodborne outbreak in the US involved soft cheese.

2-31. PATHOGENICITY OF THE GENUS KLEBSIELLA

Klebsiella pneumoniae (Friedlander's bacillus) is isolated with some frequency from the upper respiratory and intestinal tracts of normal individuals and is responsible for approximately 2 percent of the bacterial pneumonias. Pulmonary infections are characterized by extensive hemorrhagic consolidation of the lobes. The fatality rate is high in untreated cases. Klebsiella species are frequently isolated from various upper respiratory tract infections, although their presence, in many instances, is probably that of secondary invaders. The organisms have definitely been responsible for suppurative abscesses of the other visceral tissue.

2-32. PATHOGENICITY OF THE GENUS ENTEROBACTER

Several species of Enterobacter (E. cloacae, E. liquefaciens, E. aerogenes, and E. hafnia) have been recognized and exhibit a pathogenicity similar to Escherichia. Species of Enterobacter are isolated frequently in cases of septicemia and urinary tract infections.

2-33. PATHOGENICITY OF THE GENUS PROTEUS

Of the genus Proteus, four species are recognized: Proteus vulgaris, P. mirabilis, P. morganii, and P. rettgeri. Although these organisms are primarily free-living in water, soil, and sewage, they are frequently isolated from fecal specimens of normal individuals. Proteus morganii has been responsible for diarrhea of infants and children. Proteus species often cause human infections and usually do so when introduced into tissues other than the normal intestinal tract. In this connection, Proteus species rank next to E. coli as the etiological agent of cystitis. These organisms are also encountered frequently in eye and ear infections and occasionally in pleurisy, peritonitis, and suppurative abscesses in many areas of the body. Proteus are commonly associated with other bacteria in purulent wounds and may contribute to the severity of such infections.

2-34. PATHOGENICITY OF THE GENUS SALMONELLA

It is important to remember that all salmonellae are potential pathogens and may produce enteric fever, septicemia, or gastroenteritis. Such infections often originate from ingestion of contaminated food or drink.

a. **Enteric Fevers.** The enteric fevers consist of typhoid fever and paratyphoid fever.

(1) Salmonella typhi is responsible for typhoid fever, while S. paratyphi A, S. paratyphi B, and others are most often encountered in paratyphoid fever. Of these salmonellae, S. paratyphi A and S. paratyphi C are only occasionally isolated in the US.

(2) In enteric fevers, the ingested organisms enter the small intestine, spread through the intestinal lymphatics to the thoracic duct, and enter the blood stream. The resultant septicemia distributes the infection to many organs including the kidney, intestines, liver, gallbladder, and other tissues.

(3) Infections are characterized by an insidious onset, with low grade fever that ultimately becomes quite elevated during the bacteremic phase. Blood cultures are usually positive only during the first and second week of infection. Stool and urine cultures usually fail to yield the responsible Salmonella species until the third week. The duration of typhoid fever and paratyphoid fever is usually several weeks.

(4) Salmonella infections that result in septicemia are often due to S. choleraesuis. The onset of symptoms is abrupt since blood stream invasion occurs within a short period of time following oral ingestion of the organism. This is accompanied by a rapid rise in temperature that spikes during the height of infection. Wide distribution of the organisms results in focal suppuration and abscess formation in various tissues. Meningitis, osteomyelitis, endocarditis, and pneumonia are known complications of such infections. Blood cultures are most often positive when taken during the height of the fever.

b. **Gastroenteritis.** Of the many Salmonella species which produce acute gastroenteritis in man, S. typhimurium is the most frequent causative agent. S. enteritidis is possible the second most common cause. S. choleraesuis has also been implicated in gastroenteritis but to a lesser extent than either of the two previously mentioned species.

(1) Infections are characterized by fairly sudden onset (15 to 24 hours' incubation), and rather severe gastrointestinal distress with vomiting, diarrhea, and slight elevation of temperature. Recovery is rapid (1 to 3 days) since the intestinal tract is not usually invaded by the organisms.

(2) Symptoms result from the irritative action of acids and endotoxin upon the intestinal mucosa. The acids are formed by fermentation of carbohydrates by the responsible organisms. Endotoxins are released following death and cellular lysis of the etiologic agent. Only very rarely do infections develop into septicemia.

(3) Outbreaks of gastroenteritis are usually linked with consumption of certain foods and are often referred to as "food poisoning." Diseases usually originate from unsuspected subclinical cases, convalescent carriers, or healthy permanent carriers who harbor the organisms in their intestine, gallbladder, or the urinary tract. Such individuals may contaminate food or drink either directly or indirectly. The salmonellae produce no exotoxins. Upon death and lysis of the cells, endotoxins are released which largely account for the disease symptoms of man.

c. **Foods Involved in Salmonella Gastroenteritis.** Various foods have been the vehicle for transmission of Salmonella. Most of these foods are of animal origin or contaminated by foods of animal origin. The involvement of meat and meat products as vehicles of Salmonella appears to have increased in the last 10 years, and accounted for almost 50 percent of the foods from 1972 to 1976. The pasteurization of egg products has eliminated egg products as a source of Salmonella. Several outbreaks have been contributed to the consumption of precooked roast beef. These outbreaks stimulated action by the USDA to help assure the safety of this product by requiring the heating of beef roasts to at least 63ºC.

2-35. PATHOGENICITY OF THE GENUS SHIGELLA

Shigellae are the cause of bacillary dysentery.

a. Infections are usually limited to the gastrointestinal tract. The disease process is essentially an inflammation of the mucous membrane of the large intestine and terminal ileum which leads to necrosis and superficial ulceration. Symptoms occur within 1 to 2 days following ingestion of contaminated food or drink. The illness is characterized by sudden onset of abdominal pains, cramps, diarrhea, and fever. The intense irritation of the bowel is due to the release of somatic endotoxin upon autolysis of the Shigella species.

b. Infections from Sh. dysenteriae are more severe because, in addition to the endotoxin substance, an exotoxin (neurotoxin) is produced which causes paralytic symptoms. Infections from exotoxin-producing strains of Sh. dysenteriae are relatively frequent in India, Japan, China, and other parts of Asia.

c. Although some individuals recover quickly from bacillary dysentery and pass infectious bacilli in stools for only a short period, others become chronic carriers (ulcerative colitis) and may suffer relapses of the disease. The latter serve as a reservoir of infection.

d. The foods that have been involved in most outbreaks are those which are handled the most. These are salads such as potato, tuna, shrimp, macaroni, and chicken. The ingredients may be clean, but during preparation, the salad is contaminated by hand manipulation or mixing.

Section IX. YERSINIA PESTIS

2-36. MORPHOLOGY

Yersinia organisms are reasonably large (0.5 to 1.0 by 1 2 microns) gram-negative coccobacilli which are ovoid or rod-shaped. Yersinia (Pasteurella) pestis is encapsulated, nonmotile, nonsporagenous, and characteristically bipolar staining. Other species are Yersinia enterocolitica and Yersinia pseudotuberculosis.

2-37. PATHOGENICITY

Yersinia pestis is the etiological agent of plague, a disease primarily of rodents which is secondarily transmitted to man.

a. One of the more commonly affected rodents is the wild rat, but guinea pigs, squirrels, prairie dogs, and mice are also susceptible. Plague is spread among rodents through the bites of fleas, previously infected via a blood meal from an infected animal. Human plague results when an infected flea feeds on man.

b. Following entry of the plague bacilli into the body, the organisms spread by way of the lymph vessels to the regional lymph nodes. The lymphatic vessels and nodes become inflamed, hemorrhagic, and greatly enlarged, forming buboes which are usually located in the groin or axilla. Such infections are referred to as bubonic plague, and milder forms of the disease are more or less restricted to the lymphatic system. In many cases, the organisms spread to the blood stream and are distributed to all organs, particularly the spleen, liver, and lungs. The parenchymatous tissues become inflamed and hemorrhagic, ultimately leading to local necrosis. Death may result from a meningitis or overwhelming septicemia.

c. The septicemia phase is sometimes accompanied by subcutaneous hemorrhages which cause the formation of dark spots on the skin. Because of this, the plague is sometimes called the "black death."

d. Since bubonic infections may progress to involve the lungs, individuals so affected disseminate plague to the respiratory tracts of other persons by coughing or sneezing, producing a highly infectious aerosol. Primary pulmonary infections (pneumonic plague) are always fatal when untreated.

Section X. PSEUDOMONAS

2-38. INTRODUCTION

Pseudomonads are gram-negative, asporogenous, catalase-producing rods commonly found in soil and water. Most species are motile, with one polar flagellum or several polar flagella; some are nonmotile and atrichous. Unlike the Enterobacteriaceae, which only ferment carbohydrates, the pseudomonads oxidize and do not obtain energy by fermentative or photosynthetic metabolism.

2-39. PATHOGENICITY OF PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa may infect surgical wounds, severe burns, and other injuries, where it tends to produce a characteristic blue-green pus. Since it is resistant to antibiotic therapy, it tends to produce dangerous infections at the sites of previous infections eradicated by antibiotics. Infections of the eye, the ear, and the urinary tract are frequently reported. Systemic infections, which may occur in individuals with lowered resistance, tend to be fatal.

2-40. PSEUDOMONAS PSEUDOMALLEI

Melioidosis is an infectious disease caused by a gram-negative, motile bacillus, Pseudomonas pseudomallei, which is found in soil and water in Southeast Asia. The disease is manifested in a number of ways ranging from an unapparent infection to a fatal septicemia. However, the most common manifestation is an acute pneumonia or pneumonitis. Pseudomonas pseudomallei has been isolated from soil, local fruits and vegetables, well water, and a variety of surface waters. Attempts to identify a human or animal reservoir have proven unsuccessful to date. It is assumed that the organism leads a saprophytic existence in nature. Human-to-human transmission is very rare. The respiratory route of infection is most common. The disease is frequently associated with traumatic injuries.

Section XI. BRUCELLA

2-41. GENERAL TYPES

The genus Brucella includes several species known to infect man. However, man is not the primary host of any of these. Goats are infected by Br. melitensis, cattle by Br. abortus, swine by Br. suis, and dogs by Br.canis. Man can be infected with any of these organisms by direct contact or by consumption of milk or milk products. The brucellae exhibit predominantly small coccobacillary forms ranging from 0.4 microns to 3.0 microns in length and 0.4 to 0.8 microns in breadth. Fresh isolates from disease are encapsulated and form smooth mucoid colonies on agar media. The cells occur singly, in pairs, or in short chains. Brucella species do not possess flagella, nor do they form spores.

2-42. CULTURE

a. The brucellae require complex media for growth. Although many special media have been devised and recommended for cultivation of Brucella species, trypticase soy broth and agar are used with considerable success.

b. Prolonged incubation (several weeks) at 37ºC is often necessary for initial isolation of Brucella species; however, upon subculture, growth usually occurs within 3 or 4 days. Colonies of Brucella species are small, translucent, smooth, glistening, and blue-gray in color, and they possess entire (even) margins.

2-43. PATHOGENICITY

Brucella abortus, Br. melitensis, Br. suis, and Br. canis are all pathogenic for man. Following the ingestion of raw milk from infected animals, the brucellae may invade the oral mucous membranes or the lining of the alimentary tract. Infections also result from direct contact with infected tissue. Occasional cases of pulmonary brucellosis suggest that infections may be acquired by inhalation. The incidence of brucellosis is much higher among slaughterhouse attendants, veterinarians, sausage-makers, butchers, dairymen, or similar occupational groups exposed to infected animals.

a. After entry into the human host, the organisms progress by way of lymphatic channels to the thoracic duct. They enter the blood stream and are widely disseminated to various tissues including the liver, spleen, bone marrow, and other areas of the reticuloendothelial system. The organisms form multiple intracellular abscesses in the particular tissue affected. Osteomyelitis or meningitis may occur.

b. Characteristic symptoms begin insidiously (usually 10 to 14 days following infection), with slight periodic fever, weakness, and malaise. The lymph nodes and spleen gradually become enlarged; deep pain and symptoms of uncoordination may also occur. At the height of infection, acute febrile episodes occur as a result of organisms being periodically released into the blood stream. Frank symptoms usually subside within 3 months and the bacilli remain dormant in deep tissues (chronic brucellosis) as long as the general physiological well-being of the individual is maintained. Relapses of acute symptoms may occur when the resistance of the infected individual is lowered.

Section XII. BORDETELLA

2-44. INTRODUCTION

The genus Bordetella consists of three species, Bordetella pertussis, B. parapertussis, and B. bronchiseptica, which are all minute, gram-negative coccobacilli. B. pertussis is the causative agent of whooping cough.

2-45. CULTURE

The colonies are small and dome-shaped, possessing a gray metallic luster, resembling mercury droplets. Although the colonies of pertussis bacilli are beta hemolytic, the zone of hemolysis is difficult to observe since Bordet-Gengou agar contains only 15 to 20 percent blood.

Section XIII. FRANCISELLA TULARENSIS

2-46. MORPHOLOGY

Francisella tularensis (formerly known as Pasteurella tularensis), the gram-negative coccobacillus that causes tularemia, is much smaller than the plague bacillus. It is 0.3 to 0.5 by 0.2 microns upon initial isolation, but it becomes rod-like upon transfer. It may be quite pleomorphic and filamentous in old cultures. It occurs singly, is nonencapsulated in vitro and nonmotile, and may show bipolar staining. Capsulated forms occur in vivo.

2-47. CULTURAL CHARACTERISTICS

F. tularensis will not grow on ordinary media and may fail to grow even on highly enriched blood agar unless cystine or cysteine is added. Cultivation is most successful when using blood-cystine-glucose agar slants. Primary growth of F. tularensis from specimens usually requires 4 to 7 days' incubation when blood-cystine-glucose agar slants are used. In young cultures the colonies are very thin, although gram-stained smears of fluid from the base of the slant will reveal numerous cells. Later, relatively heavy growth of small, gray, transparent to translucent, mucoid colonies develop. Subcultures to blood-cystine-glucose agar usually yield abundant growth within 2 to 3 days.

2-48. PATHOGENICITY

F. tularensis is the causative agent of tularemia; which is primarily a disease of rodents, rabbits, hares, and birds. Man is only an accidental, terminal host. The reservoir of infective agents is maintained among wild animals by biting flies (Chrysops), ticks, and the rabbit louse; all of which are capable of spreading the disease from animal to animal.

a. Man can contact tularemia either directly through handling the flesh of infected animals or indirectly by an insect vector. The primary source of human infection is the rabbit. In the process of preparing the animal for food, the bacilli may enter through cutaneous abrasions or possibly through the intact skin. Aerosols of body fluids from infected animals may result in infections of the conjunctivae or lungs.

b. Following invasion of the skin and mucous membranes, an ulcerating papule usually develops at the site of entry. The bacilli spread rapidly to the regional lymph node which become enlarged and suppurative. A transient bacteremia during the first week of illness serves to distribute the organisms to various internal organs where various foci of infection develop. As the disease progresses, pneumonia and fulminating septicemia may develop, resulting in death in untreated cases.

c. Frequently the clinical signs are suggestive of the portal of entry. This is evidenced in that infections may be oculoglandular, following infection by way of the conjunctivae; ulceroglandular; following entry through the skin, or pneumonic, resulting from primary inhalation of infectious droplets. In some cases, there are no signs of localized involvement, but only the picture of a febrile systemic illness.

Section XIV. MISCELLANEOUS ORGANISMS

2-49. STREPTOBACILLUS MONILIFORMIS

Streptobacillus moniliformis is a gram-negative bacillus. The bacilli may be small and slender or may be looped or have curved filaments. The loops resemble a necklace or a string of beads (moniliform). Initial isolation is best obtained by using thioglycollate broth with the addition of ascitic fluid. Further culture may be obtained in solid media such as blood agar or serum agar with blood. The colonies growing on these media are small, smooth, and glistening, with irregular edges, and are colorless to gray. This organism is a normal inhabitant of the throat and nasopharynx of the rat. The infection in man is also known as rat-bite fever and is contracted by the bite of this animal.

2-50. VIBRIO PARAHAEMOLYTICUS

Vibrio parahaemolyticus is a gram-negative facultatively anaerobic rod. This organism is now considered to be an enteric pathogen.

a. The illness is a typical gastroenteritis, with diarrhea, abdominal cramps, nausea, vomiting and fever. The incubation period is usually 12-24 hours. The symptoms persist from a few hours to 10 days with the usual duration of 2-3 days.

b. Foods that have been incriminated in outbreaks include steamed crabs, crab salad (made from canned crabmeat), raw crab, processed lobster, boiled shrimp, roasted oysters, and raw oysters. The raw products were inadequately refrigerated. Cross contamination between cooked and raw products were also found to cause several outbreaks.

c. The control of V. parahaemolyticus should be relatively simple. It is found in marine environments and contaminates waterfoods during the warm summer months. Recontamination by raw waterfoods must be avoided. The application of good sanitary practices and good personal hygiene helps to prevent this cross contamination.

Continue with Exercises

EXERCISES, LESSON 2

INSTRUCTIONS. Answer the following exercises by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. On initial isolation, colonies of Staphylococcus aureus typically show _____ pigmentation:

   a. White.

   b. Pinkish.

   c. Golden.

   d. Reddish.

2. When grown on blood agar, Staphylococcus aureus colonies usually cause:

   a. No hemolysis of surrounding red blood cells (RBCs).

   b. An alpha type of hemolysis of the surrounding RBCs.

   c. A gamma type of hemolysis of the surrounding RBCs.

   d. Complete hemolysis of the surrounding RBCs.

3. The streptococci are characterized as being:

   a. Nonmotile and nonsporeforming.

   b. Motile and sporeforming.

   c. Spore forming in broth cultures.

   d. Motile in broth cultures.

4. In the Lancefield classification of beta hemolytic streptococci, which group is most often associated with communicable diseases in humans?

   a. A.

   b. B.

   c. C.

   d. D.

5. The most useful method for preliminary differentation of species of Streptococcus is:

   a. Gram staining reaction.

   b. Cellular morphology.

   c. Size of colonies on blood agar.

   d. Type of hemolysis produced on blood agar.

6. A smear from a colony of Corynebacterium diphtheriae should show bacilli which are:

   a. Gram-positive, spore forming.

   b. Gram-negative, spore forming.

   c. Gram-positive, in Chinese-letter patterns.

   d. Gram-negative, in Chinese-letter patterns.

7. The entry of Corynebacterium diphtheriae is usually through:

   a. The unbroken skin.

   b. An open wound.

   c. The nasopharynx.

   d. The conjunctivas.

8. Organisms causing human anthrax usually enter through the:

    a. Skin.

    b. Lungs.

    c. Conjunctivas.

    d. Gastrointestinal System.

9. Which of the following genera consists of strict anaerobes?

    a. Myobacterium.

    b. Corynebacterium.

    c. Clostridium.

    d. Bacillus.

10. Clostridium tetani gives the appearance of a microscopic drumstick due to:

    a. Bipolar staining.

    b. Metachromatic granules.

    c. Round terminal spores.

    d. Oval central spores.

11. All of the following describe clostridia except:

    a. Anaerobic organisms.

    b. Gram-positive rods.

    c. Soil inhabitants.

    d. Producers of powerful endotoxin.

    e. Spore formers.

12. The organism most commonly associated with gas gangrene is:

    a. Clostridium botulinum.

    b. Clostridium perfringens.

    c. Salmonella perfringens.

    d. Shigella pyrogenes.

13. Escherichia coli is a:

    a. Gram-positive coccus.

    b. Gram-negative coccus.

    c. Gram-positive bacillus.

    d. Gram-negative bacillus.

14. Escherichia coli is:

    a. Part of the normal flora of the intestinal tract.

    b. An organism sometimes associated with infant diarrhea.

    c. A frequent cause of urinary tract infections.

    d. All of the above.

15. Plague, known as the "black death" because of the areas of the skin darkened by subcutaneous hemorrhages, is caused by:

    a. Shigella dysenteriae.

    b. Salmonella typhi.

    c. Klebsiella pneumoniae.

    d. Yersinia pestis.

16. The causative agent of whooping cough belongs to the genus:

    a. Bordetella.

    b. Brucella.

    c. Haemophilus.

    d. Pasteurella.

17. All of the following are principal causes of foodborne diseases except:

    a. Metallic or poisonous chemicals.

    b. Pesticides.

    c. Toxicants.

    d. Microbiological agents.

18. All of the following cause foodborne illness except:

    a. Escherichia coli.

    b. Staphylococcus aureus.

    c. Clostridium mitis.

    d. Clostridium botulinum.

19. The organism producing mycotoxins in peanuts, soybeans, and corn is:

    a. Clostridium perfringens.

    b. Aspergillus flavas.

    c. Foodborne virus.

    d. Trichinella spiralis.

20. The foods that are usually involved in staphylococcal intoxications are:

    a. Low fat items improperly cooked.

    b. Raw pork.

    c. Pasteurized milk.

    d. Protein-containing products contaminated after cooking.

21. In man, the main reservoir of Staphylococcus aureus is:

    a. Skin.

    b. Lungs.

    c. Nose.

    d. Hands.

22. All of the following streptococci have been associated with food poisoning except:

    a. Streptococcus faecalis.

    b. S. viridans.

    c. S. pyogenes.

    d. S. coli.

23. An organism that produces a localized cutaneous infection seen primarily as an occupational disease of persons handling fish, animals, meat, and poultry is:

    a. Bacillus cereus.

    b. Erysipelothrix rhusiopathiae.

    c. Listeria monocytogenes.

    d. Bacillus megaterium.

24. Which of the the following foodborne illnesses have a great similarity in the foods involved in the illness?

    a. Clostridium botulinum and Bacillus cereus.

    b. Clostridium perfringens and Staphylococcus aureus.

    c. Bacillus cereus and Clostridium perfringens.

    d. Bacillus cereus and Staphylococcus aureus.

25. Canned vegetables have accounted for the majority of outbreaks of _____ in the US.

    a. Staphylococcus aureus.

    b. Clostridium botulinum.

    c. Clostridium tetani.

    d. Bacillus cereus.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 2

1. c (para 2-8e)
2. d (para 2-8e)
3. a (para 2-9a)
4. a (para 2--9c)
5. d (para 2-10)
6. c (para 2-13)
7. c (para 2-14b)
8. a (para 2-20a(3))
9. c (para 2-21)
10. c (figure 2-3)
11. d (paras 2-21a, 2-24)
12. b (para 2-24c(1))
13. d (para 2-27)
14. d (para 2-30)
15. d (para 2-37c)
16. a (para 2-44)
17. b (para 2-2)
18. c (para 2-5a)
19. b (para 2-5c)
20. d (para 2-7e)

21. c (para 2-7e(5))

22. d (para 2-11e)

23. b (para 2-16)

24. c (para 2-20d(3))

25. b (para 2-24b(3))

End of Lesson 2

LESSON ASSIGNMENT

LESSON 3	Food Spoilage Due to Microorganisms.
LESSON ASSIGNMENT	Paragraphs 3-1 through 3-13.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

3-1. Given a specific food product, list the possible bacteria responsible for spoilage of that product.

3-2. Given a genus of mold/yeast present on a food product, identify the foods that could become spoiled due to growth of the mold/yeast.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 3

FOOD SPOILAGE DUE TO MICROORGANISMS

Section I. INTRODUCTION

3-1. INTRODUCTION

As veterinary specialists, we depend upon our senses of sight, smell, taste, and touch to evaluate food quality. When based upon past experiences, these sensory evaluations are used to determine if food is spoiled. Since people have different past experiences and abilities, there are conflicting opinions concerning the point at which a food is no longer acceptable for consumption.

3-2. CAUSES OF SPOILAGE

a. The deterioration of food can be caused by rodents, insects, tissue enzymes, nonenzymatic chemical changes, physical effects, and the action of microorganisms. The microbial deterioration of a food usually is manifested by alterations in the appearance, texture, color, odor, flavor, or by slime formation.

b. The microorganisms present on food include those associated with the raw material, those acquired during harvesting, handling, and processing, or those surviving a preservation treatment and storage.

Section II. BACTERIAL SPOILAGE OF VARIOUS FOOD PRODUCTS

3-3. BACTERIAL SPOILAGE OF VEGETABLES

a. Since vegetables are harvested from or near the soil, they are subjected to a heterogeneous flora of soil, as well as airborne microorganisms. The higher water content of vegetables favors the growth of bacteria rather than molds and yeasts.

b. Bacterial soft rot is a type of spoilage that is caused by Erwinia carotovora and pseudomonads such as Pseudomonas marginalis. These organisms break down pectins, giving rise to a soft, mushy consistency, sometimes a bad odor, and a water-soaked appearance. Some of the vegetables affected by this disease are: asparagus, onions, green beans, carrots, celery, beets, lettuce, radishes, broccoli, tomatoes, and peppers.

c. Other pseudomonads cause the following spoilage conditions: bacterial blight of celery, bacterial zonate spot of cabbage and lettuce, angular leaf spot of cucumbers, black rot of cabbage and cauliflower, bacterial wilt of beans, ring rot of potatoes, and common blight of beans.

d. Canned tomato juice is subject to flat sour spoilage if not properly handled either during preparation, or final heat treatment. Flat sour spoilage is attributed to the presence of and growth of Bacillus coagulans, which either survives normal heat processes, or recontaminates the product. Detection of spoilage is made initially by flavor, pH, and odor, followed by diagnostic tests.

e. The major source of organisms on frozen vegetables is contaminated equipment. Equipment that has been especially troublesome includes choppers, slicers, conveyor and inspection belts, and filling machines.

f. A wide variety of saprophytic bacteria can be isolated from frozen vegetables. The predominant flora is influenced by vegetable type, and by geographic location. Streptococcus and Leuconostoc predominate on certain vegetables such as greens. Coliforms and enterococci are also common contaminants of frozen vegetables. It appears that they are introduced onto equipment surfaces, and become a part of the microflora of the processing line along with the more numerous contaminants such as the lactic acid bacteria.

3-4. BACTERIAL SPOILAGE OF FRUITS

a. A large and varied population of microorganisms contaminates the surface of fruits during the growing season. The ripening process increases the susceptibility of the fruit to invasion. Fruits harvested from the ground have a more varied microbial flora than those picked from the tree. Falling from the tree causes bruising, and contact with the grass and soil provides an added source of inoculum. Due to the low pH of fruits, bacteria are normally inhibited from growing on fruits. With the exception of pears, which sometimes undergo Erwinia rot, bacteria are of no known importance in the initiation of fruit spoilage.

b. The lactobacilli are important in the spoilage of fruit juice. Some strains can affect the juice by reducing the acidity, resulting in a bland, rather flat flavor and a loss in sourness. Leuconostoc species produce an unpleasant texture of fruit juices; this is a ropy texture which then becomes slimy and viscous.

3-5. BACTERIAL SPOILAGE OF FRESH AND CURED MEATS, POULTRY, AND WATERFOODS

a. Since fresh animal products are perishable, they are chilled and stored in ice or a refrigerator (0º to 4ºC). This means that psychrotrophs (psychrophiles: cold-loving bacteria) become dominant and are the primary cause of spoilage. If these fresh products are mishandled and allowed to remain at room temperature (20ºC), a more diverse flora may be present, since not only many psychrotrophs grow, but there also will be some mesophilic strains. The organisms most often involved with spoilage of refrigerated fresh meat, poultry, fish, and eggs are species of the genus Pseudomonas.

b. Spoilage of meat is due to the growth and metabolism of large numbers of microorganisms on the surface or the interior. Most spoilage is on the surface. The number of organisms that are present when spoilage is evident varies from 10^6 to 10^8 organisms per cm^2 of meat surface.

c. The most common indications of spoilage are:

(1) Off-color, off-odor, and slime, usually due to the growth of aerobic bacteria on the cut surfaces of meat.

(2) Bone-taint or deep spoilage, due to anaerobic or facultative microorganisms.

(3) Discoloration, primarily due to alterations of myoglobin, the muscle pigment. Fresh chilled meat spoilage is due to Pseudomonas, Acinetobacter and Alcaligenes. Cured meats become sour due to the activity of Micrococcus, Lactobacillus, and Microbacterium species. Fresh beef stored in air has the dominant Pseudomonas species. However, when vacuum packaged, Lactobacillus predominates and other organisms include Enterobacter and Hafnia.

d. The predominant microflora of vacuum packaged bacon is determined by the salt content. Species of Micrococcus give way to lactic acid bacteria in bacon with low salt (5 to 7 percent), while they remain dominant in bacon with higher salt (8 to 12 percent).

e. For ham, the important type of spoilage is souring. This includes various spoilage problems from mild degradation to extreme putrefaction. Clostridium putrefaciens is psychrotrophic, and prominent in the spoilage of ham. The short-cure commercial method of curing has greatly reduced the incidence of ham souring. However, gassy or puffy hams can result if the fresh meat is held too long at a moderate temperature prior to cure. This allows clostridia to grow. Proper slaughter and bleeding of hogs, prompt chilling of the meat, prompt handling, good sanitation, and use of pump and cover brines that have low microbial counts have aided in improving the microbial quality of ham. Fecal streptococci may be found in stored hams and cause a green discoloration and off-flavor.

f. Comminuted and cured sausage products are subjected to various types of spoilage. These products are generally composed of mixtures of pork, beef, salt, sugar, sodium nitrite, and spices. Hence, the flora of these products will be somewhat different than that of fresh meat. A surface slime of micrococci can occur when sufficient moisture is present. When products, such as luncheon meats, are vacuum packaged, aerobic growth is inhibited and lactic acid bacteria become dormant. The lactic acid bacteria produce CO_2 and can cause a swelling of the package. For bologna products where a casing is used, microorganisms of the genera Micrococcus, Lactobacillus, and Leuconostoc can grow and cause souring of the product during storage. The production of organic acids and reducing compounds by the bacteria can cause a fading of the pink color at the outer surface of the product, resulting in a ring of discoloration.

g. Green discolorations can occur in cured sausages. The greening may appear as rings, cores or on the surface. Lactobacillus viridescens can grow in these products and is the organism primarily involved in greening of these sausages. Other Lactobacillus and Leuconostoc species sometimes are present. The bacteria produce peroxides which react with the cured meat pigment causing the green discoloration.

h. As poultry is chilled and held in cold storage, psychrotrophic microorganisms predominate and cause deterioration. The main defects are off-odor, which appears at a bacterial load between 10^6 and 10^8 organisms per cm^2, and slime formation, which occurs soon after off-odor is noted. Species of Pseudomonas are the principal spoilage organisms. Besides Pseudomonas, other organisms, similar to those in fresh red meat spoilage, are found on spoiled poultry. These include Aeromonas, Moraxella, Alcaligenes, and Micrococcus.

i. Fish and other waterfoods are subject to contamination by microorganisms in their marine environment, as well as those acquired during catching, handling, and processing. In fish, Pseudomonas and Proteus cause putrid and ammoniacal odors, while Acinetobacter and Aeromonas are associated with unpleasant sweetish or fruity odors. Vacuum packaging of fish changes the spoilage flora in a manner similar to fresh meat. Lactobacillus and Microbacterium grow and cause souring, whereas the aerobic pseudomonads are inhibited. Frozen or fresh oysters spoil by either fermentative (characterized by a sour odor), or putrefactive mechanisms. Oysters that are well-cooked and then refrigerated spoil due to fat rancidity, rather than microbial decomposition. Various discoloration can occur in shellfish. There are several predominant organisms on iced raw shrimp. The presence of Arthrobacter and Acinetobacter may indicate inadequate cleaning. The presence of Moraxella, Flavobacterium, and Cytophaga indicates the degree of secondary contamination, and Pseudomonas indicates the potential shelf-life of processed shrimp.

j. Because of the relatively high level of glycogen, the spoilage of oysters is basically fermentative. The following pH scale can be used as a basis for determining microbial quality in oysters:

(1) pH 6.2-5.9 = Good

(2) pH 5.8 = Off condition.

(3) pH 5.7-5.5 = Musty.

(4) pH 5.2. & below = Sour or putrid.

3-6. BACTERIAL SPOILAGE OF EGGS

a. The egg is an excellent example of a product that normally is well protected by its intrinsic characteristics. Externally, a fresh egg has three structures effective to some degree in retarding the entry of microorganisms: the outer, waxy shell membrane; the shell; and the inner shell membrane. Freshly laid eggs are generally sterile. However, after eggs are laid, numerous microorganisms may be found on the outside and may enter the eggs where they can grow and cause spoilage.

b. For egg products, the shell and membranes are removed. During processing, the shelled, raw egg is subject to contamination from organisms on the egg shell, equipment, humans, added ingredients, and the final container.

c. Shell eggs will absorb odors from the storage atmosphere. Most vegetables and fruits will impart flavors and odors to shell eggs. If stored with apples, the eggs will be bitter and have a cardboard flavor and odor. If stored near gasoline or kerosene, the shell eggs will taste and smell like compounds.

d. The types of egg spoilage due to microbial growth are as follows:

(1) Black rot. When viewed with a candling light, black rot eggs are virtually opaque. When broken out, the egg content has a muddy (dark brown) appearance, a repulsive putrid odor, and H_2S is evident. The bacteria associated with this type of spoilage are species of Proteus and Aeromonas. Rots of this type are more likely to occur at room temperature (20ºC) than in cold storage (4ºC or less).

(2) White rot. Threadlike shadows may be seen in the thin white, and in later stages, the yolk appears severely blemished when the shell egg is viewed with the candling light. When opened, the egg yolk shows a crusted appearance and frequently has a fruity odor. This type of inedible egg sometimes is referred to as a colorless rot. Various organisms have been associated with this rot, including Citrobacter, Salmonella, and Alcaligenes.

(3) Sour eggs. These eggs are difficult to detect by ordinary candling, but they usually show a weak white and murky shadow around an off-center, swollen yolk. These eggs also are called fluorescent and are quite readily detected by observing with ultraviolet (UV) light. A green sheen is produced by species of Pseudomonas.

(4) Green whites. This defect is caused mainly by Pseudomonas fluorescens. Green whites of broken out eggs fluoresce when observed with UV light. Eggs with green whites may or may not have a sour odor since the green fluorescence can be observed long before any odor can be detected.

(5) Red rot. These eggs are distinguished by a red discoloration of the albumen and the surface of the yolk. An ammoniacal to putrid odor may occur. Serratia marcescens has been considered as the cause of red rot.

(6) Custard rot. In this rot, the yolk is encrusted with custard-like material and occasionally flecked with olive green pigment. The albumen becomes thin with an orange tint. There may be a slightly putrid to putrid odor. Citrobacter and Proteus vulgaris have been associated with this type of spoilage.

(7) Other rots. Alcaligenes has been accused of causing both yellow and green rots. These rots are similar in odor and in the appearance of albumen. However, the yolk is dark yellow in yellow rot and dark green in green rot. Rust red rot is associated with growth of P. vulgaris.

3-7. BACTERIAL SPOILAGE OF DAIRY PRODUCTS

a. Dairy products such as milk, butter, cream, and cheese are all susceptible to microbial spoilage because of their chemical composition. Milk is an excellent growth medium for all of the common spoilage organisms. Fresh, nonpasteurized milk generally contains varying numbers of microorganisms, depending upon the care employed in milking, cleaning, and handling of milk utensils.

b. Raw milk held at refrigerator temperatures for several days invariably shows the presence of several or all bacteria of the following genera: Streptococcus, Leuconostoc, Lactobacillus, Micrococcus, coliforms, Proteus, Pseudomonas, and Bacillus.

c. The pasteurization process eliminates all but thermoduric strains, primarily streptococci and lactobacilli, and spore forms of the genus Bacillus. The spoilage of pasteurized milk is caused by the growth of heat-resistant streptococci utilizing lactose to produce lactic acid, which depresses the pH to a point where curdling takes place.

d. The defects that can occur in milk due to microbial growth are:

(1) Off-flavors.

(2) Lipolysis with development of rancidity.

(3) Gas production.

(4) Fermentation to lactic acid with souring.

(5) Coagulation of milk proteins.

(6) Viscous or ropy texture.

(7) Discoloration.

e. A condition of raw milk due to microbial growth is referred to as ropiness. This condition is caused by the growth of Alcaligenes viscolactis and coliforms, and is flavored by low-temperature holding of raw milk for several days. The rope consists of slime-layer material produced by the bacterial cells and it gives the product a stringy consistency.

f. Bacteria cause two principle types of spoilage in butter.

(1) The first is a condition known as "surface taint" or putridity. This condition is caused by Pseudomonas putrefaciens as a result of its growth on the surface of the finished butter. The odor of this condition is apparently due to certain organic acids.

(2) The second most common bacterial spoilage condition of butter is rancidity. This condition is caused by the hydrolysis of butter fat with the liberation of free, fatty acids. The causative organism is Pseudomonas fragi although Ps. fluorescens is sometimes found.

(3) Other less common spoilage conditions in butter are malty flavor which is due to Streptococcus lactis, skunk-like odor due to Ps. mephitica, and black discoloration due to Ps. nigrifaciens.

g. The most common spoilage in cottage cheese is slimy curd. Alcaligenes species have been reported to be among the most frequent causative organisms although Pseudomonas, Proteus, Enterobacter, and Acinetobacter have been implicated. Surface discolorations may occur due to the growth of the pigmented Flavobacterium. E. coli can cause barny or unclean flavors and, if the cottage cheese is held at room temperature, the organism can cause a gassy defect.

h. A pink discoloration of Romano and other Italian cheese varieties was found to be due to Lactobacillus species. The pink discoloration occurred as a uniform band of color near the cheese surface, or as discoloration throughout the entire cheese.

3-8. BACTERIAL SPOILAGE OF CANNED FOODS

a. Although the objective in the canning of food is the destruction of microorganisms, these products still undergo microbial spoilage under certain conditions. The main reasons for this are the following: under-processing, inadequate cooling, infection of can resulting from leakage through seams, and pre-process spoilage.

b. As a guide to the type of spoilage that canned foods undergo, the following classification of canned foods based upon acidity is helpful:

(1) Low-acid: pH>4.6. Meat and marine products, milk, corn, lima beans, meat-and-vegetable mixtures. These items are spoiled by thermophilic flat-sour group (Bacillus stearothermophilus, B. coagulans), sulfide spoilers (Clostridium nigrificans, Cl. bifermentans), and/or gaseous spoilers (Cl. thermosaccharolyticum). Mesophilic spoilers include putrefactive anaerobes. Spoilage and toxin production by Cl. botulinum types A and B may also occur.

(2) Acid: pH 3.7-4.0 to 4.6. Within this category are many fruits such as tomatoes, pears, figs. Thermophilic spoilers include Bacillus coagulans. Mesophiles include B. polymyxa, B. macerans, Clostridium butyricum, lactobacilli, and others.

(3) High-acid: pH<4.0-3.0. This category includes some fruits and fruit juices such as grapefruit, rhubarb, sauerkraut, and pickles. These are generally spoiled by nonspore forming mesophiles such as lactic acid and bacteria.

c. Lactobacillus species cause a vigorous fermentation in tomato ketchup, Worcestershire sauce, and similar products. Leuconostoc species have been reported to cause gaseous spoilage of canned pineapples, and ropiness in peaches.

d. Frozen concentrated orange juice sometimes undergoes spoilage by bacteria. The orange juice is characterized as having a vinegary to buttermilk off-odor with an accompanying off-flavor. The organisms that were isolated were lactobacilli and Leuconostoc species.

e. Microbiological spoilage in canned food is usually indicated by a swelling of the container. When no abnormal external signs are present, spoilage may be indicated by abnormal odor or appearance of the product.

Section III. MOLDS

3-9. INTRODUCTION TO MOLDS AND YEASTS

Yeasts and molds are widely distributed in the environment, and may be found as part of the normal flora of a food product, on inadequately sanitized equipment, or as airborne contaminants. Although certain yeasts and molds are useful in the manufacture of various foods, such as mold-ripened cheese and bread, they also can be responsible for spoilage of many types of food. Because of their slow growth and poor competitive ability, yeasts and molds often manifest themselves on or in foods in which conditions are less favorable to bacterial growth. This section discusses molds. The next section discusses yeasts.

3-10. INTRODUCTION TO MOLDS

a. Molds are part of a larger group of microorganisms called fungi. The fungi are ubiquitous, being found everywhere. Soil, air, water, and decaying organic matter are prime sources.

b. Fungi are organisms that lack the definite root, stem, or leaves of higher plants. They are differentiated from the algae and higher plants by their lack of chlorophyll. They differ from bacteria by their more complex structure and greater size. The fungi may be multicellular or unicellular. The fundamental structural units of molds are filaments or tubes called hyphae. By formation of crosswalls or septa, some hyphae form chains of cells that are septate. The septa have pores that allow the movement of cytoplasm from one cell to another. As the hyphae elongate, they intertwine. A mass of these intertwined, branched hyphae is called a mycelium.

c. With bacteria, one colony originates from a single cell, but a fungal colony may result from a single cell, a spore, a piece of mycelium, or a number of cells. In the past, media for enumerating molds were acidified to pH 4-5. The low pH inhibited bacteria and allowed the fungi to grow. Newer media incorporate antibiotics to inherit bacteria and the media pH is near 7.0. Microscopic methods are used to enumerate mold filaments in tomato products and other canned fruits and vegetables.

d. Although important in all types of foods, molds are more apt to cause spoilage or become a health hazard in foods such as grain, flour, or nuts with low water activity or fruits with a low pH.

e. Molds can be considered as spoilage organisms in food products. Although we often associate the appearance of mold as an indication of spoilage, these organisms can degrade products before growth is evident to the naked eye.

f. Although molds associated with food are not considered to be pathogenic, some produce mycotoxins. These toxic substances pose a potential health hazard to humans.

3-11. MOLDS IN FOOD

With respect to the fungal spoilage of foods, the following genera of molds have been recovered from various spoilage conditions.

a. **Rhizopus.** These are common spoilage organisms with common bread mold being the most numerous of this genus.

b. **Thamnidium.** These grow on refrigerated meat causing a defect referred to as "whiskers."

c. **Neurospora.** These cause problems in bakeries and is known as the red or pink bread mold.

d. **Alternaria.** One of the most prevalent molds that causes black rot of tomatoes in the field. These organisms also are involved in the development of rancidity or off-flavors in dairy products.

e. **Aspergillus.** Certain strains of this genus produce aflatoxins (myocotoxins) which are a potential health hazard to humans.

f. **Botrytis.** These are the gray mold of lettuce, tomatoes, strawberries, raspberries, and grapes.

g. **Cladosporium.** These organisms can grow on the connective tissue or fat covering of meat when refrigerated for several days. Growth results in black spots on the meat.

h. **Geotrichum.** These organisms can cause spoilage problems. It has been called "Dairy Mold" since it is found growing on dairy products. G. candidum is called "machinery mold" since it will grow on equipment with attached food particles or juices. As the food product is processed, it becomes contaminated with the mold. The presence of the mold in canned foods is considered to be an adulterant and indicates inadequate sanitation in the processing plant.

i. **Penicillium.**

(1) These are important spoilage organisms. They cause green mold rot and blue mold rot in citrus fruits. They are found growing on the fatty layer or connective tissue of meat that is stored in the refrigerator for several days, and on moldy bread.

(2) Species of penicillium are also useful in various ways. Antibiotics may be produced by some species. Some species are used in cheese manufacturing.

j. **Sporotrichum.** These organisms grow at low temperature and can cause a defect called "white spot" of refrigerated meat.

Section IV. YEASTS

3-12. INTRODUCTION

a. The yeasts have been defined as fungi in which the usual dominant form is unicellular. The unicellular form gives yeast an advantage over the mycelial form of molds. There is a greater surface to volume ration that allows a higher metabolic activity.

b. Yeasts are universally recognized as beneficial due to their familiar role as a fermenter of sugars to carbon dioxide and ethyl alcohol. As one of the busiest microbial groups, yeasts impart flavor to foods and enrich human and animal diets. There are few pathogenic species of yeasts; however, no yeast has been incriminated in a clinical case of food intoxication.

c. Though few in number and less ubiquitous than bacteria, yeasts are found in a variety of natural habitats, generally living a saprophytic life in environments favorable to their growth.

d. Yeasts are microscopic organisms that may be differentiated from the common bacteria by their larger cell size, their oval, elongate, elliptical, or spherical cell shapes, and by their production of buds during the process of division. Yeasts can grow over a rather wide range of pH, alcohol, and sugar concentrations.

e. Almost any kind of food will permit yeast to grow, provided it has not been adequately heat treated. High concentrations of sugar, salt, organic acids, the exclusion of air, refrigeration, and application of other storage conditions will not safeguard a food from the action of yeasts, providing that storage is sufficiently long. The heavier the initial contamination, the sooner spoilage signs become apparent.

3-13. YEASTS IN FOOD

With respect to the fungal spoilage of foods, the following genera of yeasts have been recovered from various spoilage conditions.

a. **Debaryomyces.** These organisms have been associated with spoiled mushrooms, cheeses, cider, white wine, tomato puree, sausage, and salted beans.

b. **Saccharomyces.** These organisms have a widespread distribution. They can cause spoilage in fruit and fruit products, sugar, syrup, honey, vinegar, mayonnaise, salad dressing, dairy products (buttermilk, cheese), and fermenting foods such as cucumbers.

c. **Candida.** These organisms have been involved with the spoilage of various foods such as frankfurters, fresh fruits, vegetables, dairy products, brines, and alcoholic beverages.

d. **Torulopsis.** These yeasts are credited with causing surface slime on cottage cheese, spoilage of refrigerated beef, cream, butter, sweetened condensed milk, and various food brines.

e. **Trichosporon.** These organisms are found on various foods such as fresh shrimp, crab, beef, butter, cheese, fruit, fruit juice, and rice.

Continue with Exercises

EXERCISES, LESSON 3

INSTRUCTIONS. Answer the following exercises by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Canned tomato juice is subject to flat sour spoilage due to:

   a. Erwinia carotovora.

   b. Pseudomonas marginalis.

   c. Bacillus coagulans.

   d. Clostridium botulinum.

2. The major source of organisms on frozen vegetables is:

   a. Soil contamination.

   b. Contaminated equipment.

   c. Mold growth.

   d. Poor refrigeration.

3. A ropy texture of fruit juices is due to:

   a. Staphylococcus aureus.

   b. Erwinia ropa.

   c. Lactobacilli perfringens.

   d. Leuconostoc species.

4. The organisms that are dominant in the spoilage of fresh animal products are:

    a. Mesophiles.

    b. Thermophiles.

    c. Psychrotrophs.

    d. Thermodurics.

5. The predominant microflora of vacuum-packaged bacon is determined by the:

    a. Amount of vacuum.

    b. Type of package.

    c. Amount of water added.

    d. Salt content.

6. The most common organism in the souring of hams is:

    a. Clostridium putrefaciens.

    b. Clostridium perfringens.

    c. Bacillus cereus.

    d. Enterobacter aerogenes.

7. Green discolorations can occur in cured sausages due primarily to:

    a. Lactobacillus virulence.

    b. Bacillus subtilis.

    c. Lactobacillus viridescens.

    d. Clostridium viridescens.

8. Waterfoods are subject to contamination during:

    a. Catching.

    b. Handling.

    c. Processing.

    d. All of the above.

9. A pH determination for oysters of 5.6 indicates they are in what condition?

    a. Good.

    b. Off.

    c. Sour.

    d. Musty.

10. A fresh egg has three structures effective in retarding the entry of microorganisms. The structures are:

    a. Outer shell membrane.

    b. Inner shell membrane.

    c. Shell.

    d. All of the above.

11. Black rots in eggs may be due to:

    a. Pseudomonas.

    b. Proteus.

    c. Clostridium.

    d. Bacillus.

12. Ropiness of milk is caused by the growth of:

    a. Lactobacillus viscolactis.

    b. Alcaligens lacto.

    c. Alcaligenes viscolactis.

    d. Bacillus cereus.

13. A pink discoloration of Romano cheese was found to be due to:

    a. Lactobacillus species.

    b. Alcaligenes species.

    c. Pseudomonas species.

    d. Proteus species.

14. Microbiological spoilage of canned food is usually indicated by:

    a. Low pH of product.

    b. Dehydration of product.

    c. Rusting of the container.

    d. Swelling of the container.

15. Black rot in tomatoes is due to:

    a. Aspergillus.

    b. Proteus.

    c. Alternaria.

    d. Rhizopus.

16. Blue mold rot in citrus fruits is due to:

    a. Sporotrichum.

    b. Penicillium.

    c. Bacillus.

    d. Pseudomonas.

17. Surface slime on cottage cheese may be due to:

    a. Aspergillus.

    b. Trichosporon.

    c. Torulopsis.

    d. Rhizopus.

18. Yeasts are microscopic organisms that may be differentiated from bacteria by:

    a. Larger cell size.

    b. Production of buds.

    c. Oval cell shapes.

    d. All of the above.

19. "White spot" of refrigerated meat is due to:

    a. Sporotrichum.

    b. Penicillium.

    c. Alternaria.

    d. Aspergillus.

20. Toxic substances, produced by molds on food, are called:

    a. Enzymes.

    b. Mycotoxins.

    c. Lysozymes.

    d. Contamitoxins.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 3

1. c (para 3-3d)
2. b (para 3-3e)
3. d (para 3-4b)
4. c (para 3-5a)
5. d (para 3-5d)
6. a (para 3-5e)
7. c (para 3-5g)
8. d (para 3-5i)
9. d (para 3-5j)
10. d (para 3-6a)
11. b (para 3-6d(1))
12. c (para 3-7d)
13. a (para 3-7h)
14. d (para 3-8e)
15. c (para 3-11d)
16. b (para 3-11i)
17. c (para 3-13d)
18. d (para 3-12d)
19. a (para 3-11j)
20. b (para 3-10f)

End of Lesson 3