Laboratory Manual for

# General Microbiology

Third Edition

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# PREFACE

The earlier editions of this manual have been used in general education courses in biology and in introductory microbiology courses for students in areas of natural science over a period of about 30 years. This new edition has been extensively revised and updated, and new experiments on epidemiology and natural body defenses have been added. Each exercise has been scrutinized and modified to conform to the suggestions and experiences collected over a period of years by many teachers. Comprehensive reviews and evaluations of this edition have been submitted by Dr. Daniel Burke, Mercer University, Dr. Gary Larkin, Kent State University, and Dr. Richard Wacha, Drake University. The authors gratefully acknowledge these reviews.

This laboratory manual has been designed for use in microbiology courses taken by students who want to satisfy general education requirements or who may require only a limited laboratory experience with microorganisms. Although it is not intended primarily for use by majors in microbiology or allied fields, its wide coverage of general areas of microbiology makes it valuable as an introductory study for those students who may wish to concentrate in some areas of natural or physical science.

The authors strongly believe that some laboratory experience is most useful for students who want more fully to appreciate natural phenomena. Students will learn how to observe, culture, and identify some common types of microorganisms as well as become acquainted with some ecological, immunological, and public health associations of microbes. A major strength of the manual is its coverage of a wide spectrum of microbiological study while using only a minimal amount of equipment and supplies. The 18 exercises are designed to give students a feeling for scientific techniques while introducing those laboratory procedures that are particularly important in microbiology, for example, sterile techniques, transfer of cultures, streaking of agar plates, and pipetting. Of help to instructors as well as students is a complete listing of all culture media and staining solutions used in the exercises, together with the recipes for mixing them. There is also provided a listing of sources for the components of the culture media as well as the microbial cultures required. A table of commonly used metric units and conversion factors is also included. To assist instructors who wish to check the progress of their students, the manual now concludes with a set of review questions for each exercise that can be torn out and handed in if desired. We include several new illustrations to help guide students through the steps they will perform in laboratory.

To stimulate comments from students, we include a student survey through which students can evaluate this manual. We would appreciate it if the instructor would have the students complete this survey and see that the responses are mailed to Burgess Publishing Company or given to the Burgess representative when he or she calls.

Provo, Utah
March, 1979

J. V. B.
D. M. D.
D. H. L.
R. D. S.

# TO THE STUDENT

This manual was written to guide you in your laboratory study of microbiology. We hope that you will not only learn from it, but that you will enjoy your lab experience more for having used it. We think the following suggestions will help you get the most out of your lab and this manual.

1. Please do not bring unneeded items, such as coats or extra books, into the laboratory. Appropriate places have been provided for them outside the room.
2. Each student will be assigned a locker and a microscope. The locker contains only that equipment which you will use often. Other equipment will be provided as needed at each class meeting.
3. *You are responsible for the microscope.* Please use special care when you carry and use it and report any defect as soon as you become aware of it.
4. Most exercises are designed to be done by each student working individually; however, saving of time, materials, and equipment without loss of student achievement may be realized by having students work in teams of two or more in certain exercises. In exercises best done by teams, we suggest sizes of teams in the section titled "Procedures."
5. Before beginning each exercise, read the "Materials" and "Procedures" sections, assemble the items you will need, and familiarize yourself with the steps required. As you work, describe to yourself the purpose of the exercise and the purpose of each step you do.
6. Several exercises use living microorganisms. To prevent possible infection and transmission of these living organisms to other persons, wash your work area and your hands with a mild disinfectant and with soap and water before you leave the laboratory.
7. Spaces in which you can write laboratory notes of your choosing are provided in each exercise. To help you record results and observations, fill-in tables and outlines to guide you in making drawings are also provided. Drawings are most informative if done using colored pencils.
8. A set of review questions for each exercise is located at the end of the manual. At the option of the instructor, these questions can be answered, torn out, and handed in for grading. Even if your instructor does not require you to hand them in, we recommend that you answer them to check your own understanding. The questions test general as well as lab information, so feel free to consult your text and lecture notes as you answer the questions.

# CONTENTS

## Exercise 1

# USE OF THE COMPOUND MICROSCOPE

The **microscope** has played a more vital role than any other mechanical instrument in the discovery of microorganisms and the development of microbiology as a scientific discipline. You will use the compound microscope extensively in this course as you study form, structure, staining characteristics, and motility of different microorganisms. The microscope is a complex apparatus that is easily damaged and expensive to repair or replace. You should always handle it with extreme care. To become proficient at using a microscope, it is important that you understand the basic principles of its operation and care, as the following exercise will show.

### MATERIALS

1. Microscope, immersion oil, lens paper, glass slides, and cover slips.
2. Cotton fibers.
3. Prepared stained slides of several types of bacteria.

### PROCEDURE

The instructor will carefully demonstrate appropriate procedures for the care and use of the microscope during this exercise.

1. Examine the microscope. Figure 1-1 is provided to help you become familiar with it. The compound microscope is an instrument in which magnification occurs in two complex lens systems. Initial magnification occurs in the objective lens. Most microscopes are equipped with three objective lenses on a rotating base, and each lens may be rotated into alignment with the eyepiece or ocular lens in which the final magnification occurs. The objective lenses are identified as the low-power, high-dry, and oil immersion objectives. Each objective is sometimes designated by other terms. These terms give either the linear magnification or the focal length. The latter is about equal to but greater than the working distance between the specimen when in focus and the tip of the objective lens. Thus, the low-power objective is also called the 10X or 16-millimetre[1] (mm); the high-dry is called the 40X, 50X, or 4-mm objective; and the oil immersion is called the 90X, 100X, or 1.8-mm objective. As the magnification increases, the size of the lens at the tip of the objective becomes smaller and admits less light. This is one of the reasons that changes in position of the in-stage condenser and iris diaphragm are required when using different objectives if the specimens viewed are to be seen distinctly. The condenser concentrates the light on a small area above the stage and the iris diaphragm controls the amount of light that enters the condenser. When the oil immersion lens is used, immersion oil fills the space between the object and the objective. Because immersion oil has the same refractive index as glass, the loss of light is minimized. The eyepiece or ocular at the top of the tube magnifies the image

1. In accordance with the practice of the International Bureau of Weights and Measures, the American word *meter* will be spelled *metre*. See Paul, Martin A. 1972. International System of Units (SI). *Chemistry* 45(9):14-18.

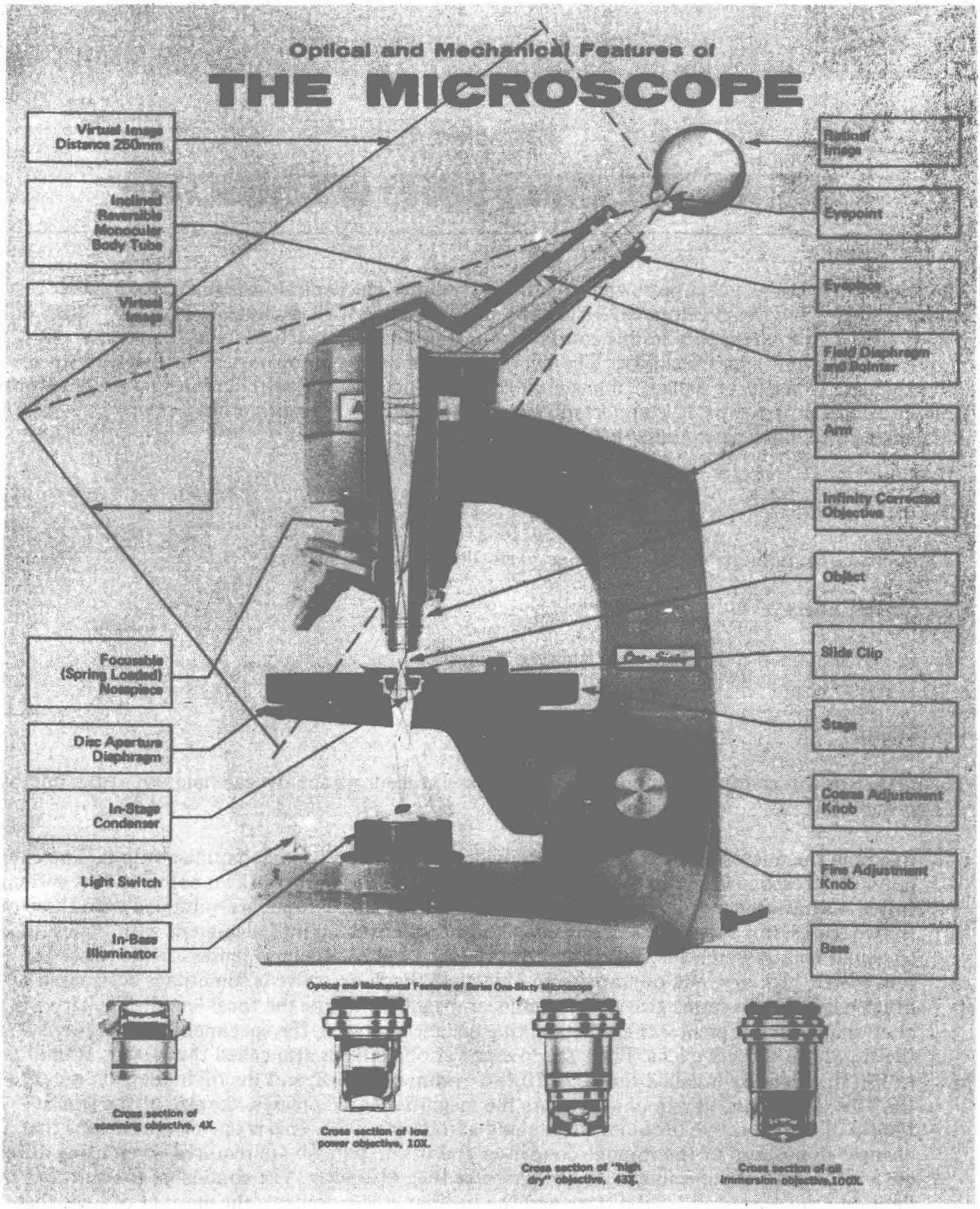



**Figure 1-1.** Compound microscope. Courtesy of American Optical Corporation, Buffalo, NY.

formed by the objective lens. Consequently, the total magnification seen by the observer is obtained by multiplying the magnification of the objective lens by the magnification of the ocular or eyepiece. For example, when using the 10X ocular and the 43X objective, total magnification = 10 X 43 = 430.

2. Place the microscope on the desk with the open part of the base away from you and over a desk lamp. *Note:* Your microscope may have a built-in source of illumination located below the condenser.

3. Using the coarse adjustment knob and looking at the microscope from the side, lower the tube[1] until the tip of the low-power objective lens is within ¼ inch of the stage. Make sure that the iris diaphragm is open and that the top of the condenser is at the level of the stage. Look into the ocular and note the amount of background illumination.

4. Repeat step 3, using the high-dry power objective in place of the low-power objective. Can you explain the difference in illumination observed through the different objectives?

5. Prepare a few cotton fibers for observation by placing them on a glass slide and covering them with a drop of water. Place a cover slip over the drop of water. This preparation is known as a wet mount. Place the glass slide on the stage of the microscope and secure it firmly using stage clips. If your microscope has a mechanical stage device, place the slide securely in it. Move the slide until the fiber is over the opening in the stage.

6. With the low-power objective in position lower the tube until the tip of the objective is within ¼ inch of the slide. Be sure that you lower the tube while looking at the microscope from the side. *Never lower the tube without observing the microscope from the side.*

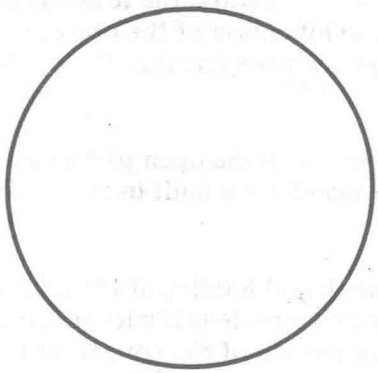
7. Look into the microscope and *slowly* raise the tube by turning the coarse adjustment knob counterclockwise until the object comes into view. *Remember–always focus upward when looking into the ocular.* It is good practice to move the slide slightly while focusing, so that you might more easily find the object on the slide. Once you bring the object into view using the coarse adjustment knob, use the more sensitive fine adjustment knob to get the desired image.

8. Open and close the diaphragm and lower and raise the condenser, noting what effect these actions have on the appearance of the object being viewed.

9. Using the coarse adjustment knob, raise the tube until the low-power objective is about 1 inch above the slide and swing the high-dry objective into place. Lower this objective until the lens almost touches the cover slip. While looking into the microscope slowly raise the tube by means of the coarse adjustment knob until the piece of cotton appears. Use the fine adjustment knob to sharpen the focus. Compare the magnification and detail afforded by the different objectives.

10. Repeat steps 6 through 9 several times until you feel familiar with the procedure. Examine several cotton fibers carefully. Draw a few fibers in the following circle. The circle represents the microscopic field.

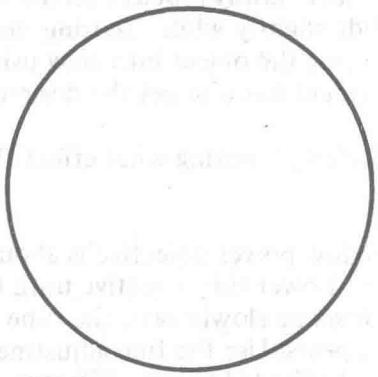
1. Your microscope may have a movable stage instead of a movable ocular tube. If so, use the coarse adjustment knob to move the stage to within ¼ inch of the tube.



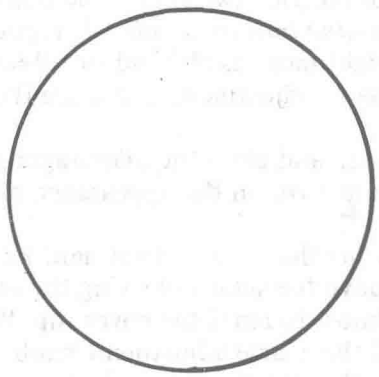
Cotton Fibers

11. Use the oil immersion lens to examine the stained bacteria that are provided. The directions for using this lens are as follows: raise the tube of the microscope; put the oil immersion objective into position; and place a small drop of immersion oil on the stained area. Lower the objective while you watch from one side until its tip is immersed in the oil and almost touches the slide. While looking into the ocular, focus upward with the coarse adjustment knob *very slowly* until the image appears. Sharpen the focus with the fine adjustment knob and use as much light as possible. An alternative technique is to locate the stained area while looking through the ocular with the low-power objective lens in place and then turning the oil immersion lens into place. The stained preparation should be very nearly in focus.

12. Make drawings of a few of the bacteria on each slide as observed through the microscope.



Bacteria



Bacteria

13. After you finish using the microscope, you should always swing the low-power objective in line with the ocular, lower the tube to its lowest position, and clean the oil from the oil immersion lens before returning the microscope to its storage place.

## DEMONSTRATION

1. Microscopes with prepared slides of microorganisms in focus.

## LABORATORY NOTES

## REVIEW QUESTIONS (PAGE 85)

## Exercise 2

# STAINING OF BACTERIA

**Bacteria** are minute, almost colorless cells invisible to the naked eye. Unstained bacteria are difficult to observe even with the aid of a microscope. To make them more easily observable, bacteria are stained with dyes. Before staining, bacteria are suspended in a drop of water on a clean microscope slide and are then spread in a thin, even film. The film is allowed to dry in the air ("air dry") and the organisms are "fixed" to the slide by gentle heat or by chemical means. The dried preparation on the slide, known as a fixed smear, can be stained by simple, Gram, or more complex staining procedures. This exercise will acquaint you with simple and Gram staining procedures.

### PART 1
### SIMPLE STAINING

The application of a single stain to a smear is termed a simple staining procedure.

### MATERIALS

1. Slant cultures of *Bacillus megaterium* (18 to 24 hours old).
2. Solutions of Gram's crystal violet.[1]
3. Inoculating loops.
4. Glass slides.
5. Toothpicks.

### PROCEDURE

1. For this and *all exercises throughout this manual,* first read the "Materials" and "Procedures" sections, assemble the items you will need, and familiarize yourself with the steps required.
2. Clean two slides by covering the entire surface of each with moist Bon Ami cleanser; let them dry and then polish each using a dry cloth towel. *If a slide is clean,* a drop of water will spread evenly over its surface with no tendency to round into droplets.
3. Place a loopful of water in the center of one clean slide.
4. Using a sterile inoculating loop, transfer a very small amount of *B. megaterium* from the surface of the agar slant to the drop of water on the slide.

1. **All staining solutions required for the exercises in this manual are listed alphabetically under "Staining Solutions" (pages 76-77), and the ingredients for each solution are given.**

5. Spread the culture over an area about the size of a dime to form a thin film. *Sterilize the needle immediately after making the smear* (why?).

6. In like manner prepare a smear of scrapings (obtained using a toothpick) from the gum margins of your rear molars.

7. Allow the slides to air dry on the desk top until the water is completely evaporated.

8. Quickly pass each slide through the warm area above the flame, film side up, two or three times. This fixes the bacterial cells onto the slide so that subsequent staining and washing will not remove them. *Important note:* Never hold the slide directly over the flame. This will overheat the preparation and destroy the cells.

9. Cover each slide with crystal violet, letting it remain 15 to 30 seconds. Then wash the stain off with a gently flowing stream of tap water.

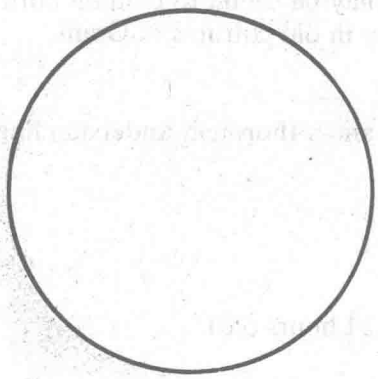
10. Gently blot the slide dry with a paper towel or allow the slide to air dry.

11. Examine each slide, using the oil immersion objective of the microscope. Shapes and groups of cells can best be observed by examining thin areas of the smear where the cells are well isolated.

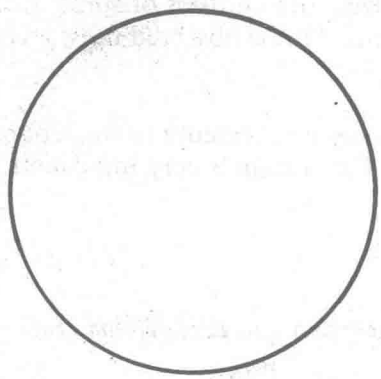
    a. When examining the slide of *B. megaterium*, note that the vegetative cells usually stain evenly whereas the spores (if present) are not stained and appear as clear spaces in the cells.

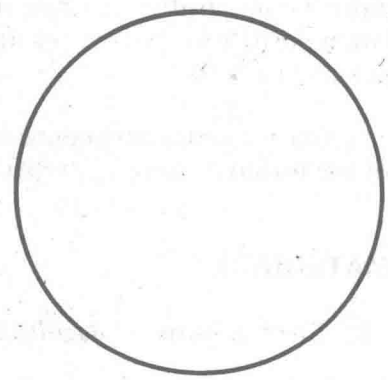
    b. When examining the smear of gum scrapings, look for the various sizes and shapes of bacteria found together with the large epithelial tissue cells.

12. Make drawings showing the typical shapes of the different bacteria. Cells of *B. megaterium* should be drawn at least 0.5 centimetres (cm) long. Other microorganisms should be drawn in proportion.



Microorganisms in Gum Scrapings





*Bacillus megaterium*

13. Have your lab instructor check your smears and drawings before you leave.

## PART 2
## GRAM STAINING PROCEDURE

The Gram stain is a differential staining procedure discovered by Christian Gram in 1884 when he was staining bacteria in tissues. It is used more than any other staining procedure in general bacteriology. It is very useful for differentiating bacteria having the same shape and size but differing in their ability to retain a crystal violet-iodine complex when washed with a decolorizer (acetone or alcohol). Microorganisms that lose the complex and become colorless after being washed by the decolorizer are called Gram negative. These latter bacteria can be more readily observed if then treated with a stain of contrasting color called a counterstain. The usual counterstain for the Gram procedure is safranin, which is red colored. Thus, at the completion of the Gram staining procedure, Gram positive bacteria are blue or violet colored and Gram negative cells are red colored. Although most bacteria are clearly either Gram positive or Gram negative, pure cultures of some bacteria may be found to contain both Gram positive and Gram negative cells. This is observed most frequently in old cultures of Gram positive bacteria.

The Gram staining procedure will be used repeatedly in the laboratory and a thorough understanding of the different steps in preparing a Gram stain is very important.

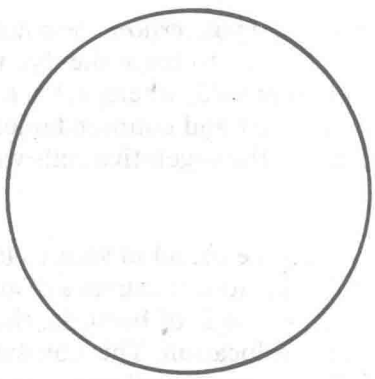
### MATERIALS

1. Slant cultures of *Bacillus megaterium* and *Escherichia coli* (18 to 24 hours old).
2. Crystal violet, iodine solution, acetone, safranin.
3. Microscope slides.

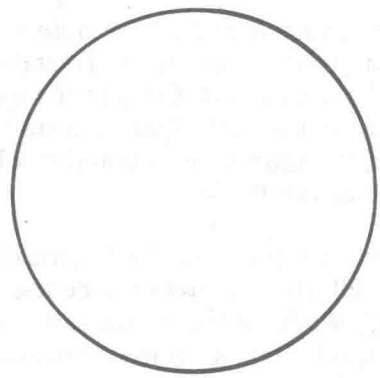
### PROCEDURE

1. On a clean slide prepare two smears, one of each of the microorganisms. Prepare the smears using the same technique you used in Part 1. Allow to air dry, and fix by warming as you did in step 8.
2. Cover both smears with crystal violet (primary stain) for 15 seconds.

3. Wash with water and shake off excess.
4. Cover with iodine solution (mordant) for 30 seconds.
5. Wash with water and shake off excess.
6. Cover the smears with acetone (decolorizer) and agitate slide gently for 10 seconds or until no more color is removed from the smear.
7. Wash with water.
8. Cover with safranin (counterstain) for 30 seconds.
9. Wash with water.
10. Carefully blot dry and examine using the oil immersion lens.
11. Make drawings showing typical shapes and colors of the bacteria.



*Bacillus megaterium*



*Escherichia coli*

## DEMONSTRATION

1. Microscopes with prepared slides of bacteria stained using both simple and Gram stains.

## LABORATORY NOTES (PART 2)

## REVIEW QUESTIONS (PAGE 87)

## Exercise 3

# STAINING OF BACTERIA (Continued)

### PART 1
### BACTERIAL ENDOSPORE STAIN

Some bacteria can form highly refractile and heat resistant bodies called **endospores.** The cell in which an endospore forms is called a sporangium. All true bacteria that form endospores are classified as members of the order Eubacteriales. The most common endospore-formers are members of the rod-shaped genera *Bacillus* (aerobic) and Clostridium (anaerobic) of the family Bacillaceae.

In Exercise 2, Parts 1 and 2, you observed that the spores of *Bacillus megaterium* were not stained by either simple or Gram stains. To stain bacterial spores, it is necessary to force the dye into the spores by the use of heat. Once in the spore, the dye is not easily removed, whereas it is easily washed out of the vegetative cell. Thus, if a stained smear is washed with water and counterstained with a second dye, the spores will retain the color of the primary stain and the vegetative cells will show the color of the counterstain.

The shape of the spore may be spherical, oval, or cylindrical. It may be found in various locations within the cell. Its diameter may be less or greater than the cell. If greater, it causes a distension of the cell wall. While all these various forms may be found in different kinds of bacteria, the spores of individual species remain constant in shape and generally in size and location. This constancy may be helpful in making a preliminary identification of an organism. This exercise will acquaint you with endospore and acid-fast staining procedures.

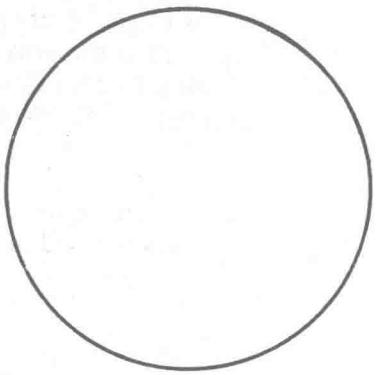
#### MATERIALS

1. Sporulation agar slants of *Bacillus megaterium* and *Bacillus brevis* (48 hours old).
2. Solutions of malachite green and alcoholic basic fuchsin.
3. Inoculating loops.
4. Glass slides.

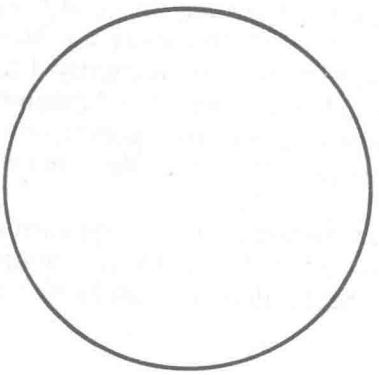
#### PROCEDURE

1. Prepare smears of *B. megaterium* and *B. brevis* on a single slide.
2. Cover the slide with malachite green; heat over a can or beaker of boiling water for 2 minutes.
3. Wash the slide with a gentle stream of tap water for 30 seconds.
4. Counterstain with alcoholic basic fuchsin for 15 to 30 seconds. (Safranin may also be used as a counterstain.)

5. Wash, blot dry, and examine under the oil immersion lens.
6. Make colored drawings of the various stages of spore formation observed with these two organisms.



*Bacillus megaterium*



*Bacillus brevis*

## LABORATORY NOTES (PART 1)