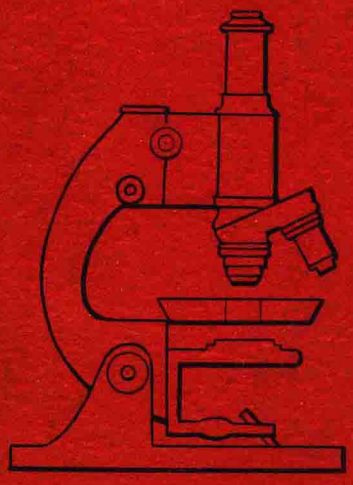
Selected Experiments in Medical Microbiology



BROOKS

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Selected Experiments in Medical Microbiology

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PREFACE

The experiments in this manual have been selected on a basis of simplicity and power to fortify the basic facts of a course in medical microbiology. In contrast to the prevailing practice of most manuals to present the greatest number of experiments, this work presents the least number. Although this is a drastic change, the author believes that he will gain more "friends" than "enemies." A large work is almost never completed in the time allotted. This means that the laboratory work is weakened in respect to its continuity and perspective.

Since each experiment is an integral part of the manual, all experiments should be performed – in the sequence suggested – either by the student, or by the instructor as a demonstration. Only in this manner will the student have a bird's-eye view of the laboratory. Although the manual was written as a companion work to Basic Facts of Medical Microbiology, it is hoped that it can be advantageously employed to accompany any microbiology text suitable to the nursing curriculum and allied areas.

The plan of the manual is simple and direct: Experiments I to X are concerned with general concepts and techniques; Experiments XI to XX cover the gamut of infectious organisms – bacteria, spirochetes, rickettsiae, viruses, fungi, protozoa, parasitic worms and ectoparasites, in that order. Pedagogic experience has indicated that this sequence is both intuitive and palatable to the student.

STEWART M. BROOKS

TO THE INSTRUCTOR

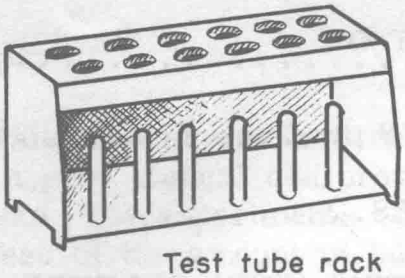
This manual was written for you as well as the student. As a matter of fact, a poor manual can prove more disconcerting to an instructor than his students. The experiments contained herein are basic and ultra simple. Regardless of the amount of laboratory time allotted to you, the entire manual can be easily completed. It is possible, however, if your course is very short, that you may have to demonstrate one or two experiments. Each experiment has a succinct preface. The purpose of this preface is to underline the fundamental nature of the experiment to be performed and its scope. Moreover, the preface should aid in the crystallization of the facts the student has acquired in the classroom. The illustrations throughout the manual have been selected to supplement the written word. In addition, they seem to "fancy up" an otherwise prosaic format. The questions at the end of each experiment have been carefully selected to inquire into the "whys" of the results and the application or implication of these results.

The appendix is strictly for you. It is astounding how much most manuals take for granted when it comes to laboratory materials. The author has tried to include every single item that is needed and to arrange it so that there is no question as to its source, preparation or place. It is hoped that both "new" and "old" instructors will find this to be a refreshing change.

TO THE STUDENT

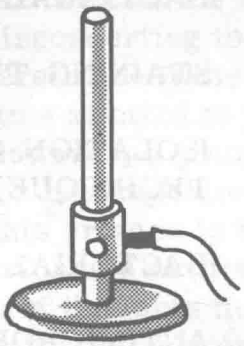
The purpose of this manual is to amplify, embellish and add spice to the theoretical information which you have acquired in the classroom. Very few students indeed do not enjoy the laboratory exercises in microbiology. Every effort has been made in this manual to further reduce this small number. Although the experiments contained herein are unbelievably simple, you should always be thoroughly familiar with each experiment before it is performed in the laboratory. Only in this way will you derive the maximum benefit from this work, enjoy yourself and take pride in your accomplishments.

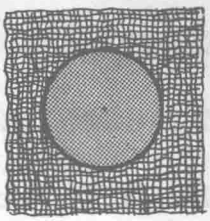
Laboratory equipment needed by the student



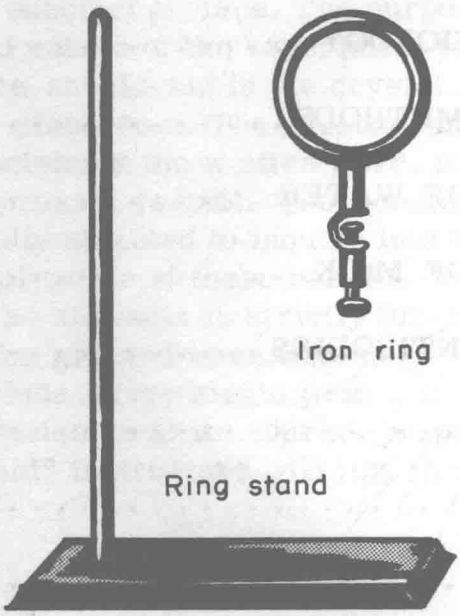
Test tube rack

Bunsen burner





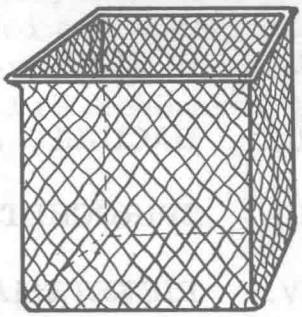
Wire gauze



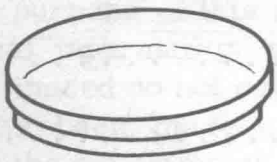
Ring stand

Iron ring

Bacteriological loop

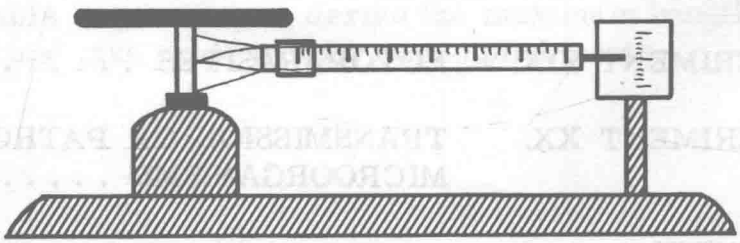


Wire basket

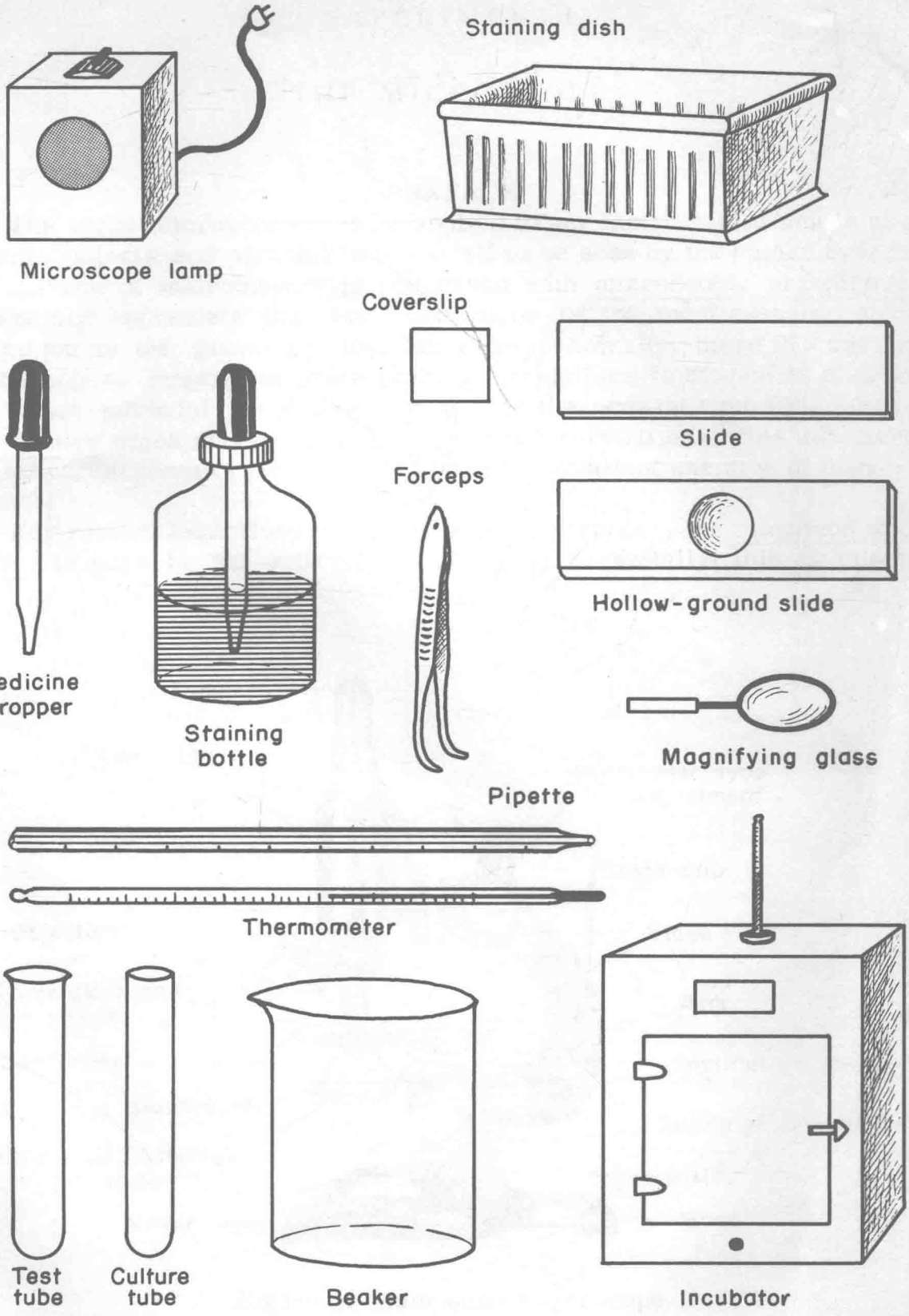


Petri dish

Gram balance



Laboratory equipment needed by the student



CONTENTS

EXPERIMENT I

THE MICROSCOPE

PREFACE

The term microscope may be applied to any instrument which is used to magnify objects and structures too small to be seen by the human eye. Since the science of microbiology is concerned with microscopic organisms, we can easily appreciate that the microscope is the most essential piece of equipment in the microbiological laboratory. Actually, there is a variety of microscopes, ranging in price from a few dollars to thousands of dollars. The most powerful microscope in use at the present time is the electron microscope which magnifies in the vicinity of 100,000 times. This unbelievable magnification permits the scientist to see the smallest enemies of man – the viruses.

For routine laboratory work, and student purposes, the compound microscope (Figure 1) is entirely satisfactory. Essentially, this is an optical

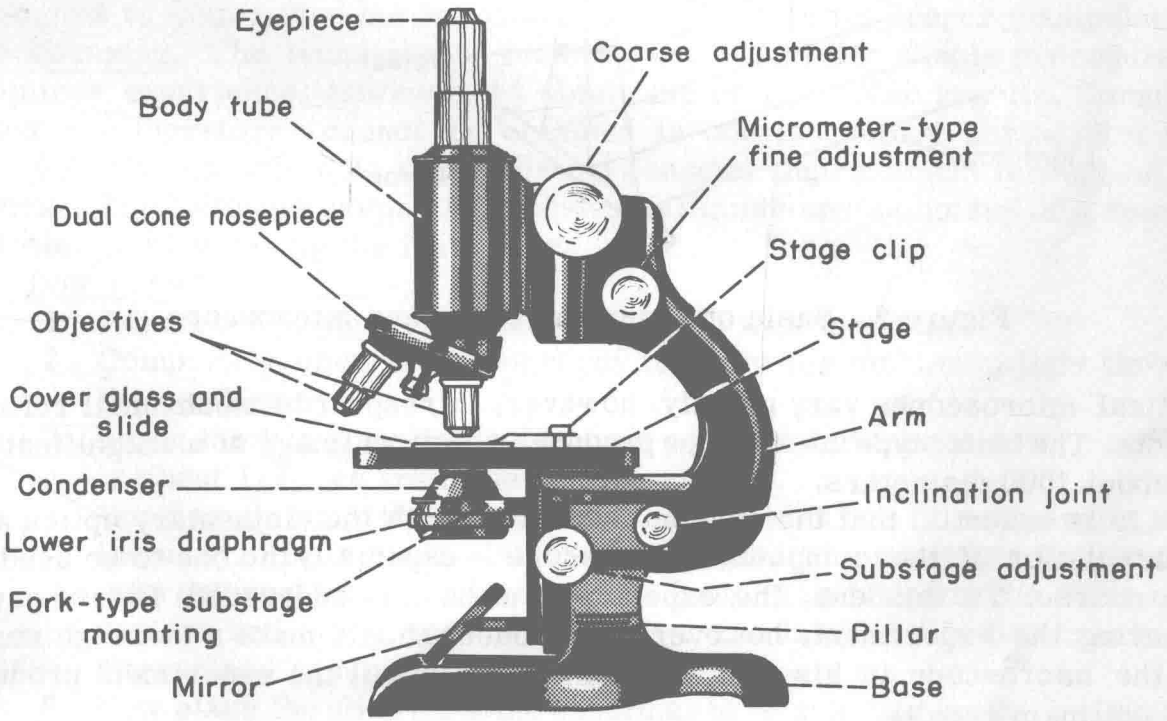


Figure 1. Compound microscope.

instrument composed of two lenses – the objective and ocular. The objective magnifies the object on the slide; the ocular magnifies the image formed by the objective. Thus, the image seen by the eye above the ocular (or eyepiece) represents a double magnification of the object on the slide (Figure 1).

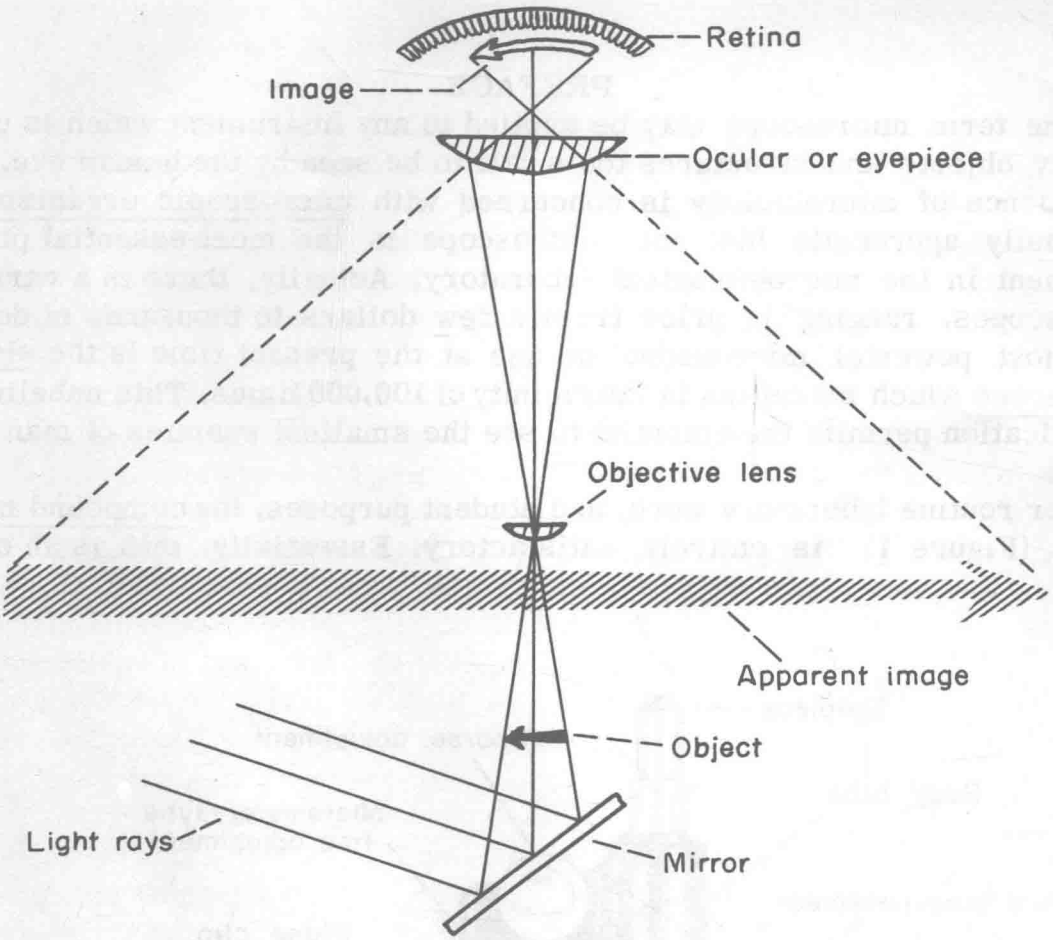


Figure 2. Basic optics of the compound microscope.

Optical microscopes vary greatly, however, in respect to mechanical refinements. The better type microscope produces a distinct image at a magnification of about 1000 diameters.

It is essential that the student understand both the elementary optics and manipulation of the compound microscope – especially the one to be used in the course. To this end, the experiment at hand is addressed. Before commencing the experiment, however, the student should make a thorough study of the microscope in his text. Only in this way will the experiment produce the optimum results.

PROCEDURE

A. Care of the Microscope. Since the compound microscope is an expensive instrument, it demands careful handling and use. The student will not encounter difficulty in this connection provided the following simple rules are judiciously exercised:

1. The body of the microscope should always be kept in an erect position when using wet preparations and when not in use.
2. The stage must always be kept clean and dry.
3. The lenses should be cleaned only with lens paper.
4. Always carry the microscope with two hands.
5. Always place the microscope gently on the desk.
6. Never run the objectives into the condenser lens or slide.
7. Always clean the microscope after using it.
8. When leaving the microscope, always place the low power objective in position over the stage.
9. Never turn the objectives with the course adjustment toward the stage unless you are watching from the side.
10. When you are finished with the microscope, make certain that it is covered either in a case or plastic bag.
11. In the event that the microscope is not functioning properly, immediately secure the aid of the instructor; also, report all damages.

B. Parts of the Microscope. Carefully study the diagram presented in Figure 1A and locate the corresponding parts on the microscope which has been assigned to you. Learn the function of each part and its proper manipulation.

C. Focusing. The technique of focusing is a relatively simple procedure. It requires experience, however, to obtain the best possible results. Complete mastery, therefore, cannot be obtained in one laboratory period. For this reason the student should strive toward constant improvement throughout the course. The following steps are intended as a guide and summary of a demonstration to be given by the instructor.

D. Low Power

1. Adjust the condenser so that it is flush with the stage.
2. Completely open the diaphragm to allow for maximum light through the condenser.
3. Swing the low power objective into position and turn it down so that it is about 1/2 inch from the condenser lens.
4. Looking through the eyepiece, adjust the mirror for maximum light intensity, i.e., a clear circle of white light. (The source of light can be indirect sunlight or a microscope lamp. The latter is the more convenient and sometimes a necessity. The lamp should be close to the mirror and squarely facing it.)
5. Now place the slide (of some specimen to be supplied by the instructor) on the stage and adjust it so that the specimen (or a portion thereof) is directly over the lens of the condenser.

6. Lower the tip of the objective close to the slide – but do not touch the slide.
7. Looking through the eyepiece, slowly move the objective away from the slide using the course adjustment. (In order to avoid eyestrain, the student must learn to keep both eyes open when examining slides.)
8. When the object is focused as clearly as possible, sharpen the focus by using the fine adjustment.
9. Now adjust the light (by adjusting the diaphragm) to obtain the best possible delineation of structures. The correct amount of light is essential.
10. In Figure 3 (below) make a drawing of the microscopic field; go on to the next procedure, leaving slide in position.

Observation:

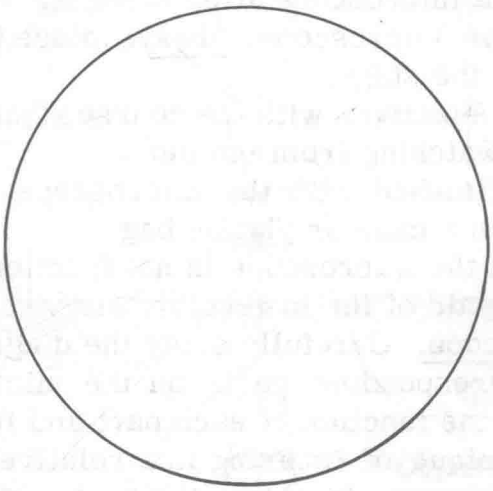


Figure 3. Low power.

Ex. High Power

1. Swing the high power objective into position.
2. Using the fine adjustment, bring the microscopic field into clear focus.
3. Adjust the diaphragm to obtain the optimum degree of illumination.
4. In the space provided below, make a drawing of the microscopic field.
5. Allow the clipped slide to remain in position.

Observation:

Figure 4. High power.

F. Oil Immersion

1. Raise the objective and place a drop of immersion oil on the slide in such a position that it will be directly under the oil-immersion objective.
2. Viewing the procedure from the side, bring the oil-immersion objective into position and carefully lower the tip into the drop of oil barely touching the slide. The tip should be completely immersed and the drop should be free from bubbles.
3. Now, using the fine adjustment, slowly raise the objective to bring the microscopic field into focus.
4. Adjust the light for optimum illumination.
5. In Figure 5, make a drawing of the microscopic field.
6. The instructor will now supply you with a bacterial slide. Starting with a clear field of light (such as explained in steps 1 to 4 under low power) focus with the oil-immersion objective. Repeat several times in order to develop your technique. Make a drawing in Figure 6.

Observation:

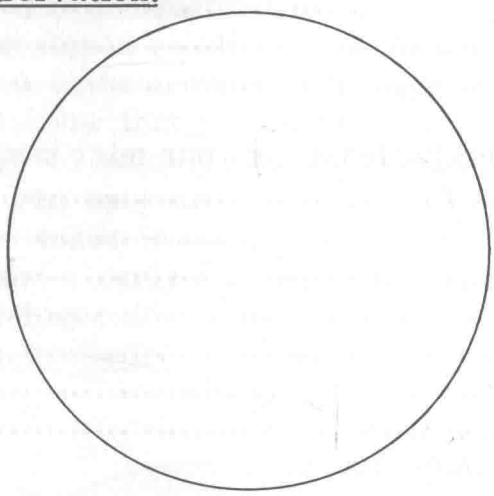


Figure 5. Oil immersion.

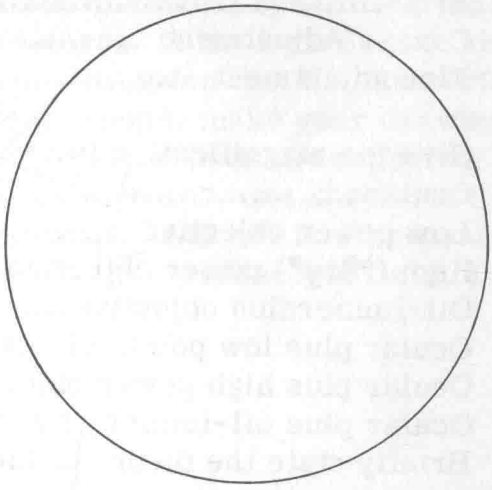


Figure 6. Bacteria (oil immersion).

G. Special Points

1. The oil-immersion lens, which has the highest power, is the most frequently used objective in microbiology.
2. Regardless of which objective is to be used, always obtain a clear field of light (using the low power objective) before starting.
3. The proper amount of illumination is an integral part of focusing. Always adjust the diaphragm, regardless of the objective being used, to obtain the clearest possible field. Often a darkened field will reveal more detail than a light field.
4. When looking at tissue slides, it is best to start with low power. This procedure insures that the microscopist will get an over-all view.
5. Once the specimen on the slide has been brought into focus, the slide should be carefully moved about to disclose as much information as

possible. Oil-immersion preparations will withstand considerable movement provided the slide is moved very slowly. If the microscope is equipped with a mechanical stage, the slide can be moved easily in four directions by turning two screws.

QUESTIONS

1. Give the function of each of the following:
   Ocular .....
   Objectives .....
   Body tube .....
   Stage .....
   Condenser .....
   Diaphragm .....
   Mirror .....
   Inclination joint .....
   Base .....
   Arm .....
   Coarse Adjustment .....
   Fine adjustment .....

2. Give the magnification for each of the following lenses of your microscope:
   Ocular .....
   Low power objective .....
   High ("dry") power objective .....
   Oil-immersion objective .....
   Ocular plus low power objective .....
   Ocular plus high power objective .....
   Ocular plus oil-immersion objective .....
3. Briefly state the theory of the compound microscope.
   .....
   .....
   .....
   .....
   .....
4. Why is oil used with the oil-immersion objective?
   .....
   .....
   .....
   .....
   .....
5. Define:
   Refractive index .....
   Micron .....
   Focal distance .....

EXPERIMENT II

MICROBIAL FORMS

PREFACE

The purpose of this experiment is to impress upon the student the variety of forms and structures found in the microscopic world. Moreover, the student will learn to recognize the principal categories of organisms which will be studied in detail later on in the manual. This experiment, then, is highly instructive. It is essential that as much time as possible be devoted to the study of each specimen.

PROCEDURE

The instructor will give you several slides of microbial specimens. Each is designed to show one or more important features. In the figures provided below make accurate drawings of what you observe. In order that the instructor will know that you have made the correct observations, make your drawings large to magnify what you see. Also, always draw more than one organism of a given specimen. To give you an idea of what is expected, one specimen has been drawn in below. Examine the slide of this organism and see how accurately and clearly the drawing shows what you see in the microscopic field. Try to achieve this type of work.

Observation:

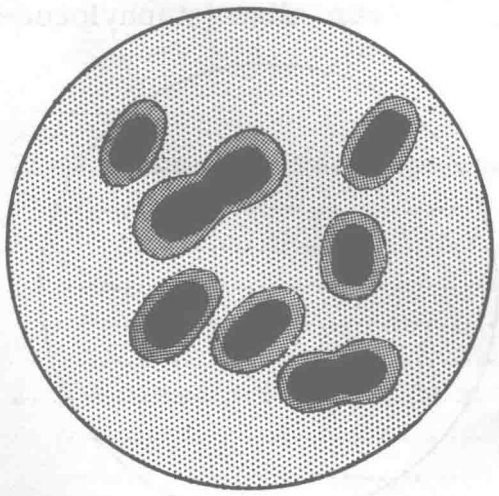


Figure 7. Klebsiella pneumoniae (capsule).

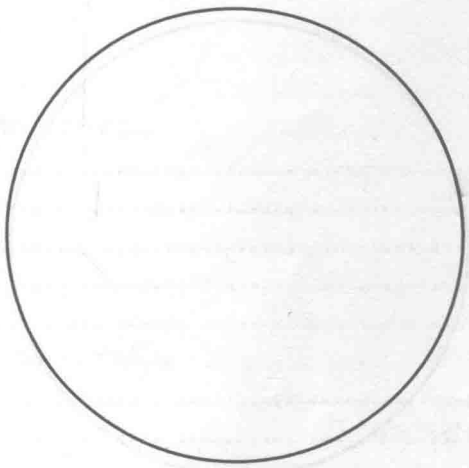


Figure 8. Bacillus subtilis (bacilli with spores).

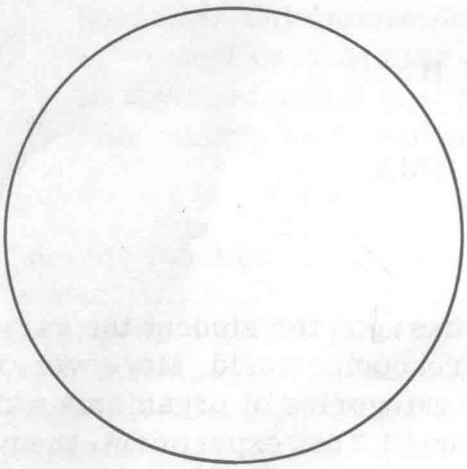


Figure 9. Streptococcus pyogenes (streptococci).

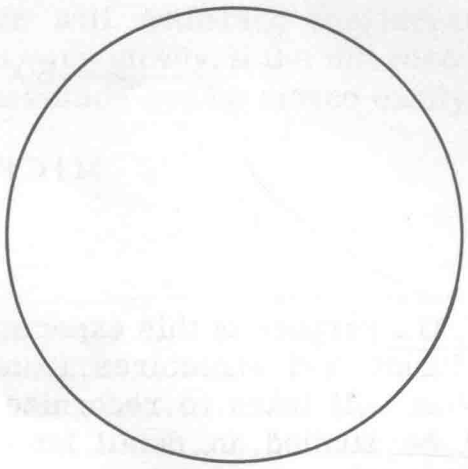


Figure 10. Salmonella typhosa (bacilli with flagella).

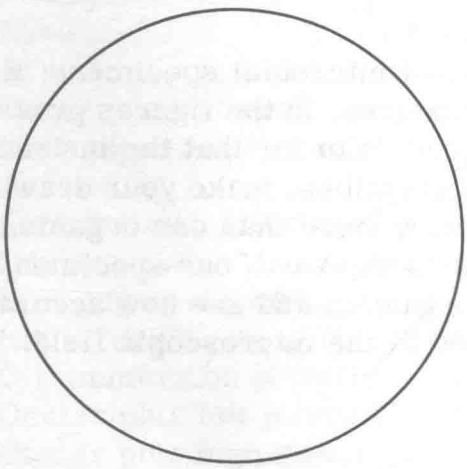


Figure 11. Diplococcus pneumoniae (diplococci).

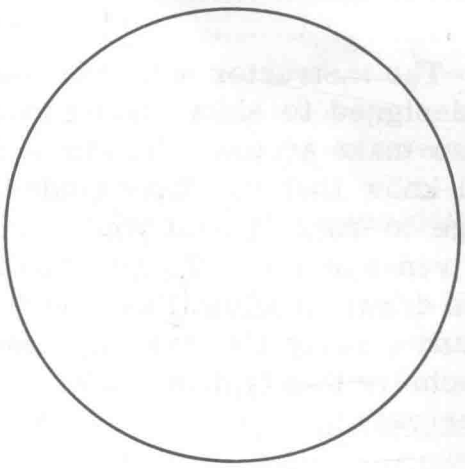


Figure 12. Micrococcus pyogenes var. albus (staphylococci).

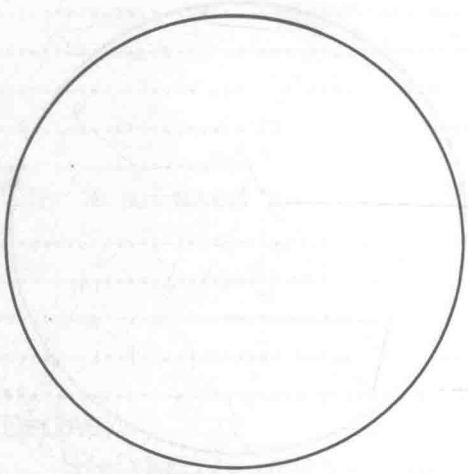


Figure 13. Vibrio comma (spirillum).

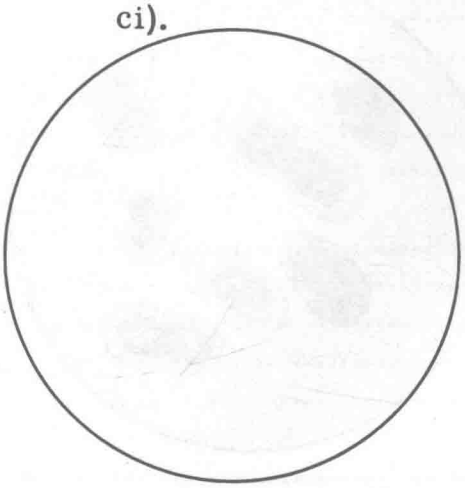


Figure 14. Treponema pallidum (spirochete).

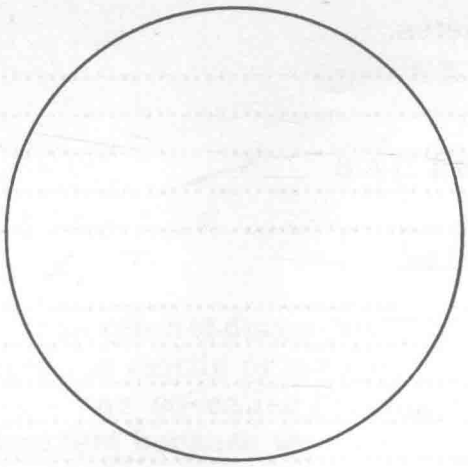


Figure 15. Mycobacterium tuberculosis (actinomycete).

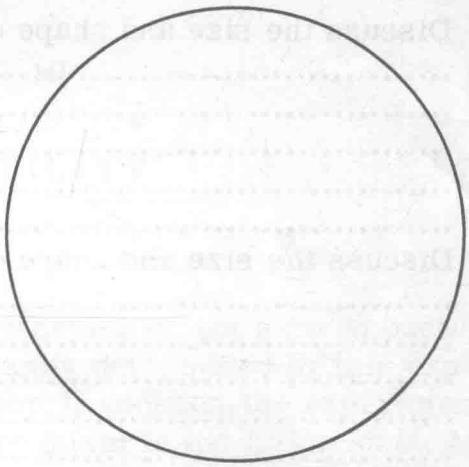


Figure 16. Trypanosoma gambiense (protozoan).

Figure 17. Candida albicans (yeast cells).

Figure 18. Penicillium notatum (mold).

QUESTIONS

1. Discuss the size, shape and groupings of the cocci.

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2. Discuss the size, shape and groupings of the bacilli.

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