PLEASE NOTE THE FOLLOWING ABBREVIATIONS THAT ARE USED IN THIS MANUAL

SL = Glass slides from black boxes in desk drawers and links to digital slides
EM = Electron micrograph in gray envelope in desk drawer (e.g. EM 20-2 means item or area designated 2 in electron micrograph #20)
J = Figure in Junqueira et al., Basic Histology, 11th Ed. (2005) (Note, there is a new, 12th edition, but this manual will reference figures from the 11th edition
R = Figure in Ross and Pawlina, Histology: A Text and Atlas, 6th Ed., (2011)

LABORATORY INSTRUCTORS

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LABORATORY MATERIALS

In addition to this laboratory manual, materials that will be used in conjunction with the laboratory are found within the desk drawer. These include the following:

1. Two black boxes containing glass slides (shared with your partner, in odd numbered drawers only).
2. A gray envelope containing electron micrographs that are referred to with the prefix **EM** in the Laboratory Manual.
3. Lens paper.

In the central drawer that you share with a 2nd year medical student, you should find:

1. a laptop computer
2. a power supply
3. a key to the locker that houses the microscope you will share with your partner and 2nd year students. **ALWAYS RETURN THIS KEY IMMEDIATELY AFTER USE!!!**

The computer will allow you access to the digital version of this laboratory manual. This **Virtual Laboratory Manual** (VLM) is essentially identical to the print version, with the exception that it contains links to images taken from our slides, links to the electron micrographs in your drawers and those that will be posted (see below), and links to digitized (**Aperio**) versions of each slide. The link to the VLM (**http://med.uc.edu/labmanuals/ma/vlm/**) should already be saved as one of your favorites after you open Internet Explorer on your computer.
To use the digitized slides, your mouse will replace the controls on your microscope.

FOR THE PRACTICAL HISTOLOGY EXAMS, YOU WILL LOOK AT GLASS SLIDES ON MICROSCOPES, AND DIGITAL VERSIONS OF SLIDES!!! THEREFORE, WE RECOMMEND THAT YOU BECOME PROFICIENT IN USING BOTH YOUR MICROSCOPE AND THE APERIO SYSTEM. YOU WILL ALSO BE ASKED TO IDENTIFY STRUCTURES ON ELECTRON MICROGRAPHS, AND, POSSIBLY, STILL IMAGES FROM YOUR SLIDES.

Other materials for the laboratory are located as follows:

1. Electron micrographs will be posted for each lab on the corkboards located in each lab. These Ems are also available through links scattered throughout the digital version of this manual.
2. Immersion oil - in each laboratory by the sink.
3. Lens cleaner for microscope lenses - in each laboratory by the sink.

In the Health Sciences Library

1. At the Circulation Desk of the Health Sciences Library there are copies of a supplementary book: An Atlas of Histology, by J. Rhodin that contains many electron micrographs. This book will be useful for review.
2. Histology Image Review, (Appleton and Lange, Electronic Media) on designated computers.

WEBSITES THAT INCLUDE USEFUL LIGHT AND/OR ELECTRON MICROGRAPHS

Internal websites used by our course:

UC-COM, Histology and Cell Biology **http://med.uc.edu/labmanuals/ma/**

http://med.uc.edu/labmanuals/ma/vlm/ (our Virtual Laboratory Manual)
http://med.uc.edu/labmanuals/ma/LabIntroPPt/ (PowerPoint previews of labs)
http://med.uc.edu/labmanuals/ma/emenv/ (micrographs from the EM envelope)

Other digital slide sets:

Virtual Slide Box, University of Iowa
http://www.path.uiowa.edu/virtualslidebox/

University of Michigan
http://histology-umms.org/

Indiana University
http://medsci.indiana.edu/junqueira/virtual/junqueira.htm

The following are websites from other universities:

LUMEN, Loyola U
http://www.lumen.luc.edu/lumen/MedEd/Histo/frames/histo_frames.html

JayDoc HistoWeb, Kansas U
http://www.kumc.edu/instruction/medicine/anatomy/histoweb/index.htm

Blue Histology, University of Western Australia
http://www.lab.anhb.uwa.edu.au/mb140/

Internet Atlas of Histology, University of Illinois at Urbana-Champaign
http://www.med.uiuc.edu/histo/medium/index.htm

Welcome to Histology at SIU SOM, Southern Illinois University
http://www.siumed.edu/~dking2/index.htm

Links to your Histology texts:

Histology: A Text and Atlas by Ross and Pawlina, 6th ed
http://meded.lwwhealthlibrary.com/book.aspx?bookid=767

Basic Histology by Junqueira and Carneiro 12th edition
http://accessmedicine.mhmedical.com/book.aspx?bookid=574

A web search may provide additional resources.

CARE AND USE OF THE MICROSCOPE

I. CARE OF THE MICROSCOPE.

All surfaces between the light source and the eye must be kept clean. The surfaces to be kept as free as possible from dust, foreign particles and grease include the eyepiece lenses, objective lenses, condenser lenses, and the filter and/or condenser of the illuminator. Remove dust and particulate matter with a small soft lens brush. Remove grease and finger marks with lens paper lightly moistened with Kodak Lens Cleaner or similar product. Eyepiece (ocular) lenses must be cleaned frequently during use, since they acquire grease and dust from eyelashes. When not in use cover microscope with a dust cover and set nosepiece on lowest magnification objective. On Olympus microscopes in cloth cases set nose piece with lowest magnification objective pointing away from stage before returning microscope to case. You may keep your microscope in the locker out of the case and store the case behind the microscope.

II. POINTERS.

There must be a pointer in one of your eyepieces if you have a binocular microscope. If you do not have a pointer, please inform the Bookstore. They will need to know your room number and the locker number of your microscope.

III. ILLUMINATION.

If you have an Olympus microscope you should place the microscope with the light switch in toward you and the cord attachment at the rear. Loosen the binocular and turn it around so that the oculars face you.

Your microscope is equipped with a built-in light source; proper direction and maximum intensity of light are already provided.

IV. USE OF THE CONDENSER AND IRIS DIAPHRAGM.

With the eyepiece removed, look down into the tube. Find the lever that controls the movement of the iris diaphragm. Move the lever in either direction to determine the extent of its excursion. When the diaphragm is partly closed, the spot of light should be in the center of the field. Finally, change the objective to 10X (low power) then adjust the size of the opening so that the image of the iris diaphragm just fills the field. Replace the eyepiece.

Next, focus the microscope on any tissue on a glass slide. Then note the effect of moving the condenser up and down with the knob under the specimen stage. The light becomes dimmer as the condenser is moved down, and brighter as it is moved up. Finally, raise the condenser to the point where the light source is in focus at the same time as the tissue on the glass slide (about 1 or 2 millimeters below the slide on the stage). Adjust the condenser slightly so that the image of the light source (as well as dust on the light source) is slightly out of focus and leave condenser in this position. You are now ready to begin your study of a section on a slide.

The above procedure may be carried out at the beginning of each period of slide study. After a few trials, it can be completed in a matter of seconds. The setting described should generally provide adequate illumination. However, it probably will be necessary to modify adjustments somewhat, depending on the magnification of the objective which is being used, the thickness of the section or slide, the intensity of the stain in the section, and other factors. The lower the magnification, the brighter will be the field of light, and vice-versa. For example, the setting as described may be optimal for use with a low power objective, but this amount of light may be too bright for the scanning objective, and too dim for the oil immersion objective. If adjustment is necessary, regulate the amount of light by the rheostat on your light source. If you do not have a variable light source, slight adjustment of the iris diaphragm and/or position of the condenser will reduce the amount of light. However, excessive adjustment of these controls will deteriorate the image. Actually,

conditions must be adjusted for every slide. Work with the light until the best possible definition of the section is obtained.

V. ADJUSTING BINOCULAR EYEPIECES**.**

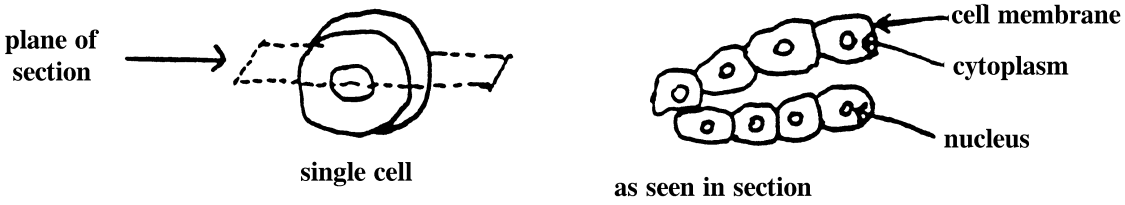
First, place a slide on the microscope stage and focus on any tissue. Next, adjust the interpupillary distance. With your eyes in position for looking into the microscope, change the distance between the two eyepieces until the two circles of light are superimposed, that is, until you see a single field. It may take a little practice to fuse the two images.

The second adjustment compensates for any difference in focusing between the two eyes. In most microscopes, one eyepiece tube is fixed and the other is adjustable. Close off the view to the adjustable eyepiece by holding a piece of paper over the top or by closing the eye. With the other eye, look into the eyepiece of the fixed tube, and focus with the coarse or fine adjustment until the image on the slide is sharp and clear. Now switch the paper to the fixed eyepiece to cover the view into the fixed tube and look at the image of the tissue through the adjustable eyepiece. By means of the adjusting mechanism on this eyepiece, focus until a sharp image is obtained with this eye. Note the setting on the scale when this is completed. Check the setting before each period of study.

VI. PROCEDURE FOR STUDYING A SECTION.

A. USE OF THE SCANNING OBJECTIVE**.** The purpose of the scanning objective is to enable the observer to view a larger field at a low magnification. It is useful for locating a desired area quickly, or for a general survey of a section to see what is present, or for an overall view of an organ. After a tissue or an organ has been studied at higher magnifications, it is advisable to return to the scanning objective and restudy the structure from this point of view.

Remove slide SL. 103 from the slide box, make sure that it is clean, and place it on the stage of the microscope right side up (cover slip on top). Place the slide so that the larger piece of tissue is under the objective. Using the scanning objective, bring the tissue into focus with the coarse adjustment. If the image is not sharp and clearly defined, adjust the light with the iris diaphragm or substage condenser. Move the slide around and note several areas that contain tissues with different appearances. The larger masses look "speckled" with blue dots. This is a thin section of liver that consists in large part of plates of hexagonal cells. This primarily two-dimensional image is composed of these cells cut into very thin slices (6 micrometers, µm).



At this magnification one sees clearly only the nuclei (numerous small blue dots) in an indistinct background.

B. USE OF THE LOW POWER**.** Move the slide so that the tissue is in the center of the microscopic field. Swing the low power objective around until it locks into place. Focus again, adjust the light, if necessary, and note that now you can see both nuclei and cell outlines.

C. USE OF THE HIGH POWER. Select a good area of the tissue and move the slide so that this area is in the center of the microscopic field. Focus carefully. Now, without changing the focus, carefully swing the high dry objective around and look at it from underneath to be sure that it does not hit the slide. Most microscopes are equipped with objectives that are parfocal, i.e. one can change from one objective to another without losing focus. Therefore, one can change objectives without refocusing the course adjustment. When the high dry objective has been locked in place, look through the eyepiece and focus (up first) with the fine adjustment until the image comes into sharp view. Adjust the light again if necessary. Cell outlines are now more sharply defined although the demarcation between cells may not be distinct in all areas, faint granules are visible in the cytoplasm, nuclear outlines are distinct, and particles (chromatin) are seen in the nuclei.

D. USE OF THE OIL IMMERSION OBJECTIVE. With the high dry objective, select the field to be studied and center it. Swing out the high dry (without changing the focus), place a drop of oil on top of the slide, swing the oil objective carefully into place, focus up, then down, if necessary, to bring the image into view. If the field is too dark, proceed as above by opening the iris diaphragm. Note increased detail of cell structures indicated above can be seen at this high magnification.

Some points to consider with failure of the object to come into focus with high power.

(a) The slide may be upside down; the cover slip should always be up.

(b) The fine adjustment is at the end of its excursion. Turn it several times in the opposite direction, refocus with the coarse adjustment and then focus the image again with the fine adjustment.

(c) If using the high dry objective, check and see that the lens has not been accidentally smeared with oil.

d) Some microscopes have a safety lock, preventing the objectives from lowering sufficiently to obtain focus. This must be released for use.

When you have completed your study with the oil immersion objective, swing the nosepiece back to the scanning position. Wipe the immersion oil off of the objective and off the slide with a clean dry cloth or lens paper.

E. **NOTE AT THIS TIME ANOTHER IMPORTANT METHOD OF OBSERVING SECTIONS.** Your eyepiece may be used as a magnifying glass in the following manner: Remove one eyepiece, invert and hold the top near the slide to be examined. Looking through the bottom of the eyepiece (the distance you hold the eyepiece from your eye will vary with the individual) you can obtain a general overall view of the section. This method is especially useful in later studies involving organ identification.

I. INTRODUCTION TO THE CELL

Basically the human body is constructed of cells that are surrounded partially or completely by microenvironments of various compositions. Cells are collections of various smaller units; some of them limited by membranes, others free in the cytoplasm. Their components, shapes, sizes, and functional conditions vary from place to place within the body as well as from time to time in the life span of the individual.

Initially, in the course we will consider cell biology in general without regard to any specific cellular type or location. Later, as specific tissues and organs are studied, you will be focusing your attention upon the particular cellular components that are especially important for each tissue or organ.

Because of the limitations of the light microscope and the rigors of techniques for tissue preparation, the student must integrate light microscopic, electron microscopic, histochemical, and biochemical information in order to gain an understanding of cell biology. You should examine your microscopic slides, your biochemistry and histology texts, and electron micrographs with this need for integration foremost in your thinking.

You already have identified those general aspects of the cell that are visible readily in a tissue stained with hematoxylin and eosin. Through the use of special techniques on a variety of cells it is possible to identify additional components. The histologist sometimes divides these components into two groups on the basis of whether or not they are parts of the cell's metabolic machinery. Those that are parts of the cell's machinery are called organelles (little organs); those that aren't are called inclusions. Before continuing with the study of the cell be sure you know what H & E, basophilia and acidophilia mean. Read the following explanation, if you are at all uncertain regarding these terms.

II. STAINING

Biological stains are used to "dye" various subunits of cells and tissues to give contrast to the various components so that they can be visualized under the microscope using ordinary light. Special microscopes and techniques are available for observing unstained living cells. By known chemical reactions and sometimes empirically by trial and error, various dyes have been shown to bind with specific cellular components. This binding is predictable when there is a particular set of conditions of pH, temperature, fixation, etc. so that by combining different stains, meaningful descriptions of histological structures may be made. If the reaction is made on the basis of a specific chemical reaction the process is called histochemistry. By this means localization of specific compounds such as carbohydrates, glycosaminoglycans, proteins, DNA, RNA, and a variety of enzymes, etc. can be made. However, a large number of staining procedures relies on the generalized ability of proteins and other compounds to dissociate either as cations or anions. Most proteins are amphoteric, dissociating as both, but within set conditions of pH one or the other will predominate. The various dyes also contain radicals that dissociate in a like manner so that electrostatic forces play a predominant role in most staining reactions. Dyes are structured such that one portion, the chromomere, is a colored compound. This is combined chemically with a radical that can dissociate, the auxochrome. The two together are often referred to as the chromogen. Thus in staining a tissue the proteins dissociating with a net positive (+) charge will combine with the auxochrome of dyes dissociating with a net negative (-) charge. Some proteins will not dissociate so another agent, usually a heavy metal, is bound to the protein first and this in turn reacts with the dye: this is called mordanting and the dye is a mordant dye.

In histology there is a common terminology that is used to refer to the interaction of dyes and structural components. Structures with proteins having a net negative charge are called acidic; those with a net positive charge are referred to as basic. Similarly, dyes with a net negative charge are designated acidic, those with a net positive charge are basic. Thus an acidic structure (net - charge) will react with a basic dye (net + charge) and the stained structure is described in histological lingo as being basophilic (base - loving). Conversely, acidophilic (acid - loving) structures are those having a net positive charge and are basic. Not all dyes react in this manner but a majority does, and it has become common usage in histology to refer to structures in this manner. Some dyes bind by other than electrostatic forces, but are predictable in their reaction so become useful markers.

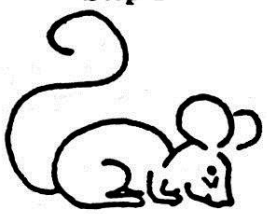
The staining of "fats" differs from the staining of other cell constituents. Since organic solvents are used in most routine histological procedures, the neutral fats and many other lipids (fat-like substances) will dissolve out of the tissue. Using ordinary staining procedures the site of lipid will be observed as unstained vacuoles. To demonstrate fats, it is usually necessary to use sections of frozen tissue (tissue frozen - sections cut - mounted) and thus avoid organic solvents. The fats can then be "stained" with dyes which are soluble in the fat to give color to the location of these fats. Examples of stains for neutral fats are osmic acid, Sudan dyes and oil red O. Special techniques must be used to demonstrate other varieties of lipids. It can be noted that in the preparation of tissues for electron microscopy osmic acid is often used as a fixative or stain; thus fats are readily visualized in electron micrographs.

Throughout the course and in pathology the predominant stains used are hematoxylin and eosin, H & E. Hematoxylin for practical purposes can be considered to be a basic dye and structures stained with it are blue and are said to be basophilic. The outstanding structure stained with hematoxylin is the nucleus. In contrast, eosin is an acid dye and stains many of the cytoplasmic components red or pink. The structures stained with eosin are referred to as being acidophilic.

This discussion serves to give a basis to the manner in which tissues can be observed. Several other techniques and special stains will be described during the course.

TISSUE PREPARATION FOR LIGHT MICROSCOPY

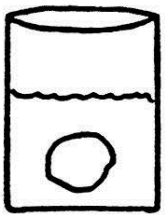
Step 1



Animal - autopsy

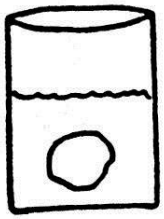
Small piece of organ cut out

Step 2



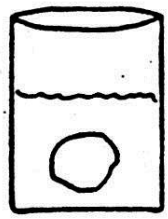
Place immediately in a fixative-designed to kill, precipitate protein and prevent autolysis

Step 3



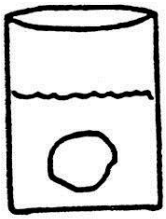
Fixative washed out with water

Step 4



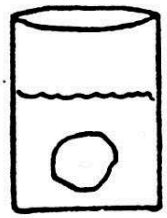
Tissue run through a series of alcohols from 50% to 70% to remove most of the water

Step 5



1/2 100% Alcohol
1/2 Organic Solvent

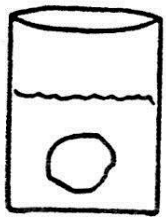
Step 6



100% Organic Solvent

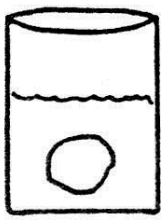
Step 5-7 replace alcohol with agent miscible with paraffin

Step 7



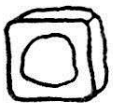
Mixture organic solvent and melted paraffin 60°C

Step 8



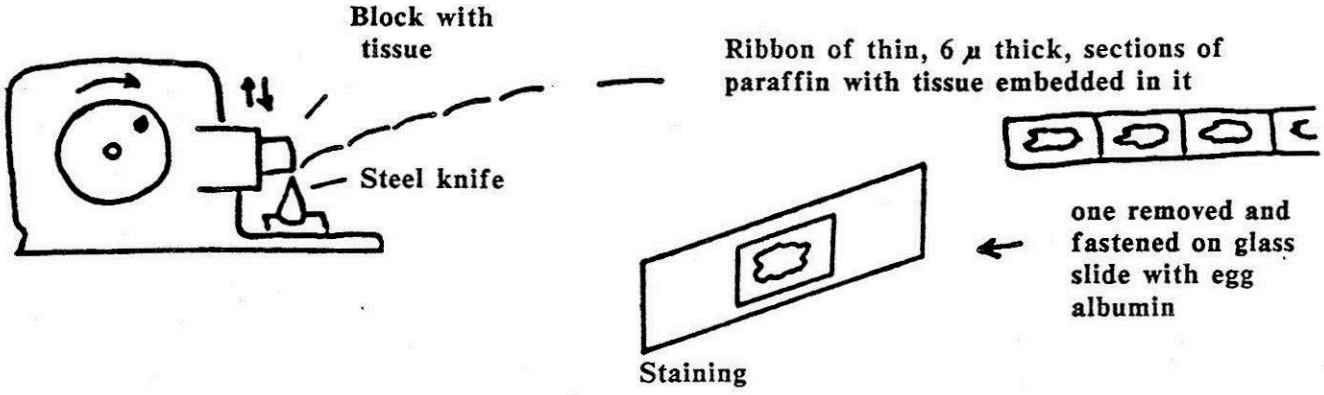
Melted paraffin

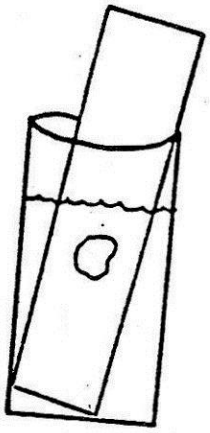
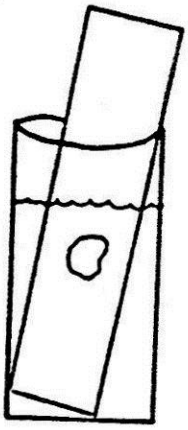
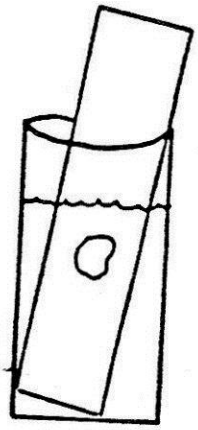
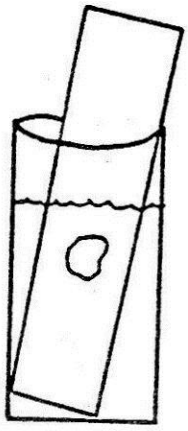
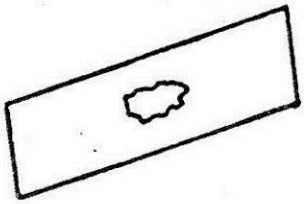
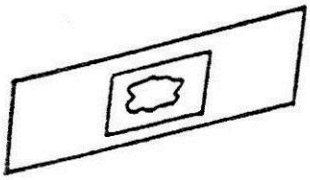
Step 9



Embed in block melted paraffin and harden in cold

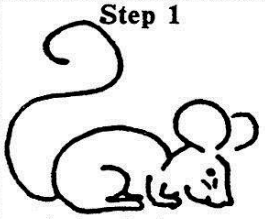
Steps 8-9 infiltrate with material hard but pliable, capable of being cut into thin slices or section



 Immersed in organic solvent to remove paraffin	 Run through a series of graded alcohols from 100% to water or 70%	 Stain one, two or more steps with washes between
 Graded alcohols 70% to 100% to organic solvent. Removes water and makes translucent to light	 Remove add drop of mounting media	 Place on coverslip and the tissue is ready to be observed. The object is the actual image to be observed or photographed

TISSUE PREPARATION FOR ELECTRON MICROSCOPY

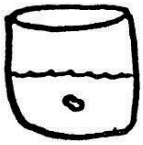
Step 1



Animal, Autopsy or Surgery

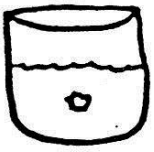
Small piece of tissue 1 x 1 mm

Step 2



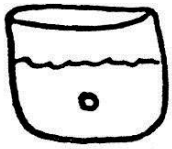
Fixative

Step 3



Series of solvents for dehydration

Step 4

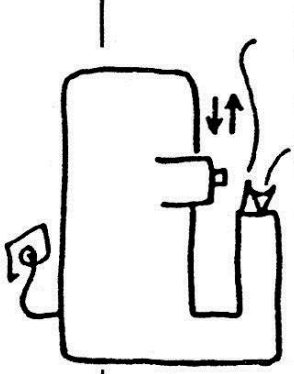


Graduate series of dilute to concentrated plastics

Step 5

Tissue in hardened plastic

Step 6



Thin sections (50-80 nm) of tissue in plastic floated on water

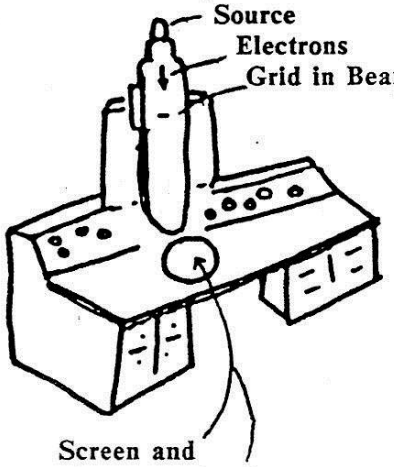
Step 7

Single sections placed on small copper grids

actual size of grid

Section coated "stained" with a heavy metal to give contrast

Step 8



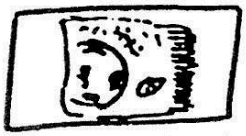
Screen and photographic plate Grid placed in microscope

Heavy metal coat ("stain") in the tissue, varying amount and distribution according to tissue, absorb or deflect electrons

Step 9

Photographic plate captures "negative" image of tissue which will be revealed when developed

Step 10



Photographic prints made and are the materials to be studied. By printing the image on photographic paper the image is converted to a "positive" image. The actual image is not elaborated in Transmission Electron Microscopy