Tuesday 12th October PY2133 + 34

BC AL/336/3/16

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General directions for practicals

Physiology is a practical subject and your practical work contributes not only to your course mark, but also to your better understanding of the theory.

Arrive promptly for the class as instructions are given at the beginning of the practical. Read any schedules which you are given before commencing the experiment. Remember that under the Health and Safety at Work Act you have a responsibility for your own and others safety whilst in the laboratory, so behave sensibly. Do not eat, drink or smoke in the laboratory unless instructed to. If you have any queries ask the Demonstrator for assistance, do not copy what someone else is doing.

During the experiment keep a protocol giving details such as age, height and weight of the subject, times at which readings are made, concentrations and volumes of any drugs given. This information must be recorded during the experiment.

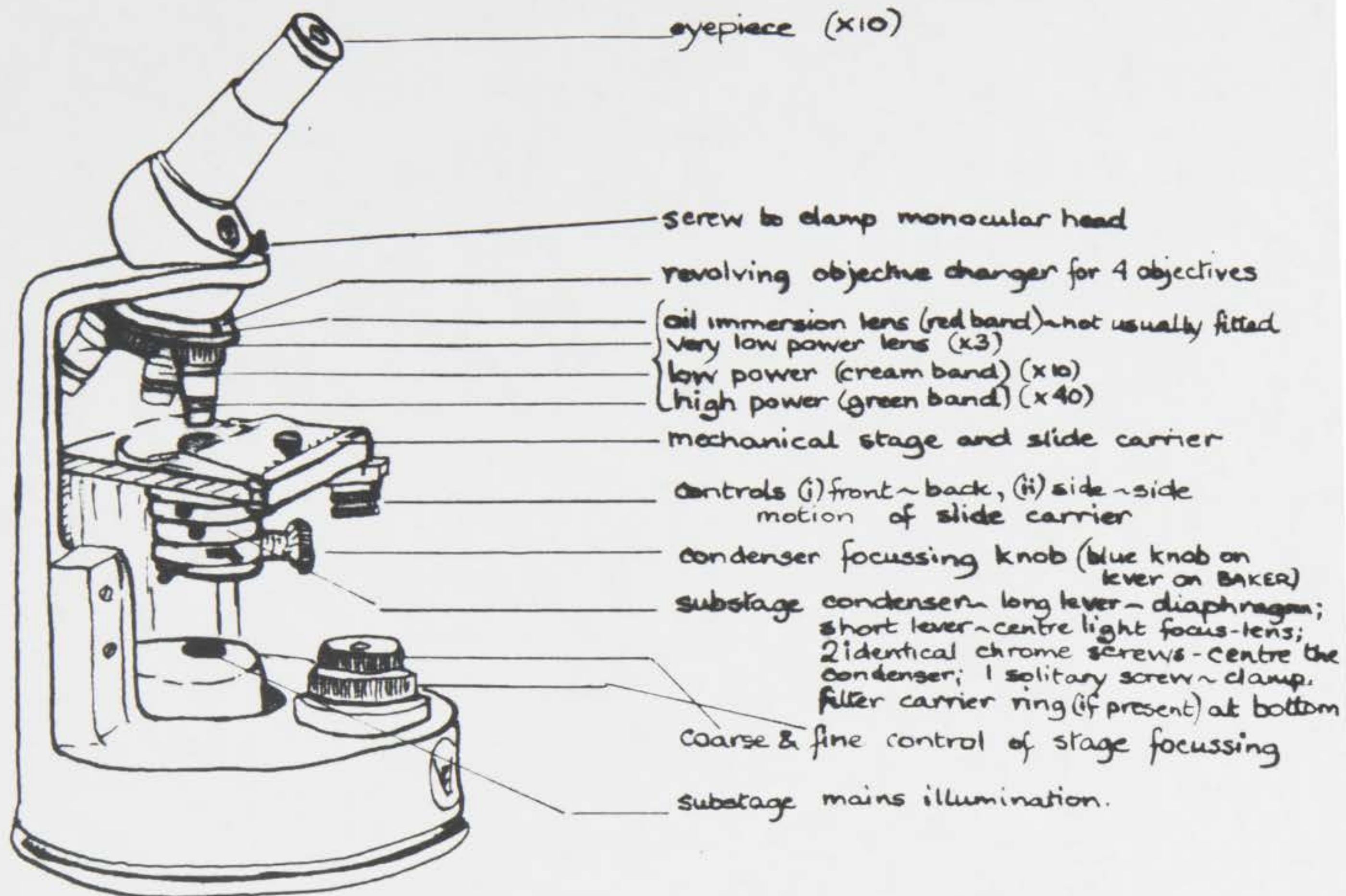
Before finishing make sure that you have all the results required of you. Where possible, you should have your own copy of any traces produced. Tidy up the bench before you leave, remove any pieces of tissue from the apparatus and pour away any solutions you have been using.

Practicals should be written up the same day as the class and books provided with squared paper should be used. The record will normally consist of:

a) Title and date of experiment.
b) Introduction - a brief description of the purpose of the experiment.
c) Apparatus - a labelled drawing of the apparatus.
d) Method - a description of the experimental procedure. If a schedule is provided this may be used instead, but any deviations from it must be noted.
e) Results - a list or table of your results together with any calculations or graphs. If you have any experimental traces, these must be stuck in securely opposite the relevant description.
f) Discussion - a consideration in your own words of the results. Were they as expected? If not, why not? What errors are there in your method? What can be deduced from the experiment? A short description of the theory behind the experiment should be given as should any accepted values for the parameters you have measured.
g) Conclusion - a summary of the experiment in two or three senstences.

N.B. Books should be placed in your tutor's pigeon-hole for marking on the dates indicated in the time-table.

MICROSCOPE (VICKERS or BAKER)



The microscopes in the Department are of the type where the focussing is achieved by movement of the stage, and the knob for this is on the base of the microscope (lower one coarse, upper one fine adjustment).

Place a slide on the stage so that it is held in place by the spring clip, and using low power (x10) objective, focus it.

ALWAYS focus specimens beginning with the stage and objective close together and rack the stage DOWN to prevent crashing objectives into specimens.

The condenser should now be brought up so that it is almost in contact with the slide and in this position, the light source filament may be seen in focus on the specimen. The condenser may then be lowered fractionally to prevent this.

If the field of view is not fully illuminated, move the top lens aside (small lever). The iris diaphragm should now be closed just enough to prevent glare. With higher power objectives, the diaphragm will have to be adjusted again. The more the diaphragm is closed down, the lower the resolution, so use it as wide open as possible.

Be careful when using condenser controls (a) not to unscrew the clamp screw thus allowing the condenser to fall out and (b) to avoid altering the centring screws.

Always select field of view with lower power objectives before proceeding to higher magnifictions.

Introduction to Histology

Histology is the study of tissues which make up the structure of the body. Specimens may be fresh, e.g. blood smear, teased nerve cells, frozen sections or sections cut from material fixed and embedded in some supporting medium. There are advantages and disadvantages in each of these and all are used as appropriate. Material is almost always stained in order to distinguish the different tissues or cells or even constituents of individual cells..

In the first practical, we shall compare two tissues in the fresh, unstained state with the fixed, stained sections of the same tissues.

1. Pancreas

A) Stained section shows an exocrine gland with secretory acini, (acinus - group of secretory cells clustered around central duct) ducts, connective tissue, blood vessels etc and also endocrine islets of Langerhans, which secrete insulin and glucagon. The exocrine secretory cells are roughly wedge-shaped and also visible are the nuclei near the base of the cells and zymogen granules near the apex. Draw H.P. of an acinus.

B) Fresh tissue - a very small piece from young rat pancreas placed on a slide with a drop of 0.9% NaCl and squashed very gently with a coverslip. Examine and draw H.P. Notice that the cells are in clusters or balls (i.e. in 3-D) and nuclei are hardly visible, since their refractive index is similar to that of the cytoplasm. Zymogen granules are visible - gathered round the ducts.

2. Ciliated Epithelial Cells

A) Stained section of trachea - innermost layer is of ciliated, pseudostratified columnar epithelium. Draw H.P.

B) Fresh cells from frog buccal cavity. Use a blunt instrument e.g. a spatula to take a scraping from the pharynx of the freshly killed frog and place on the slide with a drop of 0.6% NaCl. Cover with coverslip, examine and draw H.P. Notice the movement of the cilia which may cause the groups of cells to roll over and over. Are nuclei visible?

What advantages does fixing and staining have? What features are lost?

Staining of Paraffin Sections to show General Structure

Suitable small pieces of material taken from a freshly-killed animal are placed in fixative to prevent post-mortem changes. (Fixatives act by precipitating proteins, including enzymes). After sufficient time for the fixative to penetrate, the material is washed, then gradually dehydrated by passing through alcohols of increasing strengths and put into a clearing agent (so that light may pass through). It may then be embedded in a supporting medium like paraffin wax. Sections are cut usually around 10 µm thick.

To stain a ready-mounted paraffin section with Haematoxylin and eosin:

1. Remove wax with xylene (section may be very gently warmed on microscope lamp). Place slide on carrier over bowl, add few drops xylene, tilt and pour off.

2. "Take to water". (Gradual hydration of section). Add a few drops of absolute (100%) alcohol (section will turn opaque) tilt and pour off. If any wax remains, repeat xylene step and then absolute alcohol. Repeat with 95%, 70% and 50% alcohols and finally distilled water.

3. Stain with haematoxylin (make sure section well covered) for 10 minutes. This stains nuclei strongly and cytoplasm less so.

4. "Blue" in tap water (rinses out excess stain, and being alkaline turns colour blue and mordants it).

5. Counterstain with eosin ½-1 minute. Wash briefly in distilled water.

6. "Dehydrate" i.e. wash successively in 50%, 70%, 95% and absolute alcohol, repeating absolute alcohol.

7. "Clear" (i.e. make transparent) in xylene. If any trace of milkiness appears, water is present and the absolute alcohol step must be repeated then xylene.

8. Mount using a drop of Clearmount and being careful to exclude all air bubbles.

Nuclei - blue-purple, cytoplasm - pink, blood - red, muscle - reddish-pink.

This method can be conveniently abbreviated as follows:

i) Take to water (this includes the xylene step)
ii) Stain with haematoxylin - 10 mins and blue
iii) Counterstain in eosin - ½-1 min
iv) Dehydrate, clear and mount.

Another general stain which is often employed is Iron Haematoxylin and van Gieson where nuclei appear greyish-blue, connective tissue-brilliant magenta pink, blood-yellow and muscle-orangey:

1. Take to water.

2. Stain in fresh iron haematoxylin - 10-20 minutes and blue.

3. Counterstain van Gieson - 5-10 minutes.

4. Dehydrate, clear and mount.