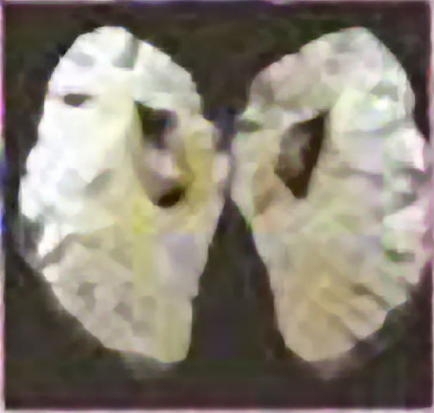
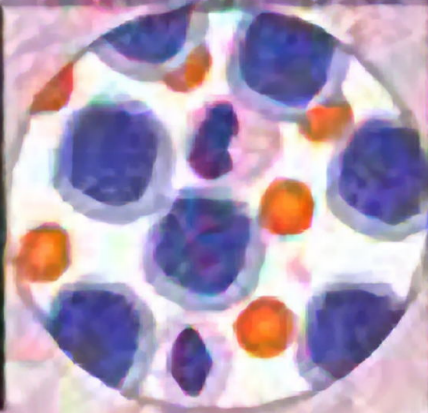
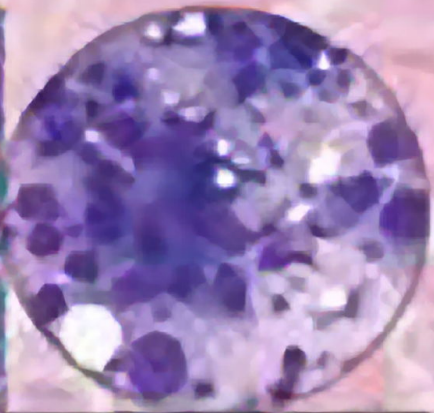
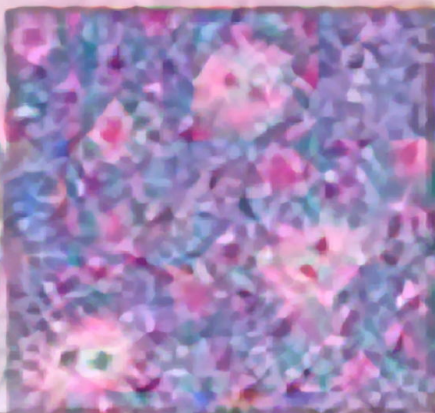
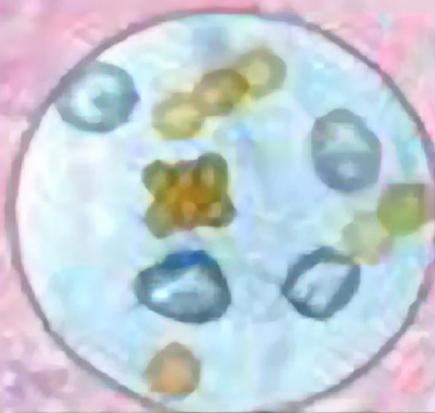


PATHOLOGY PRACTICAL BOOK

Harsh Mohan



Second Edition

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JAYPEE

PATHOLOGY PRACTICAL BOOK

PATHOLOGY PRACTICAL BOOK

SECOND EDITION

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Pathology Practical Book

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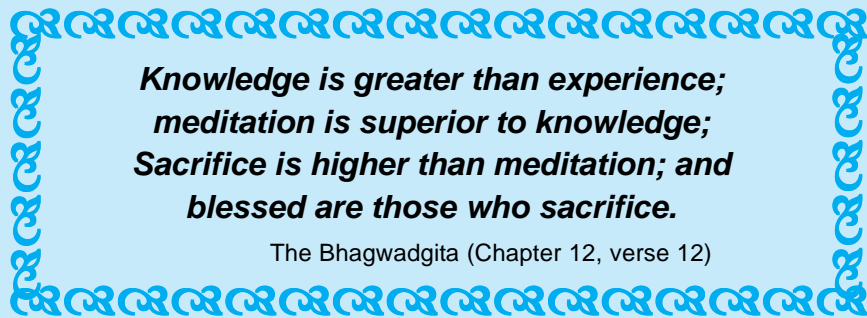
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***Knowledge is greater than experience;
meditation is superior to knowledge;
Sacrifice is higher than meditation; and
blessed are those who sacrifice.***

The Bhagwadgita (Chapter 12, verse 12)

To my wife Praveen,
for her profound love and devotion;
and our daughters: Tanya and Sugandha,
for their abiding faith.

Preface to the Second Edition

The revision of **Pathology Practical Book** (first published in 2000) had become overdue because 5th edition of my *Textbook of Pathology* has already been there with users since 2005, and it is imperative that the practical book parallels in advancements of material and presentation with its senior counterpart. The aim for the revised edition remains the same as in the previous edition of practical book and is outlined below:

Firstly, there have been several voluminous reference books and coloured atlases on various aspects of laboratory medicine separately such as on techniques, clinical pathology, cytopathology, general and systemic pathology, haematology and autopsy pathology etc; each one of them deals with these subjects in fair detail but generally remain much beyond the requirements and comprehension of undergraduates.

Secondly, I have learnt from my own experience in teaching over the years as well as known from colleagues by mutual discussion that there is quite a lack of uniformity in teaching of practical pathology to undergraduates in different institutions within the country as well as amongst different staff members within the same department in an institution— some teaching ‘too much’ in the limited time meant for learning skills while others teach ‘too little’.

Thirdly, it is observed that in order to learn practical pathology, the students bank upon main Textbook of Pathology which is in no way complete as regards requirement for practicals in pathology for undergraduates are concerned; hence the need for a comprehensive text of Pathology Practical Book.

Some of the *Key Features* of the Second Edition are as follows:

Organisation of the Book: The revised edition of the book is divided into seven sections namely: Techniques in Pathology, Clinical Pathology, General Pathology, Systemic Pathology, Cytopathology, Haematology and Autopsy Pathology, besides Appendix on Normal Values. Each section is independent and self-contained and is preceded by a page of *Section Objectives* and *Section Contents*, besides highlighting outstanding contribution of an eminent Pathologist in that subspecialty to stimulate the interest of students in the history of Pathology. The basic style of presentation in the revised edition has been retained, i.e. exercise-based teaching as would happen in the routine weekly pathology practical class of undergraduate students. These exercises are further systematically organised based on organ systems and topics.

Expanded and Updated Contents: Present edition of the book has 58 Exercises compared to 53 in the previous edition. Besides, there have been changes and insertions of newer slides in many exercises. These additions were considered essential keeping in view the contemporary concepts on learning of basic pathology of diseases. The material in each exercise has been thoroughly revised and updated laying emphasis on further clarity and accuracy of the text and images. The book lays emphasis on honing of practical skills in the students for laboratory techniques and on learning gross and microscopic pathology. Thus, the description of the topic/disease is largely on applied aspects while theoretical details have been kept out so as not to lose the main focus.

Figures: All the illustrations in the revised edition of the book are new and are more numerous now; all these are now in colour. Previous black and white line sketches of gross pictures have been replaced with clicked photographs of representative museum specimens. Likewise, all the changed photomicrographs have new corresponding coloured and labeled line sketches across them. There are also many additional photos of instruments commonly used in a modern pathology laboratory. These major changes coupled with digital technology in photography have enhanced the readability and have given a pleasing look to the book.

CD on CPCs: Another innovative feature of the revised edition is addition of a chapter on Clinico-pathologic Conferences (CPCs) and its corresponding CD containing ten structured CPCs. Since CPCs are included in the curriculum of undergraduate students, it was considered prudent to include ten CPCs pertaining to different organ

systems in CD format with the book. Each of these ten CPCs on the CD is a corollary of a case and includes its clinical data, pathologic findings at autopsy including pictures of organs and corresponding microscopic findings, and is concluded with final autopsy diagnosis and cause of death in a particular case.

In essence, the new edition provides a wholly revised material of text and illustrations, all in colour in a highly presentable and attractive format, along with bonus of CD on ten CPCs. The revised edition should meet not only the aspirations of undergraduate students of medicine and dentistry but also those pursuing alternate streams of medicine and paramedical courses. However, the present practical book certainly cannot be used as the main source material for learning Pathology since the description of diseases/topics is in no way complete, for which the readers should refer to the main textbook by the author. Thus these two books may remain complementary to each other but cannot substitute each other.

ACKNOWLEDGEMENTS

I owe gratitude to all my colleagues in general for their valuable suggestions and healthy criticism from time to time, and to my young colleagues in the department in particular who have sincerely and ably helped me in revision of some chapters. In this respect, I profusely thank Dr. Shailja (for exercises on Techniques in Pathology), Dr. Romilla (for exercises in Clinical Pathology), Dr. Annu Nanda, Dr. Sukant Garg and Dr. Neerja (for exercises in Haematology) and Dr. Tanvi Sood (on exercises in Cytopathology). Besides, Dr. Spinder Gill Samra along with Mr Satish Kaushik, both of my department, have been very helpful in making newer drawings for the revised edition which is gratefully acknowledged. I once again put on record my appreciation for the assistance rendered by Dr RPS Punia, Reader, and Ms Agam Verma, B.Sc, Senior Lab Technician, both of my department, in preparation of some exercises in the previous edition of the book.

During the completion of work on this book, the tactical support and encouragement from the Department of Medical Education & Research, Chandigarh Administration, is gratefully acknowledged.

Finally, I acknowledge sincere thanks to the staff of Jaypee Brothers Medical Publishers (P) Ltd; in general for their liberal support, and Mrs Y Kapur, Senior Desktop Operator, and Mr Manoj Pahuja, Computer Art Designer, in particular in compilation of the text, and in preparation and layout of figures as per my whims and demands. My special thanks are due to Sh. JP Vij, Chairman and Managing Director and Mr Tarun Duneja, General Manager, (Publishing) of M/s Jaypee Brothers Medical Publishers (P) Ltd, for their constant co-operation and for being so supportive in the task of getting the best output in this edition of the book.

Lastly, I have gained profitably by suggestions from users of previous edition of this book and also on my other books. I urge the students and my colleagues to continue writing to me with their suggestions and in pointing out inaccuracies which may have been there inadvertently as that would help me in improving the book further.

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SECTION ONE

TECHNIQUES IN PATHOLOGY



ANTHONY VAN LEEUWENHOEK (1632-1723)

Born in Holland, draper by profession, during his spare time invented the first ever microscope by grinding the lenses himself and made 400 microscopes. He also first introduced histological staining in 1714 by using saffron to examine muscle fibre.

SECTION I: TECHNIQUES IN PATHOLOGY

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- ✧ Learn the working of various parts of a light microscope.
- ✧ Operate and maintain the light microscope.
- ✧ Learn the broad principles of other forms of microscopy.
- ✧ Discuss fixation of tissues for routine processing in histopathology laboratory.
- ✧ To be familiar with the equipments and basic technique of routine histopathology processing of tissues in the laboratory.
- ✧ Learn the routine H & E staining for tissue sections.
- ✧ Learn the technique, applications and stains used for frozen sections.
- ✧ Learn broad principles and end results of common special (histochemical) stains.

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Microscopy of Various Types

- ◆ Light Microscope
- ◆ Other Types of Microscopy
- ◆ Recent Advances in Microscopy

Exercise

1

Microscope is the most commonly used piece of apparatus in the laboratory. It produces greatly enlarged images of minute objects.

Common light microscope is described first, followed by other special types of microscopy techniques.

LIGHT MICROSCOPE

A light microscope can be a simple or a compound microscope.

Simple microscope This is a simple hand magnifying lens. The magnification power of hand lens is from 2x to 200x.

Compound microscope This has a battery of lenses which are fitted in a complex instrument. One type of lens remains near the object (*objective lens*) and another type

of lens near the observer's eye (*eye piece lens*). The eye piece and objective lenses have different magnification. The compound microscope can be *monocular* having single eye piece (Fig. 1.1) or, *binocular* which has two eye pieces (Fig. 1.2). The usual type of microscope used in clinical laboratories is called light microscope.

A compound microscope has the following parts:

- Stand
- Body
- Optical system
- Light/illumination system

Stand

This is horse-shoe shaped in monocular microscope. It gives stability to the microscope. Binocular microscopes have a variety of ergonomic shapes of stand.



FIGURE 1.1: Monocular light microscope, Model YS 50 (Photograph courtesy of Nikon, Japan through Towa Optics India Pvt. Ltd., Delhi).



FIGURE 1.2: Binocular light microscope, Model E 200 (Photograph courtesy of Nikon, Japan through Towa Optics India Pvt. Ltd., Delhi).

Body

It consists of a limb which arises from the joint with which microscope can be moved in comfortable position. The stand and the limb carry the following:

- i. Body tubes
- ii. Stage
- iii. Knobs for coarse and fine adjustment

Body Tubes

There are two tubes: *external tube* which carries at its lower end a revolving nose piece having objective lenses of different magnification while *internal tube* is draw tube which carries at its upper end eye pieces.

Stage

This is a metallic platform which accommodates glass slide having mounted object over it to be seen. Stage is attached to the limb just below the level of objectives. It has an aperture in its centre which permits the light to reach the object. Slide on the stage can be moved horizontally or vertically by two knobs attached to slide holder. Just below the stage is *substage* which consists of condenser through which light is focused on the object. The substage can be moved up and down. The substage has an iris diaphragm, closing and opening of which controls the amount of light reaching the object.

Knobs for Coarse and Fine Adjustment

For coarse and fine adjustments, knobs are provided on either side of the body. Coarse adjustment has two bigger knobs, the movement of which moves the body tubes with its lenses. Fine adjustment has two smaller knobs on either side of the body. The fine focus is graduated and by each division objective moves by 0.002 mm.

Optical System

Optical system is comprised by different lenses which are fitted into a microscope. It consists of eye piece, objectives and condensers.

Eye Piece

In monocular microscope, there is one eye piece while binocular microscope has two. Eye piece has two plano-convex lenses. Their magnification can be 5x, 10x, or 15x.

Objectives

These are made of a battery of lenses with prisms incorporated in them. Their magnification power is 4x, 10x, 40x and 100x.

Condenser

This is made up of two simple lenses and it condenses light on to the object.

Light/Illumination System

For day light illumination, a mirror is fitted which is plane on one side and concave on the other side (Fig. 1.1). Plane mirror is used in sunlight while concave in artificial light. Currently, most of the microscopes have in-built electrical illumination varying from 20 to 100 watts (Fig. 1.2).

Magnification and Resolving Power of Light Microscope

Magnification power of the microscope is the degree of image enlargement. It depends upon the following:

- i. Length of optical tube
- ii. Magnifying power of objective
- iii. Magnifying power of eye piece

With a fixed tube length of 160 mm in majority of standard microscopes, the magnification power of the microscope is obtained by the following:

Magnifying power of objective × Magnifying power of eye piece.

Resolving power represents the capacity of the optical system to produce separate images of objects very close to each other.

$$\text{Resolving power (R)} = \frac{0.61 \lambda}{\text{NA}}$$

Where λ is wavelength of incidental light; and
NA is numerical aperture of lens

Resolving power of a standard light microscope is around 200 nm.

How to Use a Light Microscope

1. Keep the microscope in comfortable position.
2. Obtain appropriate illumination by adjusting the mirror or intensity of light.
3. When examining colourless objects, condenser should be at the lowest position and iris diaphragm closed or partially closed.
4. When using oil immersion, 100x objective should dip in oil.
5. After using oil immersion clean the lens of the objective with tissue paper or soft cloth.

OTHER TYPES OF MICROSCOPY

Dark Ground Illumination (DGI)

This method is used for examination of unstained living micro-organisms e.g. *Treponema pallidum*.

Principle

The micro-organisms are illuminated by an oblique ray of light which does not pass through the micro-organism. The condenser is blackened in the centre and light passes through its periphery illuminating the living micro-organism on a glass slide.

Polarising Microscope

This method is used for demonstration of birefringence e.g. amyloid, foreign body, hair etc.

Principle

The light is made plane polarised. Two discs made up of prism are placed in the path of light, one below the object known as *polariser* and another placed in the body tube which is known as *analyser*. Polariser sieves out ordinary light rays vibrating in all directions allowing light waves of one orientation to pass through. The lower disc (polariser) is rotated to make the light plane polarised. During rotation, when analyzer comes perpendicular to polariser, all light rays are canceled or extinguished. Birefringent objects rotate the light rays and therefore appear bright in a dark background.

Fluorescent Microscope

This method is used for demonstration of naturally-occurring fluorescent material and other non-fluorescent substances or micro-organisms after staining with some fluorescent dyes e.g. *Mycobacterium tuberculosis*, amyloid, lipids, elastic fibres etc. UV light is used for illumination.

Principle

Fluorescent microscopy depends upon illumination of a substance with a specific wavelength (UV region i.e. invisible region) which then emits light at a lower wavelength (visible region).

Electron Microscope

This is used for study of ultrastructural details of the tissues and cells. For electron microscopy, tissue is fixed in 4% glutaraldehyde at 4°C for 4 hours. Ultrathin microsections with thickness of 100 nm are cut with diamond knives.

Principle

By using an electron beam of light, the resolving power of the microscope is increased to 50,000 to 100,000 times and very small structures can be visualised. In contrast to light microscopy, resolution of electron microscopy is 0.2 nm or less.

There are two types of electron microscopy:

1. Transmission electron microscopy (TEM)
2. Scanning electron microscopy (SEM)

Transmission Electron Microscopy (TEM)

TEM helps visualize cell's cytoplasm and organelles. For this purpose, ultrathin sections are required. TEM interprets atomic rather than molecular properties of the tissue and gives two dimensional image of the tissue.

Scanning Electron Microscopy (SEM)

SEM helps in the study of cell surface. In this three-dimensional image is produced. The image is produced on cathode ray oscillograph which can also be amplified. SEM can also be used for fluorescent antibody techniques.

RECENT ADVANCES IN MICROSCOPY

In the recent times, computers and chip technology have helped in developing following advances in microscopy:

Image Analysers and Morphometry

In these techniques, microscopes are attached to video monitors and computers with dedicated software systems. Microscopic images are converted into digital images and various cellular parameters (e.g. nuclear area, cell size etc) can be measured. This quantitative measurement introduces objectivity to microscopic analysis.

Telepathology (Virtual Microscopy)

It is the examination of slides under microscope set up at a distance. This can be done by using a remote control device to move the stage of the microscope or change the microscope field or magnification called as *robobic telepathology*. Alternatively and more commonly, it can be used by scanning the images and using the high-speed internet server to transmit the images to another station termed as *static telepathology*. Telepathology is employed for consultation for another expert opinion or for primary examination.



Histopathology Techniques and Routine Staining

- ◆ Fixation
- ◆ Dehydration
- ◆ Clearing
- ◆ Impregnation
- ◆ Tissue Processors
- ◆ Embedding and Blocking
- ◆ Section Cutting (Microtomy)
- ◆ Routine Staining (H & E)

Exercise

2

Histology is the technique of examination of normal tissues at microscopic level. Histopathology is examination of tissues for presence or absence of changes in their structure due to disease processes. Both are done by examining thin sections of tissues which are coloured differently by different dyes and stains. Total or selected representative part of tissue not more than 4 mm thick is placed in steel or plastic capsules or cassettes and is subjected to the following sequential processing (tissue processing):

- Fixation
 - Dehydration
 - Clearing
 - Impregnation
 - Embedding and blocking
 - Section cutting
 - Routine staining
- } *Processing*

FIXATION

Any tissue removed from the body starts decomposing immediately because of loss of blood supply and oxygen, accumulation of products of metabolism, action of autolytic enzymes and putrefaction by bacteria. This process of decomposition is prevented by fixation. Fixation is the method of preserving cells and tissues in life-like conditions as far as possible. During fixation, tissues are fixed in complete physical and partly chemical state. Most fixatives act by denaturation or precipitation of cell proteins or by making soluble components of cell insoluble. Fixative produces the following *effects*:

- i. Prevents putrefaction and autolysis.
- ii. Hardens the tissue which helps in section cutting.
- iii. Makes cell insensitive to hypertonic or hypotonic solutions.
- iv. Acts as a mordant.
- v. Induces optical contrast for good morphologic examination.

An ideal fixative has the following properties:

- i. It should be cheap and easily available.
- ii. It should be stable and safe to handle.
- iii. It should cause fixation quickly.
- iv. It should cause minimal loss of tissue.
- v. It should not bind to the reactive groups in tissue which are meant for dyes.
- vi. It should give even penetration.
- vii. It should retain the normal colour of the tissue.

Types of Fixatives

Fixatives may be simple or compound:

- *Simple fixative* consists of one substance (e.g. formalin).
- *Compound fixative* has two or more substances (e.g. Bouin's, Zenker's).

Fixatives can also be divided into following 3 groups:

- *Microanatomical fixatives*, which preserve the anatomy of the tissue.
- *Cytological fixatives*, which may be cytoplasmic or nuclear and preserve respective intracellular constituents.
- *Histochemical fixatives*, employed for demonstration of histochemical constituents and enzymes.

Commonly used fixatives are as under:

1. Formalin
2. Glutaraldehyde
3. Picric acid (e.g. Bouin's fluid)
4. Alcohol (e.g. Carnoy's fixative)
5. Osmium tetroxide

Formalin

This is the most commonly used fixative in routine practice. Formalin is commercially available as saturated solution of formaldehyde gas in water, 40% by weight/volume (w/v). For all practical purposes, this 40% solution is considered as 100% formalin. For fixation of tissues, a 10% solution is used which is prepared by dissolving 10 ml of commercially available formalin in 90 ml of water. It takes 6-8 hours for fixation of a thin piece of tissue 4 mm thick at room temperature. The amount of fixative required is 15 to 20 times the volume of the specimen. Formalin acts by polymerisation of cellular proteins by forming methylene bridges between protein molecules.

Merits of formalin

- Rapidly penetrates the tissues.
- Normal colour of tissue is retained.
- It is cheap and easily available.
- Best fixative for neurological tissue.

Demerits of formalin

- Causes excessive hardening of tissues.
- Causes irritation of skin, mucous membranes and conjunctiva.
- Leads to formation of formalin pigment in tissues having excessive blood at an acidic pH which can be removed by treatment of section with picric alcohol in solution of NaOH.

Glutaraldehyde

This is used as a fixative in electron microscopy. Glutaraldehyde is used as 4% solution at 4°C for 4 hours for fixation of tissues.

Disadvantages of glutaraldehyde

- It is expensive.
- It penetrates the tissues slowly.

Bouin's Fluid (Picric acid)

This is used as fixative for renal and testicular needle biopsies. Bouin's fluid stains the tissues yellow. It is also a good fixative for demonstration of glycogen. It is prepared as under :

Saturated picric acid	-	375 ml
40% formaldehyde	-	125 ml
Glacial acetic acid	-	25 ml

Disadvantages

- Makes the tissue harder and brittle.
- Causes lysis of RBCs.

Carnoy's Fixative (Alcohol)

Alcohol is mainly used for fixation of cytologic smears and endometrial curettings. It acts by denaturation of cell proteins. Both methyl and ethyl alcohol can be used. Methyl alcohol is used as 100% solution for 20-30 minutes. Ethyl alcohol is used either as 95% solution or as Carnoy's fixative for tissues which contains the following:

Ethyl alcohol (absolute)	-	300 ml
Chloroform	-	150 ml
Glacial acetic acid	-	50 ml

Carnoy's is a good fixative for glycogen and dissolves fat.

Osmium tetroxide

This is used as a fixative for CNS tissues and for electron microscopy. Osmium tetroxide is best fixative for lipids. It is used as a 2% solution. It imparts black colour to tissues.

DEHYDRATION

This is a process in which water from cells and tissues is removed so that this space is subsequently taken up by wax. Dehydration is carried out by passing the tissues through a series of ascending grades of alcohol: 70%, 80%, 95% and absolute alcohol. If ethyl alcohol is not available then methyl alcohol, isopropyl alcohol or acetone can be used.

CLEARING

This is the process in which alcohol from tissues and cells is removed and is replaced by a fluid in which wax is soluble and it also makes the tissue transparent. Xylene is the most commonly used clearing agent. Toluene, benzene (it is carcinogenic), chloroform (it is poisonous) and cedar wood oil (it is expensive and very viscous) can also be used as clearing agent.

IMPREGNATION

This is the process in which empty spaces in the tissue and cells after removal of water are taken up by paraffin wax. This hardens the tissue which helps in section cutting. Impregnation is done in molten paraffin wax which has the melting point of 56°C (54-62°C).

TISSUE PROCESSORS

Nowadays all the processes of fixation, dehydration, clearing and impregnation are carried out in a special equipment which is known as *automated tissue processor*.



FIGURE 2.1: Automatic tissue processor (Photograph courtesy of Thermo Shandon, UK through Towa Optics India Pvt. Ltd., Delhi).

It can be an open (hydraulic) system or a closed (vacuum) type. In the open type, the tissue processor has 12-16 glass jars for formalin, ascending grades of alcohol, xylene and thermostatically-controlled two paraffin wax baths to keep paraffin wax in molten state. Tissue moves automatically by hydraulic mechanism from one jar to another after fixed time schedule and the whole process takes 16-22 hours (Fig. 2.1). In closed type of tissue processor, tissue cassettes are placed in a single container while different processing fluids are moved in and out sequentially according to electronically programmed cycle (Fig. 2.2). The closed or vacuum processor has the advantage that there is no hazard of contamination of the laboratory by toxic fumes unlike in open system. In addition, heat and vacuum can be applied to shorten the processing time. Thus, closed tissue processors can also be applied for short schedules or rapid processing of small biopsies.

EMBEDDING AND BLOCKING

Embedding of tissue is done in molten wax. Wax blocks are conventionally prepared using metallic L (Leuckhart's mould); nowadays plastic moulds of different colours for blocking are also available (Fig. 2.3). The moulds are



FIGURE 2.2: Vacuum tissue processor, Model Excelsior (Photograph courtesy of Thermo Shandon, UK through Labindia Instruments Pvt. Ltd., Delhi).

placed over a smooth surfaced glass tile. Molten wax is poured in the cavity in the moulds. The processed tissue pieces are put into wax with number tag and examining surface facing downward. Wax is allowed to solidify. After solidification, if L-moulds are used they are removed while plastic mould remains with the wax block. In either case, each block contains a tissue piece carrying a identification label.

Embedding and blocking can also be performed in a special instrument called *embedding centre*. It has a wax reservoir, heated area for steel moulds, wax dispenser, and separate hot and cold plates for embedding and blocking (Fig. 2.4).

SECTION CUTTING (MICROTOMY)

Microtome is an equipment for cutting sections. There are 5 types of microtomes:

1. Rotary
2. Sliding
3. Freezing
4. Rocking
5. Base-sledge

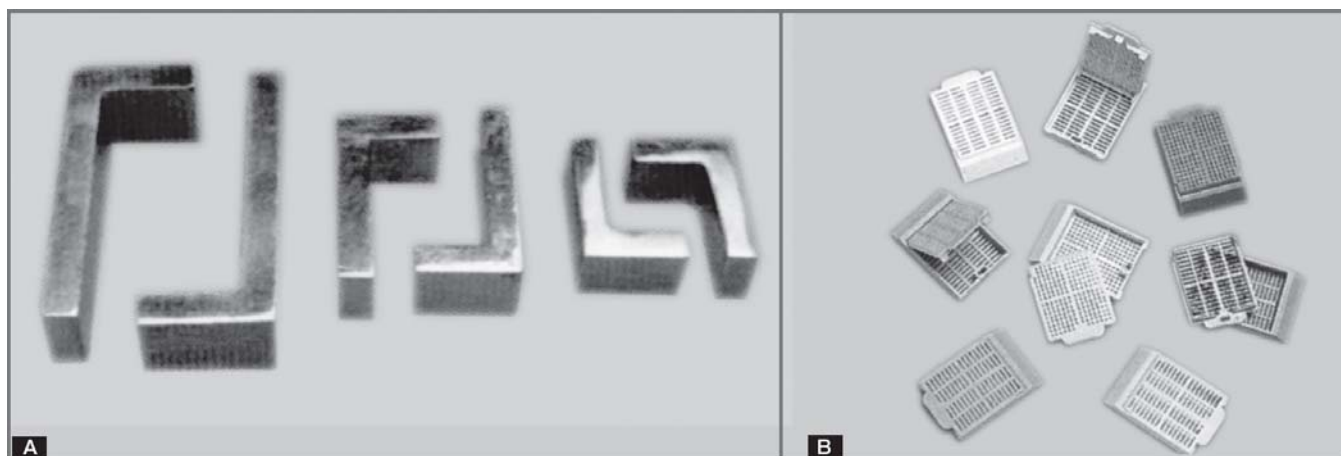


FIGURE 2.3: A, L (Leuckhart's) metal moulds. B, Plastic block moulds in different colours.

Rotary Microtome

This is the most commonly used microtome. In this, microtome knife is fixed while the tissue block is movable (Fig. 2.5). The knife in this faces upward and is wedge-shaped. The knife used is of steel but glass knife can also be used. These knives are sharpened by a process known as *honing* and *stropping*. Honing is done manually on a stone or on an electrically operated automatic hone. After honing, stropping is done which is polishing of its edge over a leather strop. The process of sharpening of microtome knife can also be done by automatic knife sharpener (Fig. 2.6). Nowadays, disposable blades for microtomy are also available.

Sliding Microtome

In this the tissue block is fixed while the knife is movable. These microtomes are used as freezing microtomes.

Freezing Microtome

See under frozen section in Exercise 3 (page 11).

Rocking Microtome

This is a simple microtome. The knife is immovable while tissue block is held in a spring-bearing rocking arm. This is more useful when cutting serial sections.

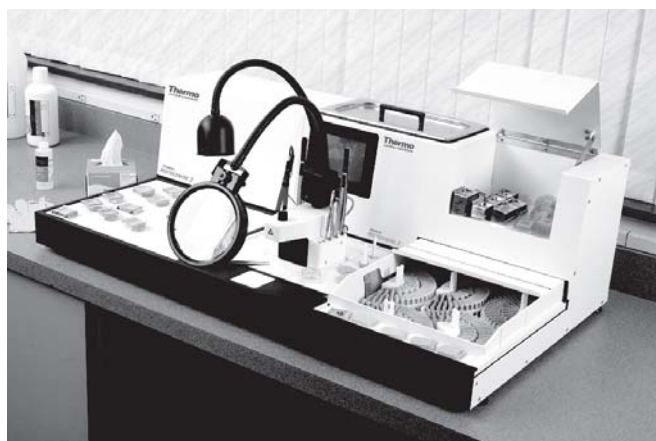


FIGURE 2.4: Tissue embedding centre, Model Histocentre (Photograph courtesy of Thermo Shandon, UK through Towa Optics (India) Pvt. Ltd., Delhi).



FIGURE 2.5: Rotary microtome, Model Finesse 325 (Photograph courtesy of Thermo Shandon, UK through Towa Optics India Pvt. Ltd., Delhi).



FIGURE 2.6: Automatic knife sharpener, Model Shandon Autosharp 5 (Photograph courtesy of Thermo Shandon, UK through Towa Optics India Pvt. Ltd., Delhi).

Base-Sledge Microtome

This type of microtome is used for very hard tissues or large blocks e.g. pieces of brain and heart.

Procedure for Microtomy

Put the paraffin block having tissue in it in the rotary microtome. Cut the section by operating the microtome manually after adjusting the thickness at 5-6 μm . Sections are picked from the knife with the help of a forceps or camel hair brush. These are made to float in a water-bath which is kept at a temperature of 40-45°C i.e. slightly below the melting point of wax. This removes folds in the section. From water-bath sections are picked on a clean glass slide. The glass slide is placed in an oven maintained at a temperature of 56°C for 20-30 minutes for proper drying and better adhesion. Coating adhesives for sections can be used before picking up sections; these include egg albumin, gelatin, poly-L-lysine etc. The section is now ready for staining.

ROUTINE STAINING (H & E)

Routine staining is done with haematoxylin and eosin (H&E).

Haematoxylin

This is a natural dye which is obtained from log-wood tree, *Haematoxylon campechianum*. This tree is nowadays commercially grown in Jamaica and Mexico. The natural extract from the stem of this tree is haematoxylin which is an inactive product. This product is oxidised to an active ingredient which is haematein. This process of oxidation is known as *ripening* which can be done naturally in sunlight, or chemically by addition of oxidant like sodium iodate, KMnO_4 or mercuric oxide. A *mordant* is added to it (e.g. potash alum) which helps in attaching the stain particles to the tissue.

Procedure for Staining

Sections are first deparaffinised (removal of wax) by placing the slide in a jar of xylene for 10-15 minutes. As haematoxylin is a water-based dye, the sections before staining are rehydrated which is done by passing the sections in a series of descending grades of alcohol and finally bringing the section to water.

- Place the slide in haematoxylin stain for 8-10 minutes.
- Rinse in water.
- *Differentiation* (i.e. selective removal of excess dye from the section) is done by putting the slide in a solution of 1% acid alcohol for 10 seconds.
- Rinse in water.
- *Blueing* (i.e. bringing of required blue colour to the section) is done by putting the section in Scott's tap water (containing sodium bicarbonate and magnesium sulfate) or saturated solution of lithium carbonate for 2-10 minutes.
- Counterstain with 1% aqueous solution of eosin for 1-3 minutes.
- Rinse in tap water.
- Before mounting, the sections have to be dehydrated which is done by passing the sections in a series of ascending grades of alcohol and finally cleared in xylene, 2-3 dips in each solution.
- Mount in DPX (dextrene polystyrene xylene) or Canada balsam.

Results :

Nuclei	:	Blue
Cytoplasm	:	Pink
Muscle, collagen,		
RBCs, keratin,	:	Pink
colloid protein		



Frozen Section and Special Stains

- ◆ Frozen Section
- ◆ Special Stains

Exercise

3

FROZEN SECTION

When a fresh tissue is rapidly frozen, the matter within the tissue turns into ice and in this state the tissue is firm, the ice acting as embedding medium. Therefore, sections are produced without the use of dehydrating solution, clearing agent or wax embedding.

Frozen section cutting is a quick diagnostic procedure for tissues before proceeding to a major radical surgery. This is also used for demonstration of some special substances in the cells and tissues e.g. fat, enzymes. This procedure can be carried out in operation theatre complex near the operating table. It has its own merits and demerits.

Merits

- This is a quick diagnostic procedure. The time needed from the receipt of tissue specimen to the study of stained sections is about 10 minutes, while in routine paraffin-sectioning at least two days are required.
- Every type of staining can be done.
- There is minimal shrinkage of tissues as compared to paraffin sections.
- Lipids and enzymes which are lost in routine paraffin sections can be demonstrated.

Demerits

- It is difficult to cut serial sections.
- It is not possible to maintain tissue blocks for future use.
- Sections cut are thicker.
- Structural details tend to be distorted due to lack of embedding medium.

Methods for Frozen Sections

There are two methods for obtaining frozen sections:

- Freezing microtome using CO₂ gas
- Refrigerated microtome (cryostat).

For frozen section, best results are produced from fresh unfixed tissue and freezing the tissue as rapidly as possible.

Freezing Microtome using CO₂ Gas

In this method freezing microtome is used which is a sliding type of microtome.

Setting of microtome and section cutting The microtome is screwed firmly to the edge of a table by means of a stout screw. A CO₂ gas cylinder is placed near the microtome. The cylinder is then connected to the microtome by means of a special tubing. The connecting tube should not have any bends or cracks. Adjust the gauze of the microtome to a required thickness of sections. The knife is inserted in its place. A few drops of water are placed over freezing stage. A selected piece of tissue is placed over stage on drops of water. Short bursts of CO₂ are applied to freeze the tissue and water till the surface of the tissue is completely covered with ice. Alternatively solid CO₂ (dry ice, cardice) can be used for freezing tissue blocks. Sections are then cut by swinging movement of knife forward and backward with a regular rhythm. The cut sections come over the knife. From the knife, sections are picked with a camel-brush and transferred to a Petri dish containing water. The sections are then placed over a glass slide with the help of a dropper. Remove the folds in the sections by tilting the slides. The slide is then passed over flame for a few seconds for fixing the sections over the slide. Section is now ready for staining with a desired stain.

Advantages

- i. It is cheap.
- ii. It requires less space.
- iii. Equipment is portable.

Disadvantages

- i. Sections cut are thick.
- ii. CO₂ gas may run out in between the procedures.
- iii. The connecting tube may be blocked due to solidified CO₂.

Refrigerated Microtome (Cryostat)

In cryostat, a microtome is fitted in a thermostatically-controlled refrigerated cabinet. A temperature of upto – 30°C can be achieved. The microtome fitted is of rotary type with an antiroll plate (Fig. 3.1).

Setting of microtome and section cutting Switch on the cryostat alongwith the knife inserted in position several hours before the procedure for attaining the operating temperature. A small piece of fresh unfixed tissue (4 mm) is placed on object disc of the deep freeze shelf of the cryostat for 1-2 minutes. The tissue is rapidly frozen. Now the object disc with tissue is inserted into microtome object clamp. Place antiroll plate in its position. By manual movement, sections are cut at desired thickness. The antiroll plate prevents folding of sections. The section is picked from the knife by opening the cabinet and taking the section directly on to the clean albuminised glass slide. A glass slide is lowered on to the knife 1 mm from section. The section comes automatically on the glass slide because of difference of temperature between the section and the slide. The section is ready for staining. The cryostat is defrosted and cleaned at weekend.

Advantages

- i. Sections cut are thin.
- ii. There is better control of temperature.
- iii. Equipment is portable.

Disadvantage

- i. Equipment is expensive.

Staining of Frozen Sections

Sections obtained by freezing microtomy by either of the methods are stained by rapid method as under:

1. Rapid H & E
2. Toluidine blue

Rapid H & E Staining

- Place the section in haematoxylin for one minute.



FIGURE 3.1: Cryostat, Model Cryotome (Photograph courtesy of Thermo Shandon, UK through Towa Optics India Pvt. Ltd., Delhi).

- Rinse in tap water.
- Differentiate in 1% acid alcohol by giving one rapid dip.
- Rinse in water.
- Quick blueing is done by passing the section over ammonia vapours or rapid dip in a blueing solution.
- Rinse in tap water.
- Counterstain with 1% aqueous eosin for 3-6 seconds.
- Rinse in tap water.
- Dehydrate by passing the section through 95% alcohol and absolute alcohol, one dip in each solution.
- Clearing is done by passing the section through xylene, one dip.
- Mount in DPX.
- Examine under the microscope.

Toluidine Blue Staining

- Place the section in toluidine blue 0.5% for ½ to 1 minute.
- Rinse in water.

- Mount in water glycerine (i.e. aqueous mountant) with coverslip.
- Examine under the microscope.

SPECIAL STAINS

These are applied for demonstration of certain specific substances/constituents of the cells/tissues. The staining depends upon either physical, chemical or differential solubility of the stain with the tissues. The principles of some of the staining procedures are well known while those of others are unknown. The various common special stains in use in the laboratory are as under:

1. Sudan black/oil red
2. van Gieson
3. Masson's trichrome
4. Reticulin
5. Verhoeff
6. Periodic acid-Schiff (PAS)
7. Methyl violet
8. Perl's reaction

Sudan Black/Oil Red O

These stains are used for demonstration of fat.

Principle Sudan black and oil red O staining are based on physical combination of the stain with fat. It involves differential solubility of stain in fat because these stains are more soluble in fat than the solvent in which these are prepared. The stain leaves the solvent and goes into the fat.

Procedure for Oil Red O Staining

- Cut frozen section of formalin-fixed tissue.
- Rinse in 60% isopropyl alcohol.
- Put in oil red O solution for 5-10 minutes.
- Rinse in 60% isopropyl alcohol.
- Wash in water.
- Counterstain with haematoxylin for 1-2 minutes.
- Blueing is done by passing the section through a solution of ammonia.
- Rinse in water.
- Mount in glycerine.

Result

With Oil red O

Fat	:	Bright red
Nuclei	:	Blue

With Sudan black

Fat	:	Black
Nuclei	:	Red

van Gieson

This stain is used for staining of collagen fibres.

Principle It is based on the differential staining of collagen and other tissues (e.g. muscle) depending upon the porosity of tissue and the size of the dye molecule. Collagen with larger pore size takes up the larger molecule red dye (acid fuchsin) in an acidic medium, while non-porous muscle stains with much smaller molecule dye (picric acid).

Result

Collagen	:	Red
Nuclei	:	Blue
Other tissues	:	Yellow
(including muscle)		

Masson's Trichrome

This stain is used for staining of muscle.

Principle Principle is the same as for van Gieson.

Result

Muscle	:	Red
Nuclei	:	Blue-black
Collagen	:	Blue-green

Reticulin

This is used for demonstration of reticulin fibres.

Principle Reticulin stain employs silver impregnation method. There is local reduction and selective precipitation of silver salt.

Result

Reticulin fibres	:	Black
Nuclei	:	Colourless
Collagen	:	Brown

Congo Red

This stain is used for demonstration of amyloid, an extracellular fibrillar proteinaceous substance.

Principle Congo red dye has selective affinity for amyloid and attaches through non-polar hydrogen bonds. It gives green birefringence when viewed by polarised light.

Result

Amyloid elastic fibres	:	Red
------------------------	---	-----

Only amyloid gives green birefringence in polarised light.

Periodic Acid-Schiff (PAS)

This stain is used for demonstration of glycogen and mucopolysaccharides.

Principle Tissues/cells containing 1,2 glycol group are converted into dialdehyde with the help of an oxidising agent which then reacts with Schiff's reagent to give bright pink colour. Normally Schiff's reagent is colourless.

Result

PAS positive substances	:	Bright pink
Nuclei	:	Blue

PAS positive substances are glycogen, amyloid, colloid, neutral mucin and hyaline cast.

Methyl Violet

This is a metachromatic stain i.e. the tissues are stained in a colour which is different from the colour of the stain itself. It is used for demonstration of amyloid in tissue.

Principle This depends upon the type of dye (stain) used and character of the tissue which unites with the dye. Tissues containing SO_4 , PO_4 or COOH groups react with basic dyes and cause their polymerization, which in turn leads to production of colour different from the original dye.

Result

Metachromatic positive tissue	:	Red to violet
Other tissues	:	Blue

Other metachromatic stains used are crystal violet, toluidine blue.

Prussian Blue/Perl's Reaction

This is used for demonstration of iron.

Principle Ferric ions present in the tissue combine with potassium ferrocyanide forming ferric-ferrocyanide.

Result

Iron	:	Blue
Cytoplasm and nuclei	:	Red to pink



SECTION TWO

CLINICAL PATHOLOGY



PAUL EHRLICH (1884-1915)

'IMMUNOLOGIST AND CLINICAL PATHOLOGIST'

German Physician, winner of Nobel Prize for his work in immunology;
described Ehrlich's test for urobilinogen; staining
techniques of cells and bacteria, and laid the foundations of haematology.

SECTION II: CLINICAL PATHOLOGY

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- ✧ Learn the physical characteristics of urine.
- ✧ Learn the technique to perform, interpret and importance of most of the chemical tests of urine examination.
- ✧ Perform and interpret microscopic examination of urine.
- ✧ Learn the technique of semen examination.
- ✧ Gain knowledge of normal values of semen analysis in healthy males and in infertility.
- ✧ Technique and interpretation of CSF examination.

Section Contents

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Urine Examination I: Physical and Chemical

- ◆ Adequacy of Specimen
- ◆ Physical Examination
- ◆ Chemical Examination
- ◆ Automated Urinalysis

Exercise

4

Examination of urine is important for diagnosis and assistance in the diagnosis of various diseases. Routine (complete) examination of urine is divided into four parts:

- A. Adequacy of specimen
- B. Physical/gross examination
- C. Chemical examination
- D. Microscopic examination

The last named, microscopic examination, is discussed separately in the next exercise.

A. ADEQUACY OF SPECIMEN

The specimen should be properly collected in a clean container which should be properly labelled with name of the patient, age and sex, identity number with date and time of collection. It should not show signs of contamination.

Specimen Collection

For routine examination a clean glass tube is used; for bacteriologic examination a sterilized tube or bottle is required. A mid-stream sample is preferable i.e. first part of urine is discarded and mid-stream sample is collected. For 24 hours sample, collection of urine is started in the morning at 8 AM and subsequent samples are collected till next day 8 AM.

Methods of Preservation of Urine

Urine should be examined fresh or within one hour of voiding. But if it has to be delayed then following preservatives can be added to it which prevent its decomposition:

- i. *Refrigeration* at 4°C.
- ii. *Toluene*: Toluene is used 1 ml per 50 ml of urine. It acts by forming a surface layer and it preserves the chemical constituents of urine.

iii. *Formalin*: 6-8 drops of 40% formalin per 100 ml of urine is used. It preserves RBCs and pus cells. However, its use has the disadvantage that it gives false-positive test for sugar.

iv. *Thymol*: Thymol is a good preservative; 1% solution of thymol is used. Its use has the disadvantage that it gives false-positive test for proteins.

v. *Acids*: Hydrochloric acid, sulfuric acid and boric acid can also be used as preservative.

B. PHYSICAL EXAMINATION

Physical examination of urine consists of volume, colour, odour, reaction/pH and specific gravity.

Volume

Normally 700-2500 ml (average 1200 ml) of urine is passed in 24 hours and most of it is passed during day time.

i) *Nocturia* Nocturia means when urine is passed in excess of 500 ml during night. This is a sign of early renal failure.

ii) *Polyuria* Polyuria is when excess of urine is passed in 24 hours (> 2500 ml). Polyuria can be physiological due to excess water intake, may be seasonal (e.g. in winter), or can be pathological (e.g. in diabetes insipidus, diabetes mellitus).

iii) *Oliguria* When less than 500 ml of urine is passed in 24 hours, it is termed as oliguria. It can be due to less intake of water, dehydration, renal ischaemia.

iv) *Anuria* When there is almost complete suppression of urine (< 150 ml) in 24 hours. It can be due to renal stones, tumours, renal ischaemia.

Colour

Normally urine is clear, pale or straw-coloured due to pigment urochrome.

i) *Colourless* in diabetes mellitus, diabetes insipidus, excess intake of water.

ii) *Deep amber colour* due to good muscular exercise, high grade fever.

iii) *Orange colour* due to increased urobilinogen, concentrated urine.

iv) *Smoky urine* due to small amount of blood, administration of vitamin B₁₂, aniline dye.

v) *Red* due to haematuria, haemoglobinuria.

vi) *Brown* due to bile.

vii) *Milky* due to pus, fat.

viii) *Green* due to putrefied sample, phenol poisoning.

Odour

Normally urine has faint aromatic odour.

i) *Pungent* due to ammonia produced by bacterial contamination.

ii) *Putrid* due to UTI.

iii) *Fruity* due to ketoacidosis.

iv) *Mousy* due to phenylketonuria.

Reaction/pH

It reflects ability of the kidney to maintain H⁺ion concentration in extracellular fluid and plasma. It can be measured by pH indicator paper or by electronic pH meter.

Freshly voided normal urine is slightly acidic and its pH ranges from 4.6-7.0 (average 6.0).

- *Acidic urine* is due to the following:
 - i. High protein intake, e.g. meat.
 - ii. Ingestion of acidic fruits.
 - iii. Respiratory and metabolic acidosis.
 - iv. UTI by *E. coli*.
- *Alkaline urine* is due to following:
 - i. Citrus fruits.
 - ii. Vegetables.
 - iii. Respiratory and metabolic alkalosis.
 - iv. UTI by *Proteus*, *Pseudomonas*.

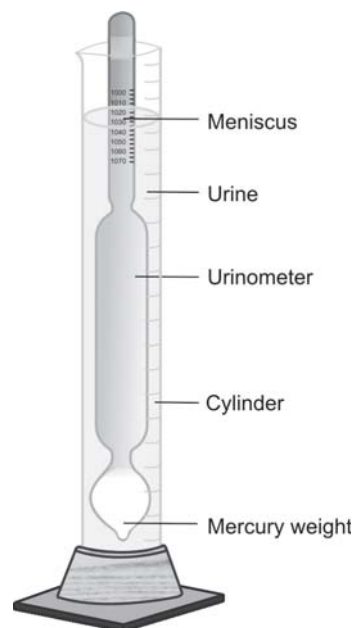


FIGURE 4.1: Urinometer and the container for floating it for measuring specific gravity.

Specific Gravity

This is the ratio of weight of 1 ml volume of urine to that of weight of 1 ml of distilled water. It depends upon the concentration of various particles/solutes in the urine. Specific gravity is used to measure the concentrating and diluting power of the kidneys. It can be measured by urinometer, refractometer or reagent strips.

1. Urinometer

Procedure

- Fill urinometer container 3/4th with urine.
- Insert urinometer into it so that it floats in urine without touching the wall and bottom of container (Fig. 4.1).
- Read the graduation on the arm of urinometer at lower urinary meniscus.
- Add or subtract 0.001 from the final reading for each 3°C above or below the calibration temperature respectively marked on the urinometer.

2. Refractometer

It measures the refractive index of urine. This procedure requires only a few drops of urine in contrast to urinometer where approximately 100 ml of urine is required.

3. Reagent Strip Method

This method employs the use of chemical reagent strip (see Fig. 4.3, page 20).

Significance of Specific Gravity

The normal specific gravity of urine is 1.003 to 1.030.

Low specific gravity urine occurs in:

- i. Excess water intake
- ii. Diabetes insipidus

High specific gravity urine is seen in:

- i. Dehydration
- ii. Albuminuria
- iii. Glycosuria

Fixed specific gravity (1.010) urine is seen in:

- i. ADH deficiency
- ii. Chronic nephritis

C. CHEMICAL EXAMINATION

Chemical constituents frequently tested in urine are: proteins, glucose, ketones, bile derivatives and blood.

Tests for Proteinuria

If urine is not clear, it should be filtered or centrifuged before testing. Urine may be tested for proteinuria by qualitative tests and quantitative methods.

Qualitative Tests for Proteinuria

1. Heat and acetic acid test
2. Sulfosalicylic acid test
3. Heller's test
4. Reagent strip method.

1. Heat and Acetic Acid Test

Heat causes coagulation of proteins. The procedure is as under:

- Take a 5 ml test tube.
- Fill 2/3rd with urine.
- Acidify by adding 10% glacial acetic acid if urine is alkaline.
- Boil upper portion for 2 minutes (lower part acts as control).
- If precipitation or turbidity appears add a few drops of 10% acetic acid.

Interpretation If turbidity or precipitation disappears on addition of acetic acid, it is due to phosphates; if it persists after addition of acetic acid then it is due to

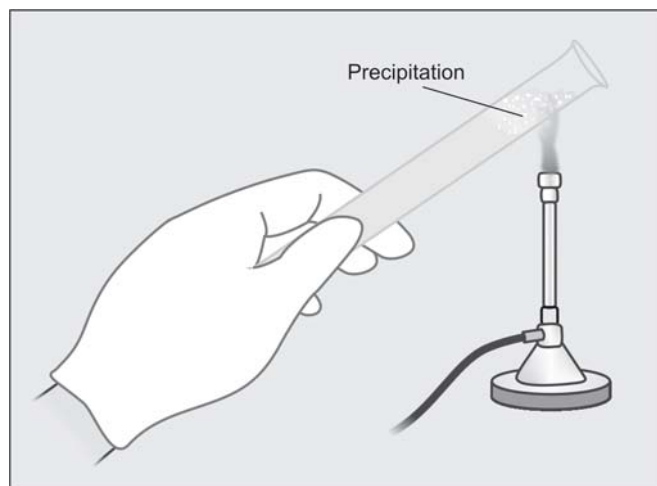


FIGURE 4.2: Heat and acetic acid test for proteinuria. Note the method of holding the tube from the bottom while heating the upper part.

proteins. Depending upon amount of protein the results are interpreted as under (Fig. 4.2):

No cloudiness = negative.

Cloudiness against dark background = traces (less than 0.1 g/dl).

Cloudiness without granularity = +(0.1 g/dl).

Granular cloudiness = ++ (0.1-0.2 g/dl)

Precipitation and flocculation = +++ (0.2-0.4 g/dl).

Thick solid precipitation = ++++ (0.5 g/dl).

2. Sulfosalicylic Acid Test

This is a very reliable test. The procedure is as under:

- Make urine acidic by adding acetic acid.
- To 2 ml of urine add a few drops (4-5) of 20% sulfosalicylic acid.

Interpretation Appearance of turbidity which persists after heating indicates presence of proteins.

3. Heller's Test

- Take 2 ml of concentrated nitric acid in a test tube.
- Add urine drop by drop by the side of test tube.

Interpretation Appearance of white ring at the junction indicates presence of protein.

4. Reagent Strip Method

Bromophenol coated strip is dipped in urine. Change in colour of strip indicates presence of proteins in urine and is compared with the colour chart provided for semiquantitative grading (Fig. 4.3).



FIGURE 4.3: Strip method for testing various constituents in urine. Multistix 10 SG, and Uristix (Photograph courtesy of Bayer Diagnostics, Baroda, India).

Quantitative Estimation of Proteins in Urine

1. Esbach's albuminometer method
2. Turbidimetric method.

1. Esbach's albuminometer method

- Fill the albuminometer with urine upto mark U.
- Add Esbach's reagent (picric acid + citric acid) upto mark R (Fig. 4.4).
- Stopper the tube, mix it and let it stand for 24 hours.
- Take the reading from the level of precipitation in the albuminometer tube and divide it by 10 to get the percentage of proteins.

2. Turbidimetric method

- Take 1 ml of urine and 1 ml standard in two separate tubes.
- Add 4 ml of trichloroacetic acid to each tube.
- After 5 minutes take the reading with red filter (680 nm).

Causes of Proteinuria

Normally, there is a very scanty amount of protein in urine (< 150 mg/day).

- **Heavy proteinuria** (> 3 gm/day) occurs due to:
 - i. Nephrotic syndrome
 - ii. Renal vein thrombosis
 - iii. Diabetes mellitus
 - iv. SLE

- **Moderate proteinuria** (1-3 gm/day) is seen in:
 - i. Chronic glomerulonephritis
 - ii. Nephrosclerosis

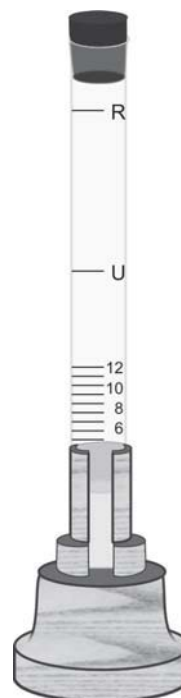


FIGURE 4.4: Esbach's albuminometer for quantitative estimation of proteins (U = urine; R = Esbach's reagent).

- iii. Multiple myeloma
- iv. Pyelonephritis

■ **Mild proteinuria** (< 1.0 gm/day) occurs in:

- i. Hypertension
- ii. Polycystic kidney
- iii. Chronic pyelonephritis
- iv. UTI
- v. Fever.

■ **Microalbuminuria** is excretion of 20-200 mg/L of albumin (20-200 µg/minutes) and is indicative of early and possibly reversible glomerular damage.

Test for Bence-Jones Proteinuria

Bence-Jones proteins are light chains of γ -globulin. These are excreted in multiple myeloma and other paraproteinaemias. In heat and acetic acid test performed under temperature control, these proteins are precipitated at lower temperature (56°C) and disappear on further heating above 90°C but reappear on cooling to lower temperature again. In case both albumin as well as Bence-Jones proteins are present in urine, the sample of urine is heated to boiling. Precipitates so formed due to albumin are filtered out and the test for Bence-Jones proteins is repeated under temperature control as above.

Test for Glucosuria

Glucose is by far the most important of the sugars which may appear in urine. Normally approximately 130 mg of glucose per 24 hours is passed in urine which is undetectable by qualitative tests.

Tests for glucosuria may be qualitative or quantitative.

Qualitative Tests

These are as under:

1. Benedict's test
2. Reagent strip test

1. *Benedict's Test*

In this test cupric ion is reduced by glucose to cuprous oxide and a coloured precipitate is formed.

Procedure

- Take 5 ml of Benedict's qualitative reagent in a test tube.
- Add 8 drops (or 0.5 ml) of urine.
- Heat to boiling for 2 minutes (Fig. 4.5).
- Cool in water bath or in running tap water.

Interpretation

No change of blue colour	= Negative
Greenish colour	= traces (< 0.5 g/dl)
Green/cloudy green ppt	= + (1g/dl)
Yellow ppt	= ++ (1-1.5g/dl)
Orange ppt	= +++ (1.5-2g/dl)
Brick red ppt	= ++++ (> 2g/dl)

Since Benedict's test is for reducing substances excreted in the urine, the test is positive for all reducing sugars (glucose, fructose, maltose, lactose *but not* sucrose) and other reducing substances (e.g. ascorbic acid, salicylates, antibiotics, L-dopa).

2. *Reagent Strip Test*

These strips are coated with glucose oxidase and the test is based on enzymatic reaction. This test is specific

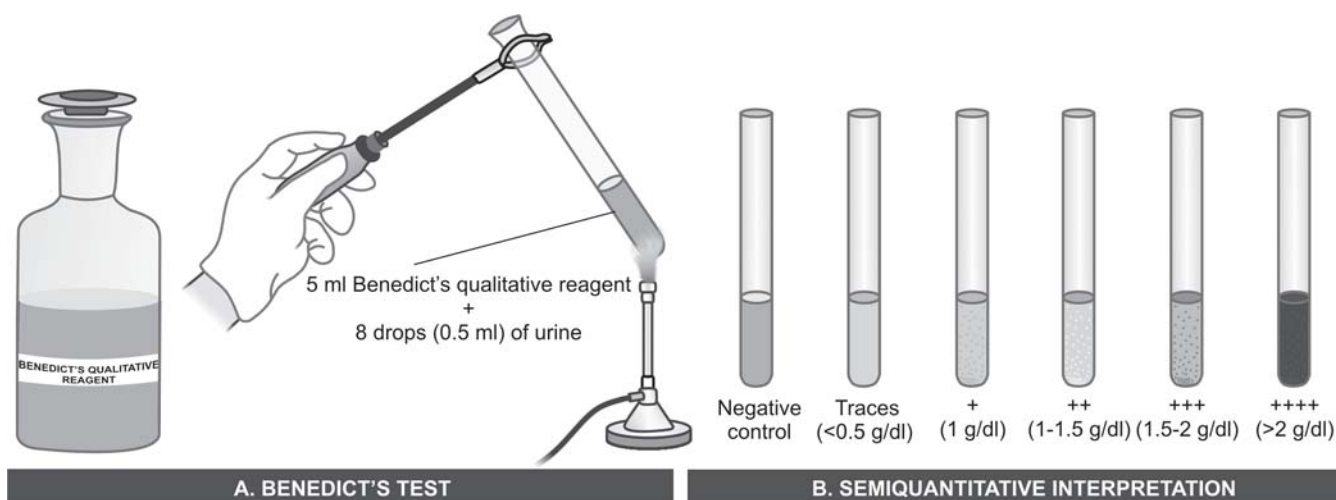


FIGURE 4.5: A, Method for Benedict's test (qualitative) for glucosuria. The test sample shows brick red precipitation (++++). B, Semiquantitative interpretation of glucosuria by Benedict's test.

for glucose. The strip is dipped in urine. If there is change in colour of strip it indicates presence of glucose. The colour change is matched with standard colour chart provided on the label of the reagent strip bottle (see Fig. 4.3).

Quantitative Test for Glucose

Procedure Take 25 ml of quantitative Benedict's reagent in a conical flask. Add to it 15 gm of sodium carbonate (crystalline) and some pieces of porcelain and heat it to boil. Add urine to it from a burette slowly till there is disappearance of blue colour of Benedict's reagent. Note the volume of urine used. Calculate the amount of glucose present in urine as under:

$$\frac{0.05 \times 100}{\text{Amount of urine}}$$

(0.05 gm of glucose reduces 25 ml of Benedict's reagent).

Causes of Glucosuria

- i. Diabetes mellitus
- ii. Renal glucosuria
- iii. Severe burns
- iv. Administration of corticosteroids
- v. Severe sepsis
- vi. Pregnancy

Tests for Ketonuria

These are products of incomplete fat metabolism. The three ketone bodies excreted in urine are: acetoacetic acid (20%), acetone (2%), and β -hydroxybutyric acid (78%).

Tests for Ketonuria

1. Rothera's test
2. Gerhardt's test
3. Reagent strip test

1. Rothera's Test

Principle Ketone bodies (acetone and acetoacetic acid) combine with alkaline solution of sodium nitroprusside forming purple complex.

Procedure

- Take 5 ml of urine in a test tube.
- Saturate it with solid ammonium sulphate salt.
- Add a few crystals of sodium nitroprusside and shake.
- Add liquor ammonia from the side of test tube.

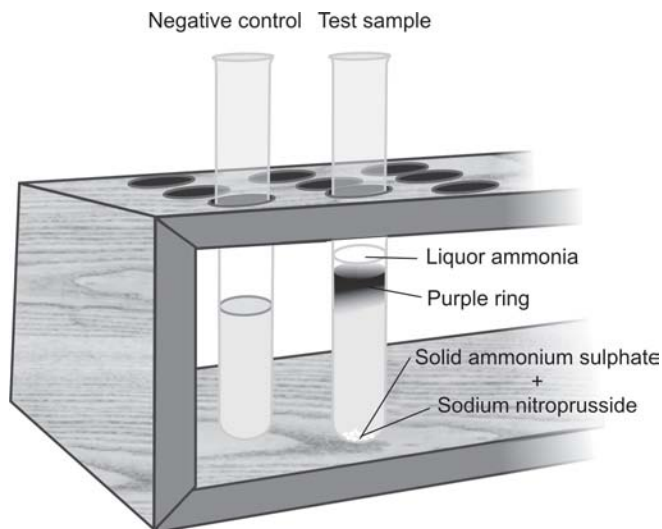


FIGURE 4.6: Rothera's test for ketone bodies in urine showing purple coloured ring in positive test.

Interpretation Appearance of purple coloured ring at the junction indicates presence of ketone bodies (Fig. 4.6).

2. Gerhardt's Test

It is not a very sensitive test.

Procedure

- Take 5 ml of urine in a test tube.
- Add 10% ferric chloride solution drop by drop.
- Filter it and add more ferric chloride.

Interpretation Brownish red colour indicates presence of ketone bodies.

3. Reagent Strip Test

These strips are coated with alkaline sodium nitroprusside. When strip is dipped in urine it turns purple if ketone bodies are present (See Fig. 4.3).

Causes of ketonuria

- i. Diabetic ketoacidosis
- ii. Dehydration
- iii. Hyperemesis gravidarum
- iv. Fever
- v. Cachexia
- vi. After general anaesthesia

Test for Bile Derivatives in Urine

Three bile derivatives excreted in urine are: urobilinogen, bile salts and bile pigments. While urobilinogen is

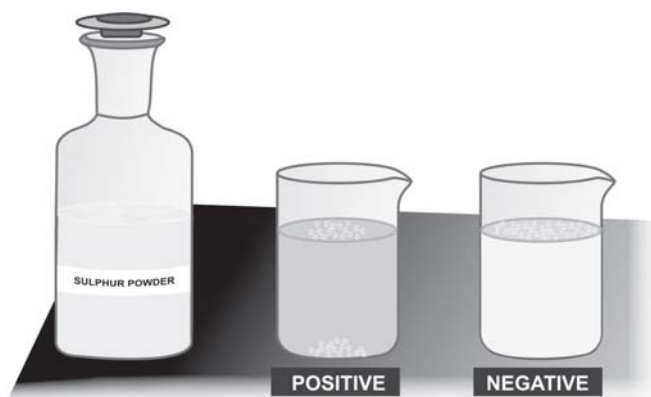


FIGURE 4.7: Hay's test for bile salts in urine. The test is positive in beaker in the centre contrasted with negative control in beaker on right side.

normally excreted in urine in small amounts, bile salts and bile pigments appear in urine in liver diseases only.

Tests for Bile Salts

Bile salts excreted in urine are cholic acid and chenodeoxycholic acid. Tests for bile salts are Hay's test and strip method.

1. Hay's Test

Principle Bile salts if present in urine lower the surface tension of the urine.

Procedure

- Fill a 50 or 100 ml beaker 2/3rd to 3/4th with urine.
- Sprinkle finely powdered sulphur powder over it (Fig. 4.7).

Interpretation If bile salts are present in the urine then sulphur powder sinks, otherwise it floats.

2. Strip Method

Coated strips can be used for detecting bile salts as for other constituents in urine (see Fig. 4.3).

Causes for bile salts in urine:

- i. Obstructive jaundice

Tests for Urobilinogen

Normally a small amount of urobilinogen is excreted in urine (4 mg/24 hr). The sample should always be collected in a dark coloured bottle as urobilinogen gets oxidised on exposure to light.

Tests for urobilinogen in urine are Ehrlich's test and reagent strip test.

1. Ehrlich's Test

Principle Urobilinogen in urine combines with Ehrlich's aldehyde reagent to give a red purple coloured compound.

Procedure

- Take 10 ml of urine in a test tube.
- Add 1 ml of Ehrlich's aldehyde reagent.
- Wait for 3-5 minutes.

Interpretation Development of red purple colour indicates presence of urobilinogen. A positive test is subsequently done in dilutions; normally it is positive in upto 1:20 dilution.

2. Reagent Strip Test

These strips are coated with p-dimethyl-amino-benzaldehyde. When strip is dipped in urine, it turns reddish-brown if urobilinogen is present (see Fig. 4.3).

Significance

Causes of increased urobilinogen in urine

- i. Haemolytic jaundice and haemolytic anaemia

Causes for absent urobilinogen in urine

- ii. Obstructive jaundice

Tests for Bilirubin (Bile Pigment) in Urine

Bilirubin is breakdown product of haemoglobin. Normally no bilirubin is passed in urine.

Following tests are done for detection of bilirubin in urine:

1. Fouchet's test
2. Foam test
3. Reagent strip test

1. Fouchet's Test

Principle Ferric chloride oxidises bilirubin to green biliverdin.

Procedure

- Take 10 ml of urine in a test tube.
- Add 3-5 ml of 10% barium chloride.
- Filter through filter paper.
- To the precipitate on filter paper, add a few drops of Fouchet's reagent (ferric chloride + trichloroacetic acid).

Interpretation Development of green colour indicates bilirubin.

2. Foam Test

It is a non-specific test.

Procedure

- Take 5/10 ml of urine in a test tube.
- Shake it vigorously.

Interpretation Presence of yellow foam at the top indicates presence of bilirubin.

3. *Reagent Strip Test*

Principle It is based on coupling reaction of bilirubin with diazonium salt with which strip is coated. Dip the strip in urine; if it changes to blue colour then bilirubin is present (see Fig. 4.3).

Causes of bilirubinuria

- i) Obstructive jaundice
- ii) Hepatocellular jaundice

Tests for Blood in Urine

Tests for detection of blood in urine are as under :

1. Benzidine test
2. Orthotoluidine test
3. Reagent strip test

1. *Benzidine Test**Procedure*

- Take 2 ml of urine in a test tube.
- Add 2 ml of saturated solution of benzidine with glacial acetic acid.
- Add 1 ml of H_2O_2 to it.

Interpretation Appearance of blue colour indicates presence of blood. Benzidine is, however, carcinogenic and this test is not commonly used.

2. *Orthotoluidine Test**Procedure*

- Take 2 ml of urine in a test tube.
- Add a solution of 1 ml of orthotoluidine in glacial acetic acid.
- Add a few drops of H_2O_2 .

Interpretation Blue or green colour indicates presence of blood in urine.

3. *Reagent Strip Test*

The reagent strip is coated with orthotoluidine. Dip the strip in urine. If it changes to blue colour then blood is present (see Fig. 4.3).

Causes of blood in urine

- i. Renal stones
- ii. Renal tumours
- iii. Polycystic kidney
- iv. Bleeding disorders
- v. Trauma.

AUTOMATED URINALYSIS

Currently, fully automated urine chemistry reagent strip analysers are available which are equipped to perform automatic pipetting or test strip dipping, as well as carry out photometric measurement of reagent strip fields. The end result readings can be taken as a print-out.



Urine Examination II: Microscopy

- ◆ Collection of Sample
- ◆ Preparation of Sediment
- ◆ Examination of Sediment
- ◆ Automation in Urine Analysis

Exercise

5

Microscopic examination of urine is discussed under three headings:

- A. Collection of sample
- B. Preparation of sediment
- C. Examination of sediment
- D. Automation in urine analysis

COLLECTION OF SAMPLE

Early morning sample is the best specimen. It provides an acidic and concentrated sample which preserves the formed elements (RBCs, WBCs and casts) which otherwise tend to lyse in a hypotonic or alkaline urine. The specimen should be examined fresh or within 1-2 hours of collection. But if some delay is anticipated, the sample should be preserved as described in the preceding exercise.

PREPARATION OF SEDIMENT

- Take 5-10 ml of urine in a centrifuge tube.
- Centrifuge for 5 minutes at 3000 rpm.
- Discard the supernatant.
- Resuspend the deposit in a few ml of urine left.
- Place a drop of this on a clean glass slide.
- Place a coverslip over it and examine it under the microscope.

EXAMINATION OF SEDIMENT

Urine is an unstained preparation and its microscopic examination is routinely done under reduced light using the light microscope. This is done by keeping the condenser low with partial closure of diaphragm. First examine it under low power objective, then under high power and keep on changing the fine adjustment in order to visualise the sediments in different planes and

report ascells/HPF (high power field). Phase contrast microscopy may be used for more translucent formed elements. Rarely, polarizing microscopy is used to distinguish crystals and fibres from cellular or protein casts.

Following categories of constituents are frequently reported in the urine on microscopic examination:

1. Cells (RBCs, WBCs, epithelial cells)
2. Casts
3. Crystals
4. Miscellaneous structures

1. Examination of Cells

RBCs

These appear as pale or yellowish, biconcave, double-contoured, disc-like structures, and when viewed from side they have an hour-glass appearance. In hypotonic urine, RBCs swell up while in hypertonic urine they are crenated. They can be confused with WBCs, yeast and air bubbles/oil droplets but can be distinguished as under (Fig. 5.1):

- i. The WBCs are larger in size and are granular.
- ii. Yeast cells appear round but show budding.
- iii. Air bubbles and oil droplets vary in size. When edge of the coverslip is touched with a pencil, oil droplets tumble while RBCs do not.

Significance Normally 0-2 RBCs/HPF may be passed in urine. RBCs in excess of this number are seen in urine in the following conditions:

Physiological

- i. Following severe exercise
- ii. Smoking
- iii. Lumbar lordosis

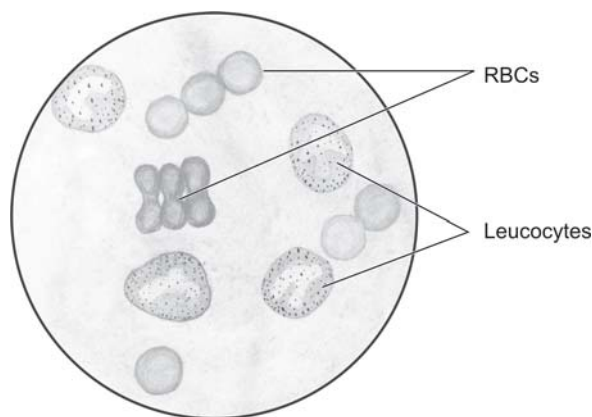


FIGURE 5.1: RBCs and WBCs in the urine sediment.

Pathological

- i. Renal stones
- ii. Tumours
- iii. Glomerulonephritis
- iv. Polycystic kidney
- v. UTI
- vi. Trauma

WBCs

These appear as round granular 12-14 μm in diameter. In fresh urine nuclear details are well visualised (Fig. 5.1). WBCs can be confused with RBCs. For differentiating, add a drop of dilute acetic acid under coverslip. RBCs are lysed while nuclear details of WBCs become more clear. WBCs can also be stained by adding a drop of crystal violet or safranin stain.

Significance Normally 0-4 WBCs/HPF may be present in females. WBCs are seen in urine in following conditions:

Pathological

- i. UTI
- ii. Cystitis
- iii. Prostatitis
- iv. Chronic pyelonephritis
- v. Renal stones
- vi. Renal tumours

Epithelial Cells

These are round to polygonal cells with a round to oval, small to large nucleus. Epithelial cells in urine can be squamous epithelial cells, tubular cells and transitional cells i.e. they can be from lower or upper urinary tract, and sometime it is difficult to distinguish between different types of these cells. At times, these cells can be confused with cancer cells.

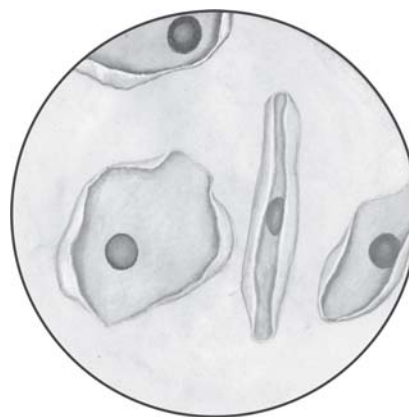


FIGURE 5.2: Squamous epithelial cells in urine, frequently seen in females.

Significance Normally a few epithelial cells are seen in normal urine, more common in females and reflect normal sloughing of these cells (Fig. 5.2).

When these cells are present in large number along with WBCs, they are indicative of inflammation.

2. Casts

These are formed due to moulding in renal tubules of solidified proteins. Their shape depends upon their site of origin. In general casts are cylindrical in shape with rounded ends. The basic composition of casts is Tamm-Horsfall protein which is secreted by tubular cells. Casts appear in urine only in renal diseases.

Depending upon the content, casts are of following types (Fig. 5.3):

- i. Hyaline cast
- ii. Red cell cast
- iii. Leucocyte cast
- iv. Granular cast
- v. Waxy cast
- vi. Fatty cast
- vii. Epithelial cast
- viii. Pigment cast

Hyaline Cast

Hyaline cast is basic protein cast. These are cylindrical, colourless homogeneous and transparent (Fig. 5.3,A). They are passed in urine in the following conditions:

- i. Fever
- ii. Exercise
- iii. Acute glomerulonephritis
- iv. Malignant hypertension
- v. Chronic renal disease

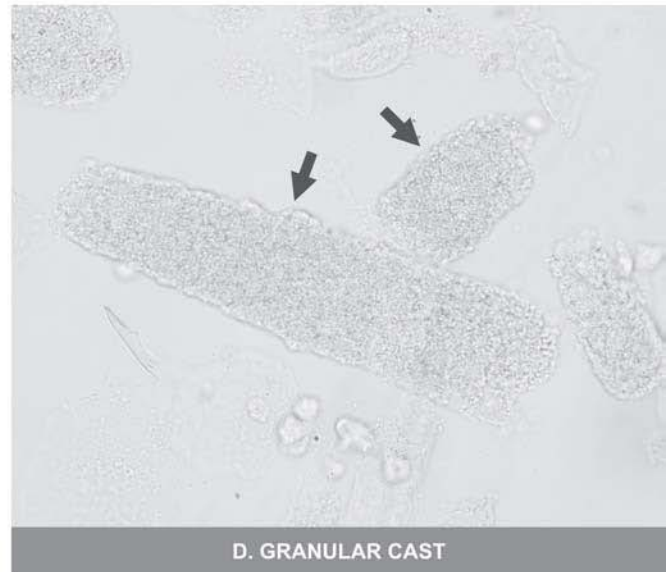
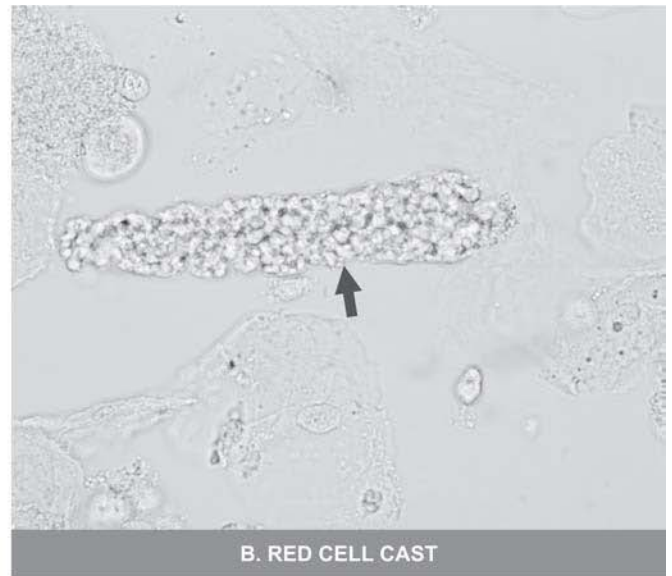


FIGURE 5.3: Various types of casts in urine.

Red Cell Cast

These casts contain RBCs and have a yellowish-orange colour (Fig. 5.3,B). Glomerular damage results in appearance of RBCs into tubules. They are passed in urine in the following conditions:

- i. Acute glomerulonephritis
- ii. Renal infarct
- iii. Goodpasture syndrome
- iv. Lupus nephritis

Leucocyte Cast

These contain granular cells (WBCs) in a clear matrix. WBCs enter the tubular lumina from the interstitium (Fig. 5.3,C). They are passed in urine in the following conditions:

- i. Acute pyelonephritis
- ii. Acute glomerulonephritis
- iii. Nephrotic syndrome
- iv. Lupus nephritis
- v. Interstitial nephritis

Granular Casts

Granular casts have coarse granules in basic matrix. Granules form from degenerating cells or solidification of plasma proteins (Fig. 5.3,D). They are passed in urine in the following conditions:

- i. Pyelonephritis
- ii. Chronic lead poisoning
- iii. Viral diseases
- iv. Renal papillary necrosis

Waxy Casts

Waxy casts are yellowish homogeneous with irregular blunt or cracked ends and have high refractive index. These are also known as renal failure casts. They are passed in urine in the following conditions:

- i. Chronic renal failure
- ii. End-stage kidney
- iii. Renal transplant rejection

Fatty Cast

They contain fat globules of varying size which are highly refractile. Fat in the cast is cholesterol or triglycerides. These are passed in urine in the following conditions:

- i. Nephrotic syndrome
- ii. Fat necrosis

Epithelial Cast

Epithelial casts contains shed off tubular epithelial cells and appear as two parallel rows of cells. Sometimes these are difficult to differentiate from WBC casts. They are passed in urine in following conditions:

- i. Acute tubular necrosis
- ii. Heavy metal poisoning
- iii. Renal transplant rejection

Pigment Cast

Pigment casts include haemoglobin casts, haemosiderin casts, myoglobin casts, bilirubin cast, etc.

Crystals

Formation and appearance of crystals in urine depends upon pH of the urine, i.e. acidic or alkaline.

Crystals in Acidic Urine

These are as under (Fig. 5.4):

- i. Calcium oxalate
- ii. Uric acid

- iii. Amorphous urate
- iv. Tyrosine
- v. Cystine
- vi. Cholesterol crystals
- vii. Sulphonamide

i) Calcium Oxalate

These are colourless refractile and have octahedral envelope-like structure. They can also be dumb-bell shaped (Fig. 5.4,A).

ii) Uric Acid

They are yellow or brown rhomboid-shaped seen singly or in rosettes. They can also be in the form of prism, plates and sheaves (Fig. 5.4,B).

iii) Amorphous Urate

They appear as yellowish brown granules in the form of clumps (Fig. 5.4,C). They dissolve on heating. When they are made of sodium urate, they are needle-like in the form of thorn-apple. They are passed more often in patients having gout.

iv) Tyrosine

They are yellowish in the form of silky needles or sheaves (Fig. 5.4,D). They are passed in urine in jaundice.

v) Cystine

They are colourless, hexagonal plates which are highly refractile (Fig. 5.4,E). They are passed in urine in an inborn error of metabolism, cystinuria.

vi) Cholesterol Crystals

These are rare and are seen in urinary tract infection, rupture of lymphatic into renal pelvis or due to blockage of lymphatics (Fig. 5.4,F).

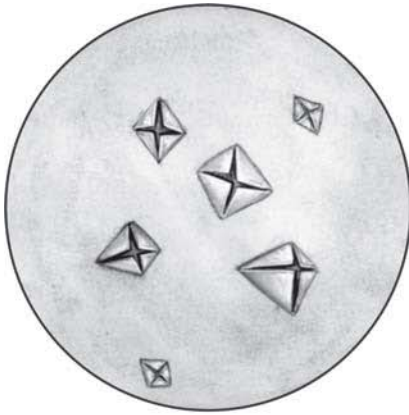
vii) Sulphonamide

They appear as yellowish sheaves, rosettes, or rounded with radial striations (Fig. 5.4,G). They appear in urine after administration of sulphonamide drugs.

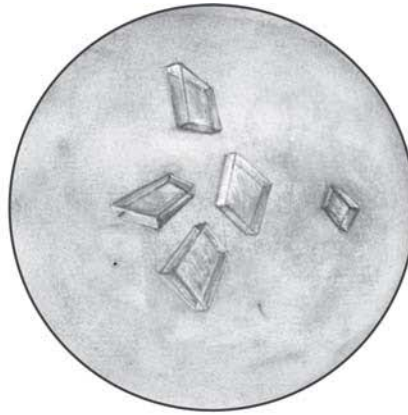
Crystals in Alkaline Urine

These are as under (Fig. 5.5):

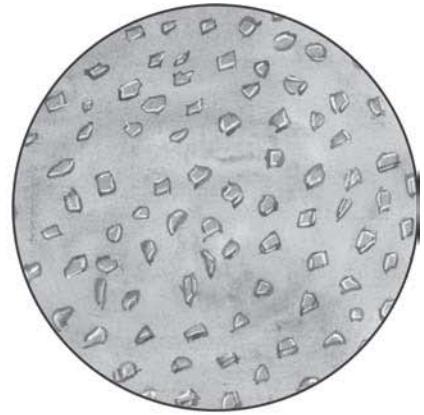
- i. Amorphous phosphate
- ii. Triple phosphate
- iii. Calcium carbonate
- iv. Ammonium biurate



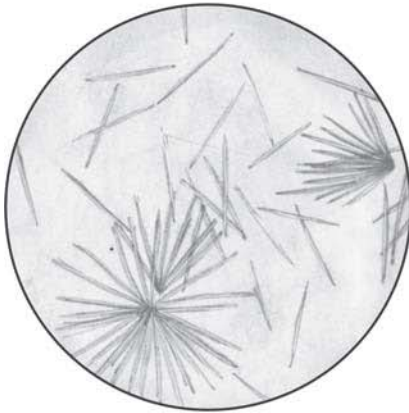
A, CALCIUM OXALATE



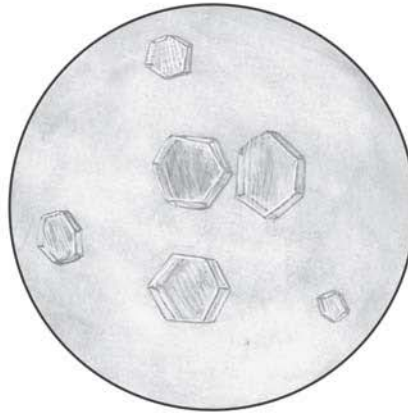
B, URIC ACID



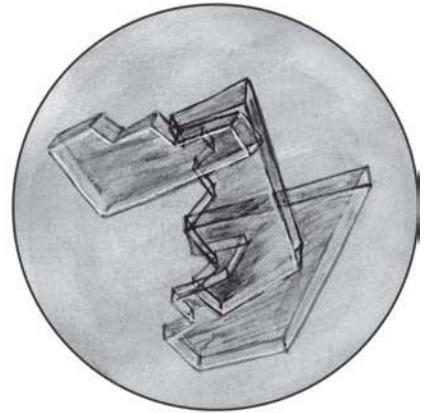
C, AMORPHOUS URATES



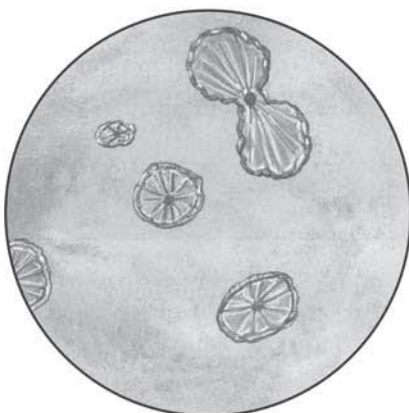
D, TYROSINE



E, CYSTINE

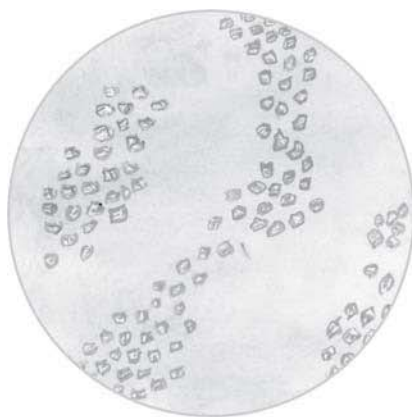


F, CHOLESTEROL

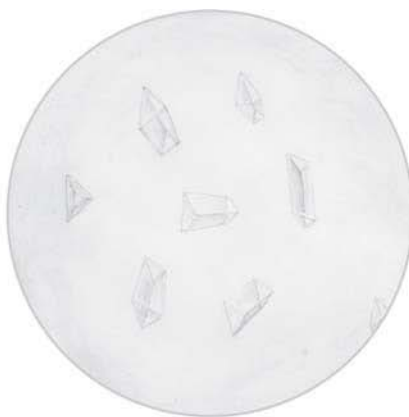


G, SULPHONAMIDE

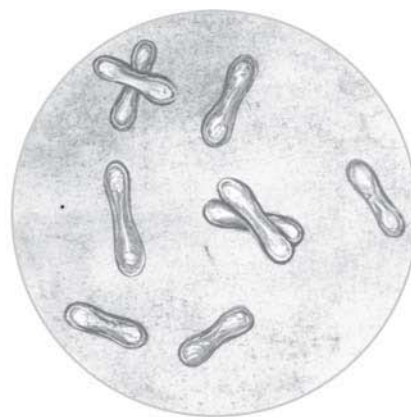
FIGURE 5.4: Various types of crystals in acidic urine.



A, AMORPHOUS PHOSPHATES



B, TRIPLE PHOSPHATES



C, CALCIUM CARBONATE



D, AMMONIUM BIURATES

FIGURE 5.5: Various types of crystals in alkaline urine.*i) Amorphous Phosphate*

They are seen as colourless granules in the form of clumps or irregular aggregates (Fig. 5.5,A). They dissolve when urine is made acidic.

ii) Triple Phosphate

They are in the form of prisms and sometimes in fern leaf pattern (Fig. 5.5,B). They dissolve when urine is made acidic.

iii) Calcium Carbonate

They are in the form of granules, spheres or rarely dumbbell-shaped (Fig. 5.5,C). They again dissolve in acidic urine.

iv) Ammonium Biurate

They are round or oval yellowish-brown spheres with thorns on their surface giving 'thorn apple' appearance (Fig. 5.5,D). They dissolve on heating the urine or by making it acidic.

4. Miscellaneous Structures in Urine

These include the following (Fig. 5.6):

- i. Spermatozoa
- ii. Parasite
- iii. Fungus
- iv. Tumour cells

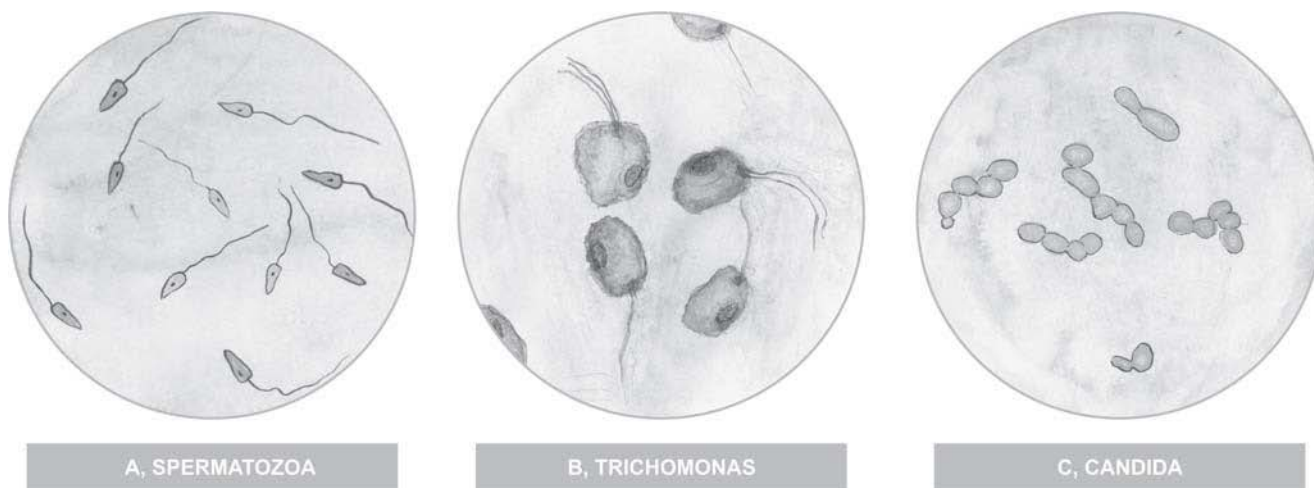


FIGURE 5.6: Miscellaneous structures in urine.

i) Spermatozoa

They can be seen in normal urine in males. They have a head and tail and can be motile (Fig. 5.6,A).

ii) Parasites

Urine may contain *Trichomonas vaginalis* which is more common in females (Fig. 5.6,B). Eggs of *Schistosoma hematobium* or *Entamoeba histolytica* can also be seen in urine.

iii) Fungus

Candida which are budding yeast cells can be seen in urine in patients with UTI or as contaminant (Fig. 5.6,C).

iv) Tumour Cells

Tumour cells having all the characteristics of malignancy may be seen singly or in groups in urine. These tumour cells could be from kidney, ureter, bladder or urethra. These cells are examined after staining of urine

sediment. This subject is discussed in detail later in Exercise 43 of cytology.

AUTOMATION IN URINE ANALYSIS

In the recent times, automated urine analysis has been made possible by one of the following techniques:

Urine Strip Analysers

These are commercially available electronic urine strip readers. These strips may include various parameters of physical, chemical and microscopic constituents. After analysis, the results are obtained as print-out.

Flow Cytometry

Just as flow cytometry is used for blood and other body fluids, urine can be analysed by flow cytometry. In this method, DNA and membranes of formed elements are stained and pass as a laminar flow through a laser beam and the light scatter is measured by fluorescence impedance.



Semen Analysis

- ◆ Sample Collection
- ◆ Gross Examination
- ◆ Microscopic Examination
- ◆ Chemical Examination
- ◆ Immunological Assays
- ◆ Microbiological Assays
- ◆ Sperm Function Tests

Exercise

6

Semen examination is done for the investigation of the following:

1. Infertility
2. Success of vasectomy
3. Medicolegal cases e.g. rape

Analysis of semen consists of the following:

- A. Sample collection
- B. Gross examination
- C. Microscopic examination
- D. Chemical examination
- E. Immunological assays
- F. Sperm function tests
- G. Microbiological assays.

A. SAMPLE COLLECTION

Patient is instructed to collect the specimen by masturbation after 4-7 days of sexual abstinence. The sample is collected in a clean glass tube, wide-mouthed container or in a properly washed dry condom. The sample is submitted in the laboratory immediately but preferably within one hour of collection for examination. Two specimens collected at 2-3 weeks interval are used for evaluation.

B. GROSS EXAMINATION

Semen is examined grossly for the following features:

1. Colour
2. Volume
3. Viscosity
4. Reaction
5. Liquefaction

Colour Normally it is whitish, grey-white or slightly yellowish.

Volume Normally, volume of semen is between 2.5 and 5 ml. The volume is slightly more in patients of infertility. The volume does not vary with the period of abstinence.

Viscosity When ejaculated, semen is fairly viscid and it falls drop by drop.

Reaction Normally it is slightly alkaline with pH between 7 and 8.

Liquefaction Liquefaction occurs because of presence of fibrinolysin. Normally liquefaction occurs within 10-30 minutes.

C. MICROSCOPIC EXAMINATION

Semen is examined microscopically for the following:

1. Motility
2. Count
3. Morphology

Motility

Place a drop of liquefied semen on a clean glass slide. Put a coverslip over it and examine it under the microscope, first under low power and then under high power. Normally within 2 hours of ejaculation more than 60% of spermatozoa are vigorously motile, and in 6-8 hours 25-40% are still motile. If motility is less than 50%, a stain for viability such as eosin Y with nigrosin as counterstain can be done. Red dye accumulates in the heads of non-motile sperms.

Count

This is done in Neubauer's chamber using a WBC pipette. Draw liquefied semen in WBC pipette upto mark 0.5 and then draw the diluting fluid upto mark 11. The composition of diluting fluid is as under:

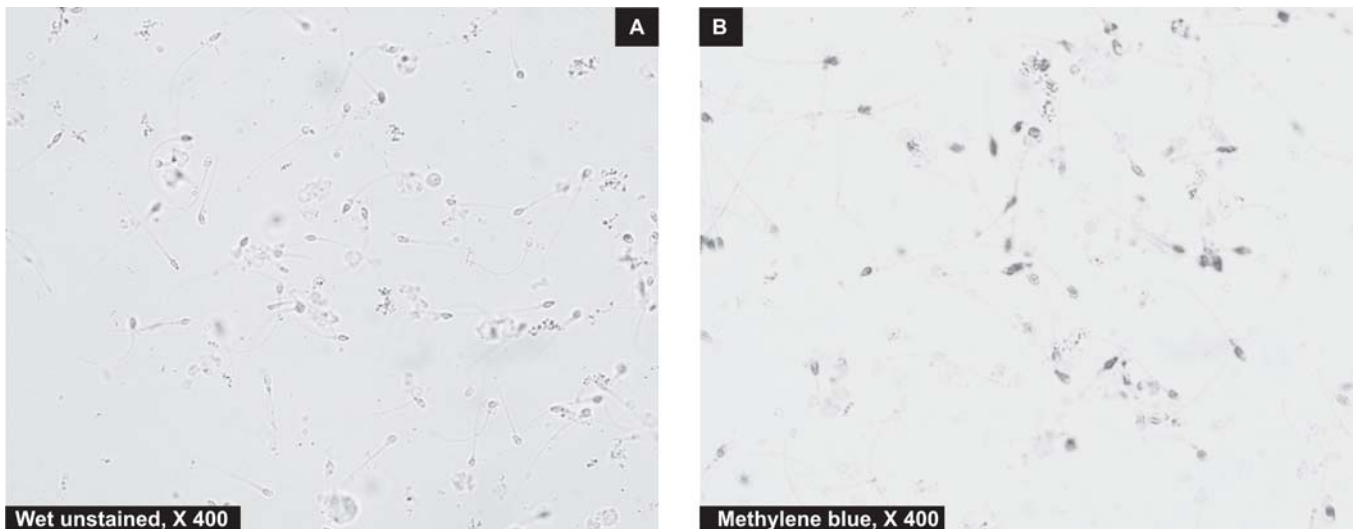


FIGURE 6.1: Appearance of spermatozoa. **A**, Wet unstained **B**, Wet preparation stained with methylene blue.

Sodium bicarbonate	5 gm
Formalin (neutral)	1 ml
Distilled water	100 ml

After mixing it properly, charge the Neubauer's chamber. Allow the spermatozoa to settle down in 2 minutes. Examine under microscope and count the number of spermatozoa in two large peripheral squares (used for TLC counting) and multiply the number by 1 lakh (100,000) which gives number of spermatozoa per millilitre:

$$\begin{aligned}
 &\text{In } 1 \times 1 \times 0.1 \mu\text{l volume,} \\
 &\quad \text{number of spermatozoa} = n \times 10 \\
 &\text{But dilution factor is 10} \\
 \therefore \text{Number of spermatozoa per } \mu\text{l} &= n \times 10 \times 10 \\
 \text{or Number of spermatozoa per ml} &= n \times 10 \times 10 \times 1000 \\
 &= n \times 100,000 \\
 &\text{i.e. } n \times 1 \text{ lakh}
 \end{aligned}$$

(where n is the average number of spermatozoa counted in two squares).

Normal value = > 60 million/ml
Abnormal value = < 20 million/ml.

Morphology

Prepare a thin smear from liquefied semen on a glass slide. Stain it with any of the Romanowsky stains, Pap or

H&E stain. Observe at least 200 spermatozoa for any abnormality in their morphology. Normally 80% of spermatozoa are normal (Fig. 6.1). The abnormal forms of spermatozoa are with double head, swollen and pointed head, double tail and rudimentary forms. Also look for the presence of RBCs or WBCs, if any. Computer-mediated morphologic screening is particularly useful in samples with very low numbers of normal sperm count which may otherwise remain undetected.

D. CHEMICAL EXAMINATION

Chemical analysis of semen consists of the following tests:

1. Fructose test
2. Acid phosphatase test

Fructose Test

This test determines androgen deficiency or ejaculatory obstruction to semen; the level of seminal fructose is low in both these conditions. Normal seminal fructose level is 150-600 mg/dl.

Fructose is measured qualitatively by resorcinol test.

Procedure

- Take 5 ml of dilute HCl in a test tube.
- Add 1 ml of semen.
- Add 5 mg of resorcinol.
- Boil.

Interpretation Appearance of red colour indicates presence of fructose which can be measured by spectrophotometer.

Acid Phosphatase Test

This test is used for seminal stain and on vaginal aspirate in medicolegal cases. Normally semen has 2500 KA units/ml of acid phosphatase.

E. IMMUNOLOGICAL ASSAYS

The presence of sperm antibody binding to head or tail antigens is considered specific for immunologic infertility. The antibodies are usually of immunoglobulin A (IgA) or IgG, and rarely of IgM class. These are detected by direct or indirect mixed agglutination reaction tests.

F. MICROBIOLOGICAL ASSAYS

Genital tract infections by bacteria, yeast and sexually-transmitted diseases may have significant adverse effect on male infertility. If the concentration of bacteria exceeds 1000 CFUs per ml, the colonies should be identified and tested for antibiotic sensitivity.

G. SPERM FUNCTION TESTS

Defective sperm function may affect various fertilising activities such as the transport of sperm in the male and female reproductive tracts and thus events directly related to fertilization such as specific zona binding, penetration and formation of male pronucleus, and accordingly function assays are devised.



Examination of CSF

- ◆ Normal Composition of CSF
- ◆ Specimen Collection
- ◆ Microscopic Examination
- ◆ Chemical Examination
- ◆ Microbiological Examination
- ◆ Immunological Examination

Exercise

7

Seventy per cent of CSF is produced by choroid plexus in the lateral, third and fourth ventricle while the remainder is produced by the surface of brain and spinal cord. CSF examination is an important part of neurologic evaluation in non-neoplastic and neoplastic diseases of CNS.

NORMAL COMPOSITION OF CSF

Appearance	:	Clear and colourless
Rate of production	:	500 ml/day
Total volume	:	120-150 ml in adults, 10-60 ml in neonates
Specific gravity	:	1.006-1.008
Normal pressure	:	60-150 mm of water in adults, 10-100 mm water in infants
Sugar	:	50-80 mg/dl (i.e. 60% of plasma value)
CSF: Plasma glucose ratio	:	0.3-0.9
Proteins	:	15-45 mg/dl
Chloride	:	720-760 mg% (i.e. same as in plasma)
Cells	:	0-4 leucocytes/ml (0-30 leucocytes/ml in neonates)
Bacteria	:	Nil

Examination of CSF is discussed under the following headings:

- A. Specimen collection
- B. Microscopic examination
- C. Chemical examination
- D. Microbiological examination
- E. Immunological examination

A. SPECIMEN COLLECTION

CSF is obtained by:

- i. Lumbar puncture

- ii. Cisternal puncture
- iii. Ventricular cannulas or shunts
- iv. Lateral cervical puncture

Normally upto 2 ml of CSF is withdrawn. Most often, CSF tap is done by lumbar puncture for which indications can be divided into following 4 categories:

- a. Meningeal infection
- b. Subarachnoid haemorrhage
- c. CNS malignant tumours
- d. Demyelinating diseases.

The specimen should be transported to the laboratory immediately and processed within one hour otherwise cellular degradation occurs giving incorrect results. In case delay in examination of CSF is anticipated, the sample may be refrigerated, except for microbial culture. Various parameters of normal CSF in comparison with various types of meningitis are given in Table 7.1.

B. MICROSCOPIC EXAMINATION

It involves TLC and DLC in the CSF.

Total Leucocyte Count

TLC in CSF can be done by:

- i. Manual method
- ii. Automated method

Manual Method

Diluting fluid It has the following composition:

Crystal violet	0.1 gm
Glacial acetic acid	5 ml
Distilled water	45 ml

Take a RBC pipette; draw diluting fluid upto mark 1 and then draw CSF upto mark 101. Charge the Fuchs-Rosenthal chamber and count the cells in all 9 squares

(used for counting RBCs as well as WBCs). The result is expressed as:

$$\text{Cells per } \mu\text{l} = \text{Number of cells} \times 1.1$$

Automated Method

With automated method if counts are low the results are not good. The method employs use of electronic particle counter as for TLC in blood.

Differential Leucocyte Count

Centrifuge or cytocentrifuge a small amount of CSF; prepare smears from the sediment. Stain one of the smears with any of the Romanowsky stain and examine under high power and oil immersion of microscope for the presence of various cells. The various cells which may be seen in CSF are:

- i. neutrophils,
- ii. lymphocytes,
- iii. plasma cells,
- iv. monocytes, and
- v. malignant cells.

Conditions Causing Increased Neutrophils in CSF

- i. Bacterial meningitis
- ii. Brain abscess
- iii. Brain infarct
- iv. Repeated lumbar puncture

Conditions Causing Increased Lymphocytes in CSF

- i. Viral meningitis

- ii. Tuberculous meningitis
- iii. Parasitic meningitis
- iv. Fungal infections

Conditions Causing Plasma Cells in CSF

- i. Tuberculous meningitis
- ii. Syphilitic meningoencephalitis
- iii. Multiple myeloma
- iv. Malignant brain tumours

Conditions Causing Lymphocytes and Monocytes in CSF

- i. Viral meningitis
- ii. Degenerative brain disorders
- iii. Tuberculous meningitis
- iv. Fungal meningitis
- v. Sarcoidosis of meninges

Conditions Causing Malignant Cells in CSF

- i. Metastatic cancers
- ii. Leukaemias
- iii. Lymphomas
- iv. Medulloblastoma
- v. Ependymoma

C. CHEMICAL EXAMINATION

Sugar, proteins, chloride and enzymes, ammonia and amines, electrolytes and acid-base balance and tumour markers can be estimated in CSF. CSF findings in various types of meningitis are summarised in Table 7.1.

Table 7.1: CSF findings in health and various types of meningitis.

Feature	Normal	Acute pyogenic (bacterial meningitis)	Acute lymphocytic (viral meningitis)	Chronic (tuberculosis meningitis)
1. Naked eye appearance	Clear and colourless	Cloudy or frankly purulent	Clear or slightly turbid	Clear or slightly turbid, forms fibrin coagulum on standing
2. CSF pressure	60-150 mm water	Elevated (above 180 mm water)	Elevated (above 250 mm water)	Elevated (above 300 mm water)
3. Cells	0-4 lymphocytes/ml	1,000-100,000 neutrophils/ml	10-100 mononuclears/ml	100-1000 mononuclears/ml
4. Proteins	15-45 mg/dl	Raised	Raised	Raised
5. Glucose	50-80 mg/dl	Reduced (usually less than 40 mg/dl)	Normal	Reduced (usually less than 45 mg/dl)
6. Bacteriology	Sterile	Causative organisms present	Sterile	Tubercle bacilli present

D. MICROBIOLOGICAL EXAMINATION

Smears from CSF can also be stained with Gram's stain for bacteria and Ziehl Neelsen's stain for AFB and India ink for the capsule of Cryptococcus.

CSF can be subjected to culture of the following:

- i. Bacteria
- ii. Tubercle bacilli
- iii. Fungus

E. IMMUNOLOGICAL EXAMINATION

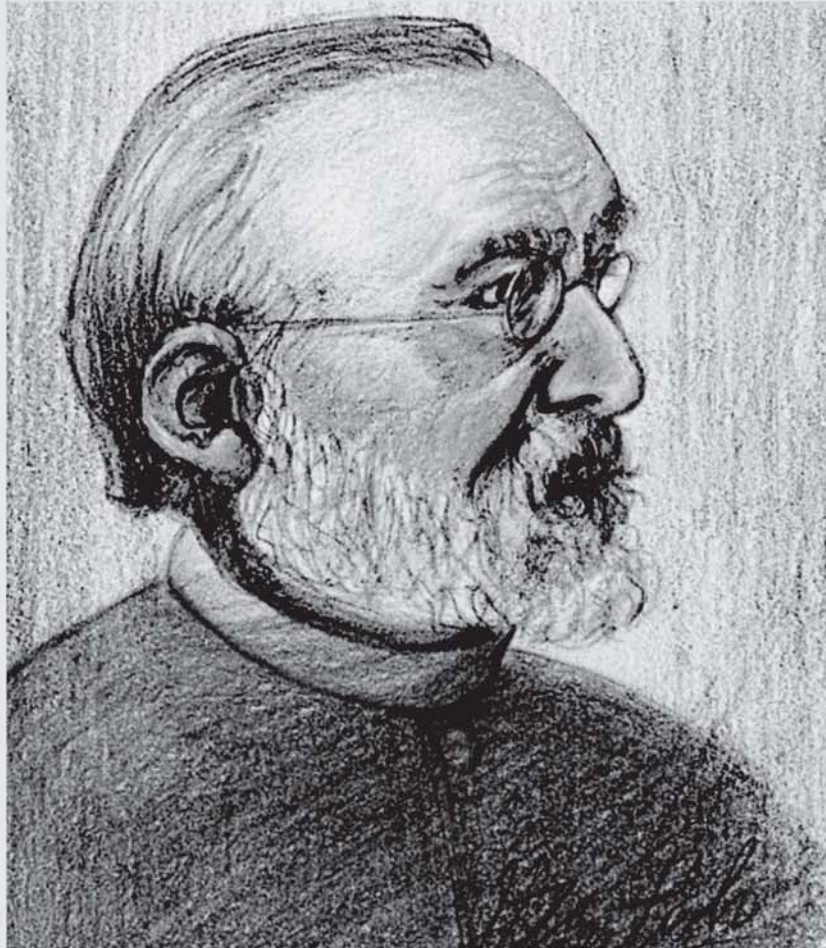
CSF can be required for demonstration of the following:

- i. Viral inclusions by immunostains
- ii. PCR for viral DNA and tuberculosis
- iii. ELISA for tuberculosis
- iv. VDRL for syphilis.



SECTION THREE

GENERAL PATHOLOGY



RUDOLF VIRCHOW (1821-1902)
'FATHER OF MODERN PATHOLOGY'

German physician, who described cellular basis of disease and introduced histopathology as a diagnostic branch. Important discoveries going by his name include: Virchow cells (lepra cell), Virchow node (enlarged left supraclavicular lymph node in cancer of the stomach), Virchow space in brain tissue, and Virchow's triad in pathogenesis of thrombosis.

SECTION III: GENERAL PATHOLOGY

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- * Learn to describe and identify museum specimens of common conditions of general pathology encountered by an undergraduate student.
- * Learn to describe, draw and identify common conditions of general pathology on microsections.

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Degenerations

- ◆ Vacuolar Nephropathy
- ◆ Hyaline Change in Leiomyoma
- ◆ Myxoid Degeneration in Ganglion

Exercise

8

VACUOLAR NEPHROPATHY

Vacuolar nephropathy is the initial or reversible change seen grossly as "cloudy change" and microscopically as well-defined "hydropic vacuoles" in the convoluted tubules in the kidney. Most commonly, it is due to hypokalaemia e.g. due to intrinsic renal disease, ulcerative colitis, diabetic ketoacidosis, use of diuretics etc.

G/A The kidneys are slightly enlarged and pale due to compression of blood vessels. The cut surface has cloudy, opaque appearance.

M/E

- The epithelial cells of proximal convoluted tubules

show small clear well-defined vacuoles in their cytoplasm.

- These vacuoles are watery in character (hydropic change) and do not stain for fat or glycogen; they represent distended endoplasmic reticulum.
- The tubular epithelial cells are ballooned out and the nuclei are pushed to the base of cells (Fig. 8.1).

HYALINE CHANGE IN LEIOMYOMA

The word hyaline simply refers to morphologic appearance of the material that has glassy, pink, homogeneous appearance when routinely stained with haematoxylin and eosin. Hyaline change (or hyalinisation) represents

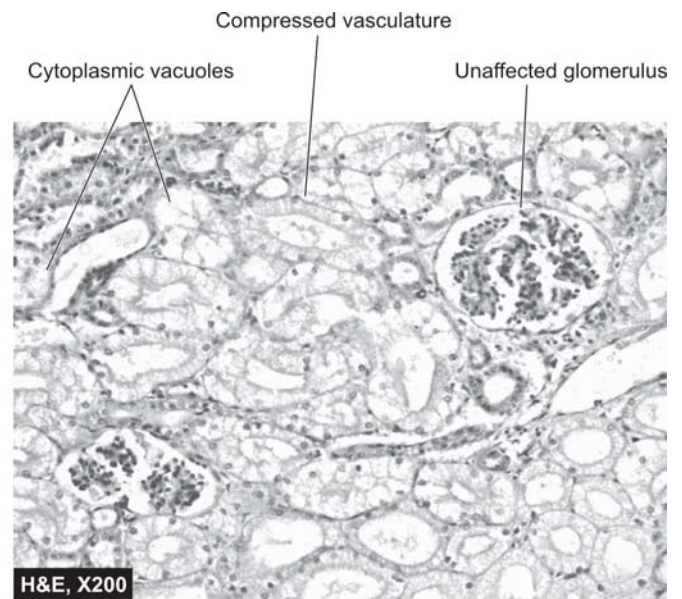
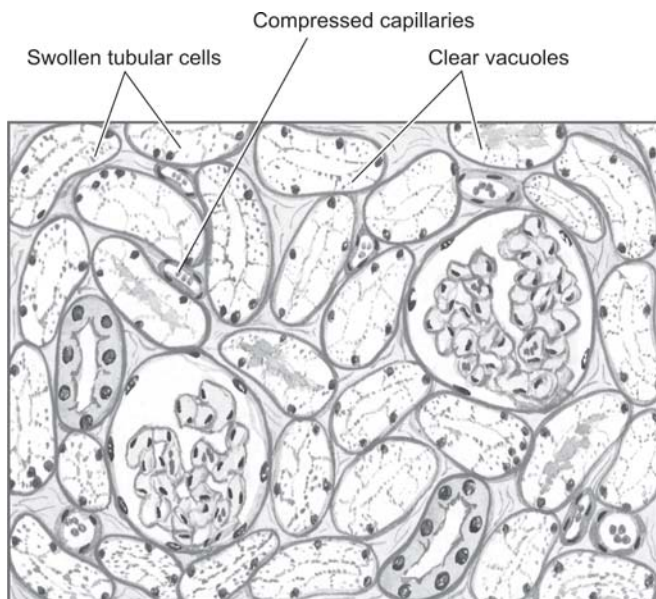


FIGURE 8.1: Vacuolar nephropathy. The tubular epithelial cells are distended with cytoplasmic vacuoles while the interstitial vasculature is compressed.

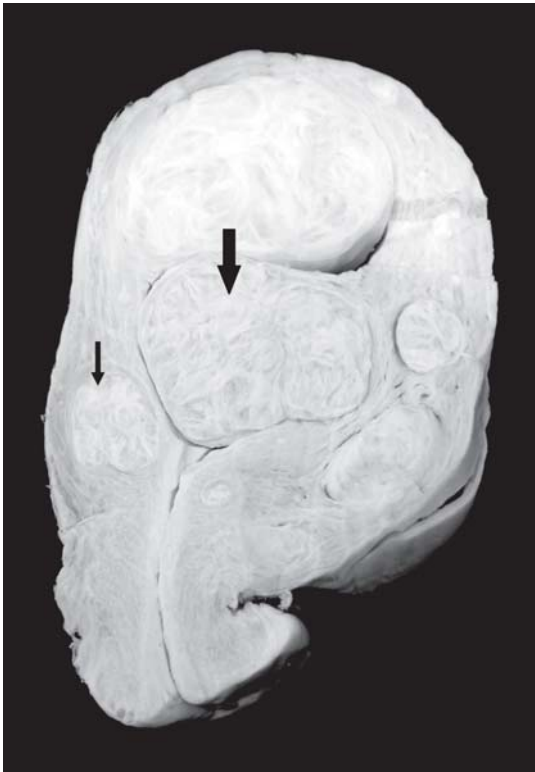


FIGURE 8.2: Leiomyomas uterus. Sectioned surface shows multiple circumscribed, firm nodular masses of variable sizes, submucosal (thick arrow) and intramural (thin arrow) in location.

an end-stage of many diverse and unrelated lesions. It may be intracellular or extracellular. Hyaline degeneration in leiomyoma, a benign smooth muscle tumour, is an example of extracellular hyaline in the connective tissue. Uterine leiomyomas may be subserosal, intramural or submucosal.

G/A The tumour is circumscribed, firm to hard. Cut surface presents a whorled appearance. The hyalinised area in the tumour appears glassy and homogeneous (Fig. 8.2).

M/E

- There is mixture of smooth muscle fibres and fibrous tissue in varying proportions. Some of the muscle fibres may be cut longitudinally and some transversely.
- Whorled arrangement of muscle fibres admixed with fibrous tissue is seen at places.
- Nuclei of the smooth muscle fibres are short, plump and fusiform while those of the fibroblasts are longer, slender and curved (Fig. 8.3).
- Hyaline degeneration which is the commonest change due to insufficient blood supply appears as pink, homogeneous and acellular.

MYXOID DEGENERATION IN GANGLION

While mucus is the normal watery secretion of mucous glands (epithelial mucin) or by certain connective tissue

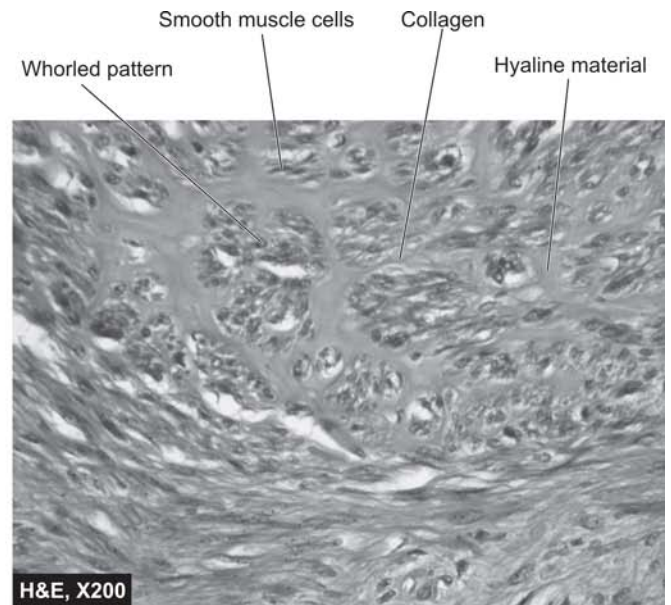
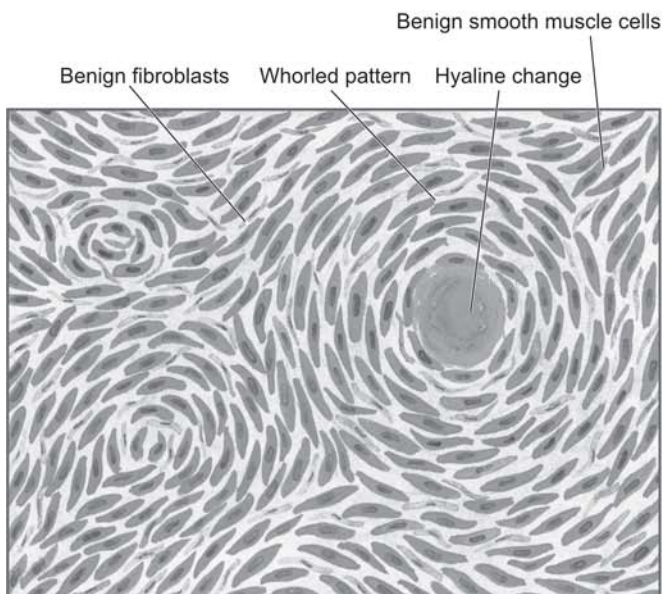


FIGURE 8.3: Leiomyomas. Microscopy shows whorls of smooth muscle cells which are spindle-shaped, having abundant cytoplasm and oval nuclei and mixed with fibrous tissue. The hyaline material appears homogeneous, pink and glassy.

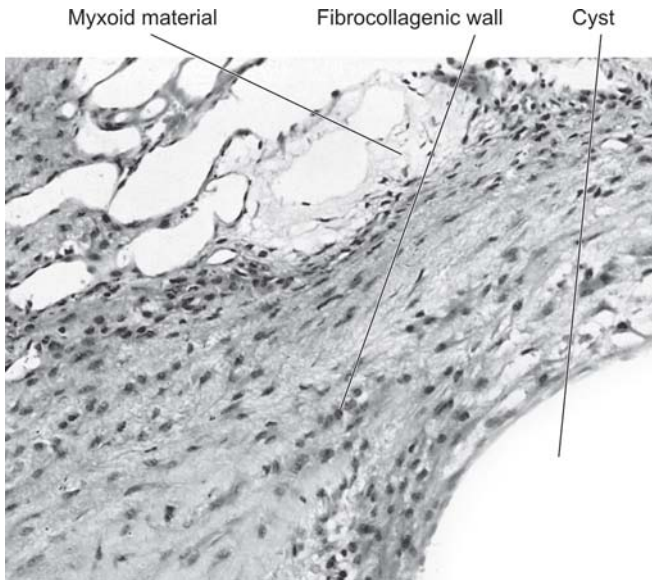


FIGURE 8.4: Cyst of ganglion. It shows a fibrous wall without any distinct lining. The wall shows areas of basophilic myxoid material.

cells, especially in foetus (connective tissue mucin), myxoid or muroid degeneration refers to exaggerated form of the process and may involve both these types of mucin. In the early stages of a ganglion of the wrist, connective tissue mucin develops in the synovial membrane in connection with the tendon sheath.

G/A The lesion appears most commonly as a cyst containing soft muroid material.

M/E

- i. The cyst of ganglion is composed of fibrous wall devoid of any specialised lining.
- ii. Centre of the cyst contains a mass of acellular basophilic myxoid material (Fig. 8.4).
- iii. Connective tissue mucin stains positively with colloidal iron and alcian blue.



Intracellular Accumulations

- ◆ Fatty Change Liver
- ◆ Melanin Pigment in Naevus
- ◆ Anthracotic Pigment in Lung
- ◆ Brown Atrophy Heart

Exercise

9

FATTY CHANGE LIVER

Fatty change (steatosis) is seen most commonly in the liver since it is the major organ involved in fat metabolism. The causes include alcohol abuse (most common cause in industrialised world), protein malnutrition, obesity, diabetes mellitus, anoxia, and various toxins (carbon tetrachloride, chloroform, ether, etc).

G/A The liver is enlarged and yellow with tense, glistening capsule and rounded margins. The cut surface bulges slightly and is pale-yellow and greasy to touch (Fig. 9.1).

M/E

- Fat in the cytoplasm of the hepatocytes is seen as clear area which may vary from minute droplets in the cytoplasm of a few hepatocytes (*microvesicular*) to distention of the entire cytoplasm of most cells by coalesced droplets (*macrovesicular*) pushing the nucleus to periphery of the cell.
- When the steatosis is mild, centrilobular hepatocytes are mainly affected, while the progressive accumulation of fat involves the entire lobule.
- Occasionally, the adjacent cells containing fat rupture producing fatty cysts (Fig. 9.2).
- Infrequently, lipogranulomas may appear consisting of collection of macrophages, lymphocytes and multinucleate giant cells.
- Special stains such as Sudan III, Sudan IV, Sudan black and oil red O can be employed to demonstrate fat in the tissue.

MELANIN PIGMENT IN NAEVUS

Common moles or naevi (more appropriately termed nevocellular naevi) are the common benign neoplasms of the skin arising from melanocytes. There are numerous

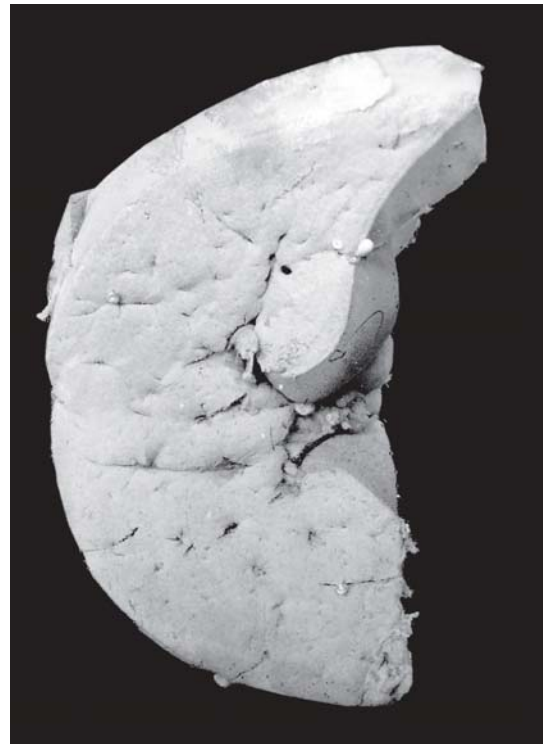


FIGURE 9.1: Fatty liver. The organ is enlarged. The external surface shows tense capsule, rounded margins and pale-yellow colour. Cut surface shows yellow parenchyma greasy to touch.

clinical and histologic types of nevocellular naevi and have variable clinical appearance.

G/A Grossly and clinically, a mole initially appears as a small tan dot 0.1-0.2 cm in diameter but subsequently enlarges to a uniform coloured tan to brown area which may be flat or slightly elevated and having regular, circular or oval outline.

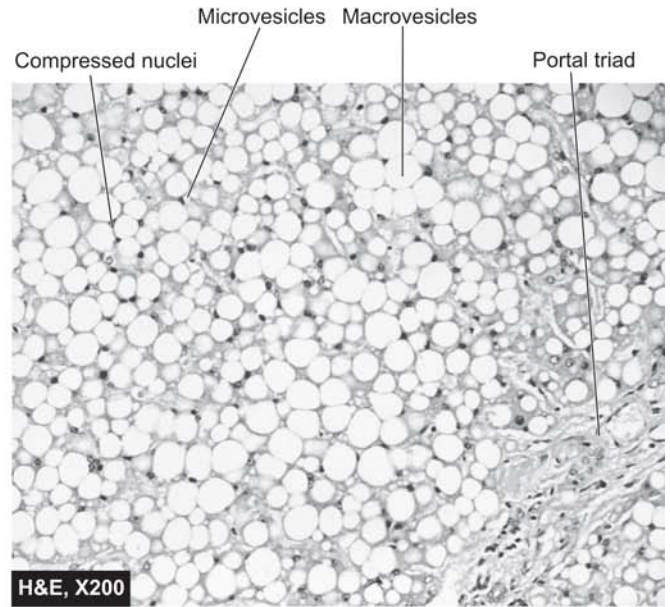
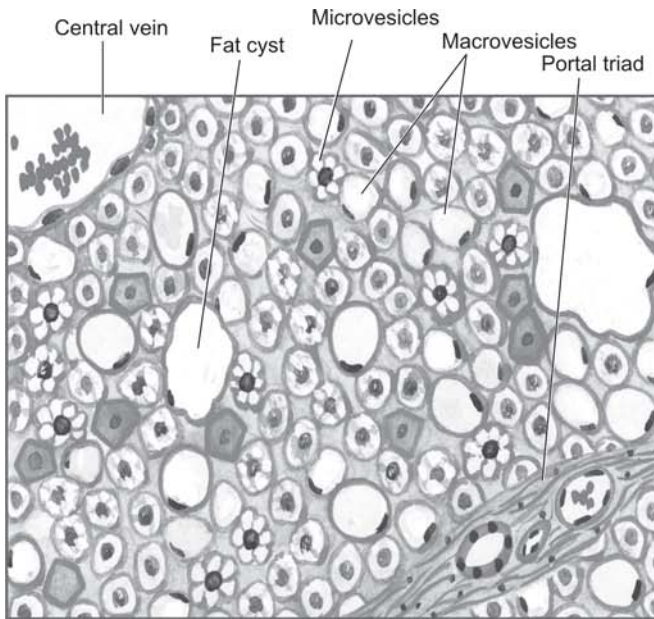


FIGURE 9.2: Fatty change liver. Many of the hepatocytes are distended with fat vacuoles pushing the nuclei to the periphery, while others show multiple small vacuoles in the cytoplasm.

M/E

- i. The lesion is composed of melanocytes forming aggregates or nests at the dermo-epidermal junction (junctional naevus) which subsequently migrate to the underlying dermis (compound naevus). The older lesions may be entirely confined to dermis (dermal naevus).
- ii. The melanocytes forming naevi are round to oval cells and have round or oval nuclei. The cytoplasm of naevus cells is homogeneous and contains abundant granular brown-black melanin pigment (Fig. 9.3).
- iii. The pigment is more marked in the naevus cells in the lower epidermis and upper dermis but the cells

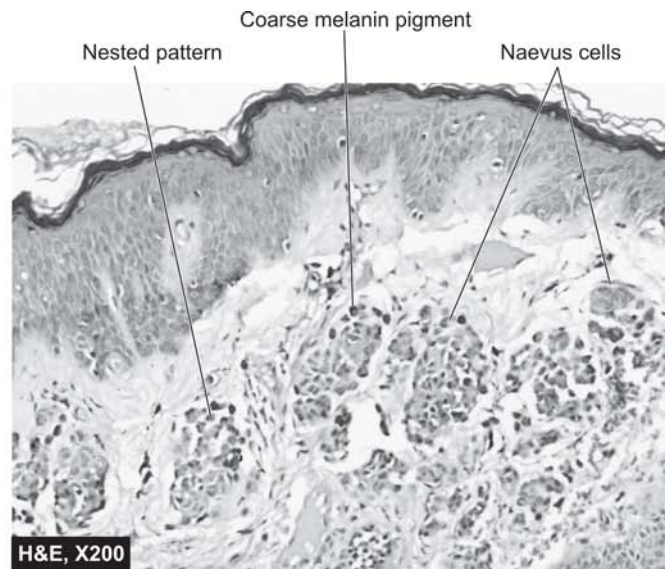
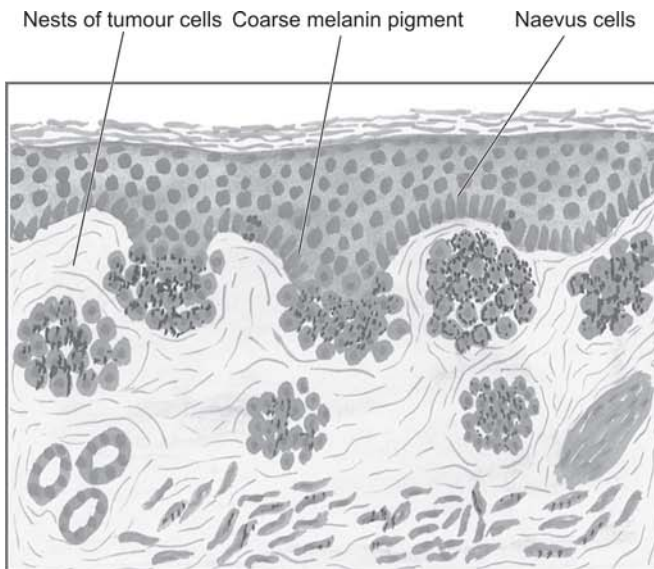


FIGURE 9.3: Intradermal naevus. Melanin-containing naevus cells form clusters in the upper dermis.

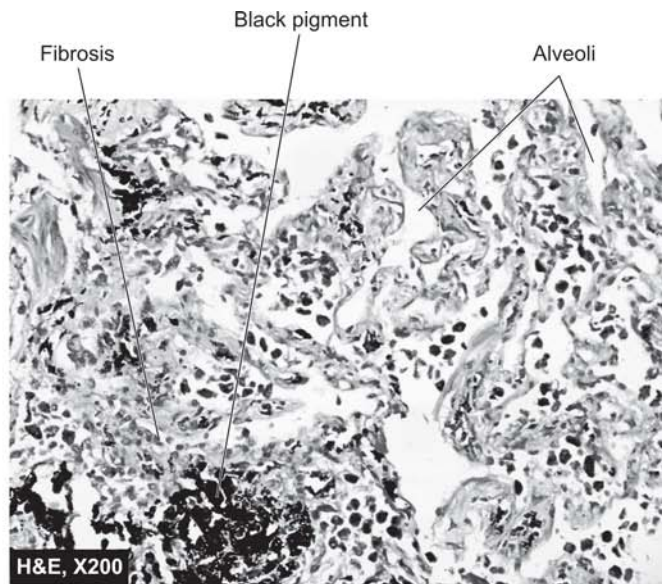
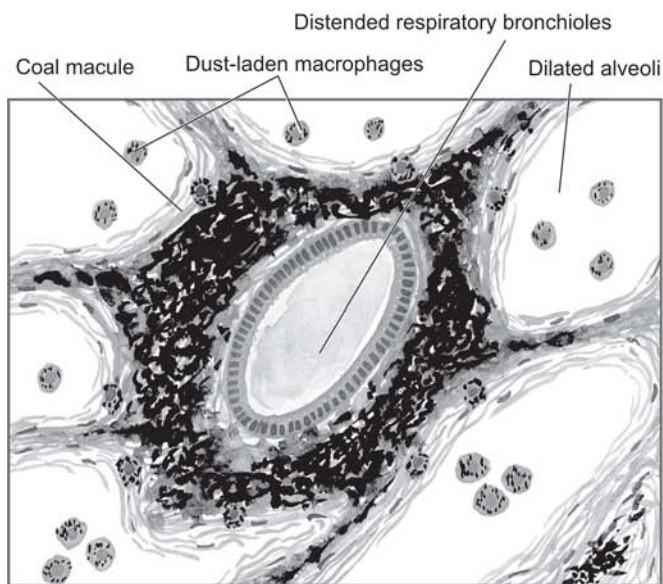


FIGURE 9.4: Histologic appearance of lung in coal-workers' pneumoconiosis. A coal macule composed of aggregates of dust-laden macrophages is seen surrounding a respiratory bronchiole. The alveoli and respiratory bronchioles surrounding the coal macule are distended.

in the mid-dermis and lower dermis hardly contain any pigment.

ANTHRACOTIC PIGMENT IN LUNG

The common, benign and asymptomatic deposition of carbon dust in the lungs of most urban dwellers due to

atmospheric pollution and cigarette smoke is termed anthracosis of the lung. Anthracosis is thus not a lung disease in true sense. However, coal-miners may inhale very large amount of particulate carbon and develop a severe form of anthracosis called coal-miners' pneumoconiosis.

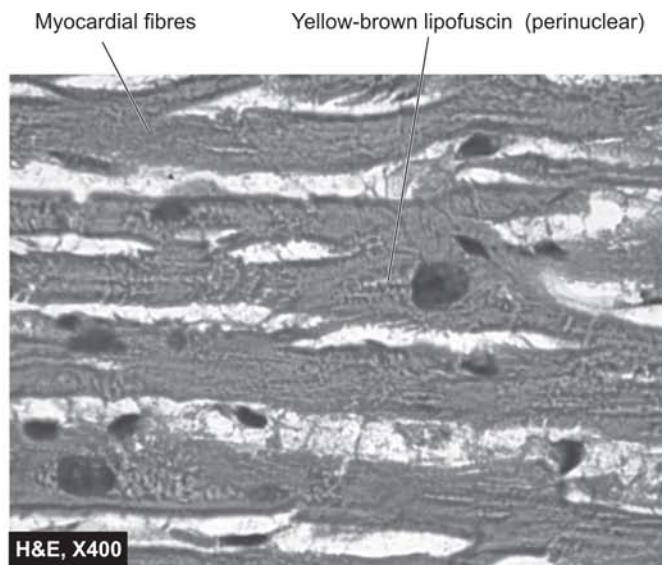
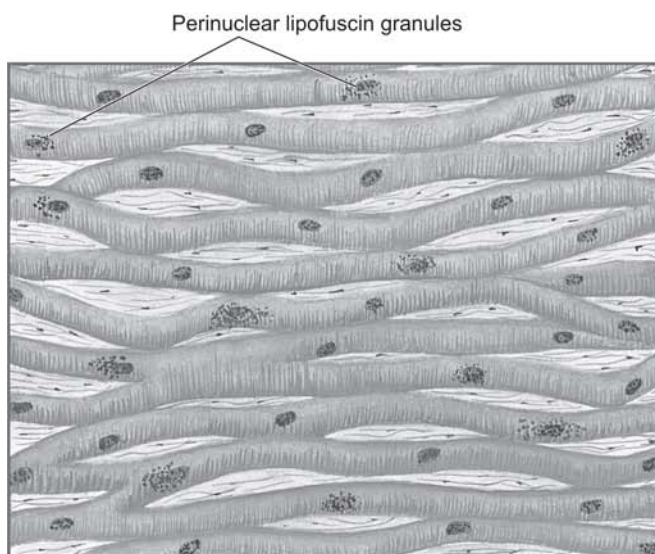


FIGURE 9.5: Brown atrophy of the heart. The lipofuscin pigment granules are seen in the cytoplasm of the myocardial fibres, especially at the poles of nuclei.

G/A The lungs as well as the involved hilar lymph nodes are black in colour. The cut section shows blackish mottling due to aggregates of anthracotic pigment.

M/E

- i. Anthracotic pigment, black in colour, is deposited in the macrophages around respiratory bronchioles, beneath the pleura, and in the hilar lymph nodes.
- ii. While anthracosis is not associated with any significant loss of pulmonary function, coal-workers' pneumoconiosis is characterised by coal macule and coal nodule consisting of carbon-laden macrophages admixed with some amount of collagen (Fig. 9.4).

BROWN ATROPHY HEART

Brown atrophy of the heart is the term used for intracellular accumulation of yellowish-brown lipid

pigment called lipofuscin (*lipo* = fat, *fuscus* = brown) in the myocardial fibres. This pigment is also known as lipochrome or wear and tear or aging pigment.

G/A The change is seen in the heart of aging patients or patients with severe malnutrition and cancer cachexia. The heart is small in weight and light brown in colour.

M/E

- i. The myocardial fibres contain yellow-brown, finely granular, intracytoplasmic pigment, often in perinuclear location.
- ii. The myocardial fibres show changes of atrophy (Fig. 9.5).
- iii. Lipofuscin can be stained by fat stains but differs from other lipids in being fluorescent and acid fast.



Amyloidosis

- ◆ Amyloidosis Kidney
- ◆ Amyloidosis Spleen
- ◆ Amyloidosis Liver

Exercise

10

AMYLOIDOSIS KIDNEY

Amyloidosis of the kidney is most common and most serious because of its ill-effects on renal function. The deposits in the kidneys are found in most cases of secondary amyloidosis and in about one-third cases of primary amyloidosis.

G/A The kidneys may appear normal, enlarged or terminally contracted due to ischaemic effect of narrowing of vascular lumina. The cut surface is pale, waxy and translucent (Fig. 10.1).

M/E

- The amyloid is seen as amorphous, eosinophilic, hyaline extracellular material. It is deposited mainly in the *glomeruli*, initially on the glomerular basement membrane but later extends to produce luminal

narrowing and distortion of the glomerular capillary tuft.

- The amyloid deposits in the *tubules* begin close to the tubular epithelial basement membrane. Subsequently, the deposits may produce degenerative changes in the tubular epithelial cells and amyloid casts in the tubular lumina.
- The walls of small *arteries and arterioles* in the interstitium of the kidney are narrowed due to amyloid deposit (Fig. 10.2).
- Congo red staining* imparts pink or red colour to the amyloid when seen in ordinary light but demonstrates green birefringence when viewed under polarising microscopy (Fig. 10.3).

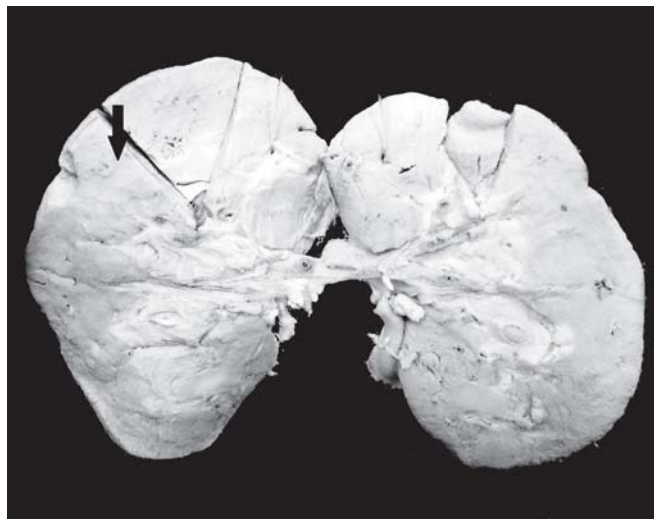


FIGURE 10.1: Amyloidosis of kidney. The kidney is small and pale in colour. Cut section shows pale waxy translucency with loss of cortico-medullary distinction (arrow).

AMYLOIDOSIS SPLEEN

Splenic amyloid may have two patterns—one associated primarily with deposition in the stroma of the red pulp (*lardaceous spleen*) and the second with in the stroma of the white pulp (*sago spleen*).

G/A The spleen may be normal-sized or may cause moderate to marked splenomegaly. The cut surface of the spleen shows one of the two patterns of deposition—*lardaceous spleen* characterised by diffuse map-like areas of pale, waxy translucency (Fig. 10.4) or, alternatively, *sago spleen* seen as multiple pale foci corresponding to the regions of splenic follicles.

M/E

- In *lardaceous spleen* the amyloid deposits are seen in the walls of splenic sinuses and the region of the red pulp.
- In *sago spleen*, the amyloid deposits begin in the walls of the arterioles of the white pulp and eventually replacing the splenic follicles (Fig. 10.5).

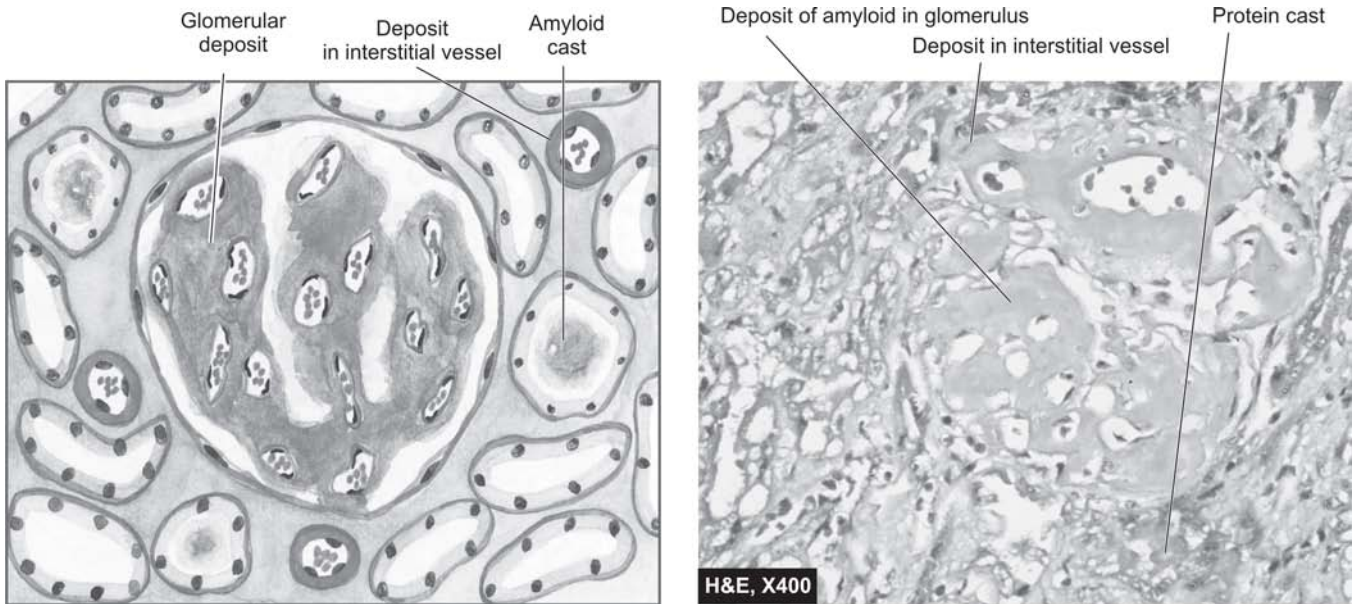


FIGURE 10.2: Amyloidosis kidney. The amyloid deposits are seen mainly in the glomerular capillary tuft.

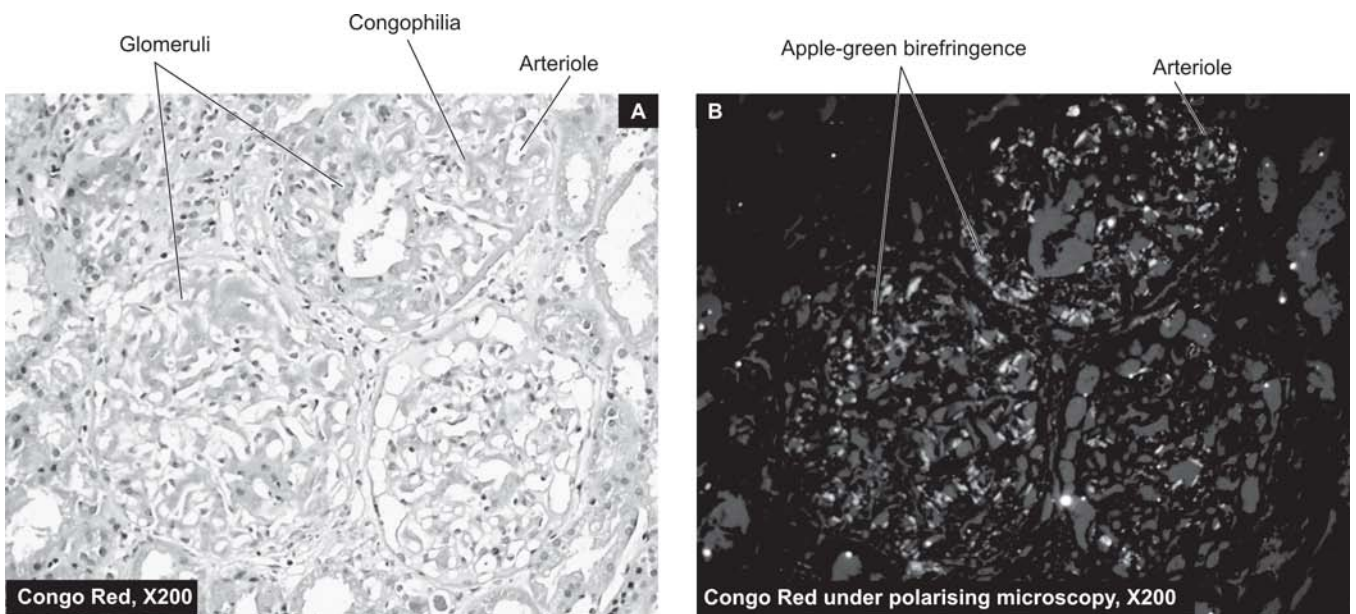


FIGURE 10.3: Amyloidosis kidney, Congo Red stain. **A**, The amyloid deposits are seen mainly in the glomerular capillary tuft showing red pink color. **B**, When viewed under polarising microscopy, it shows green birefringence.



FIGURE 10.4: Lardaceous amyloidosis spleen. The sectioned surface shows presence of pale waxy translucency seen as map-like areas (arrow).

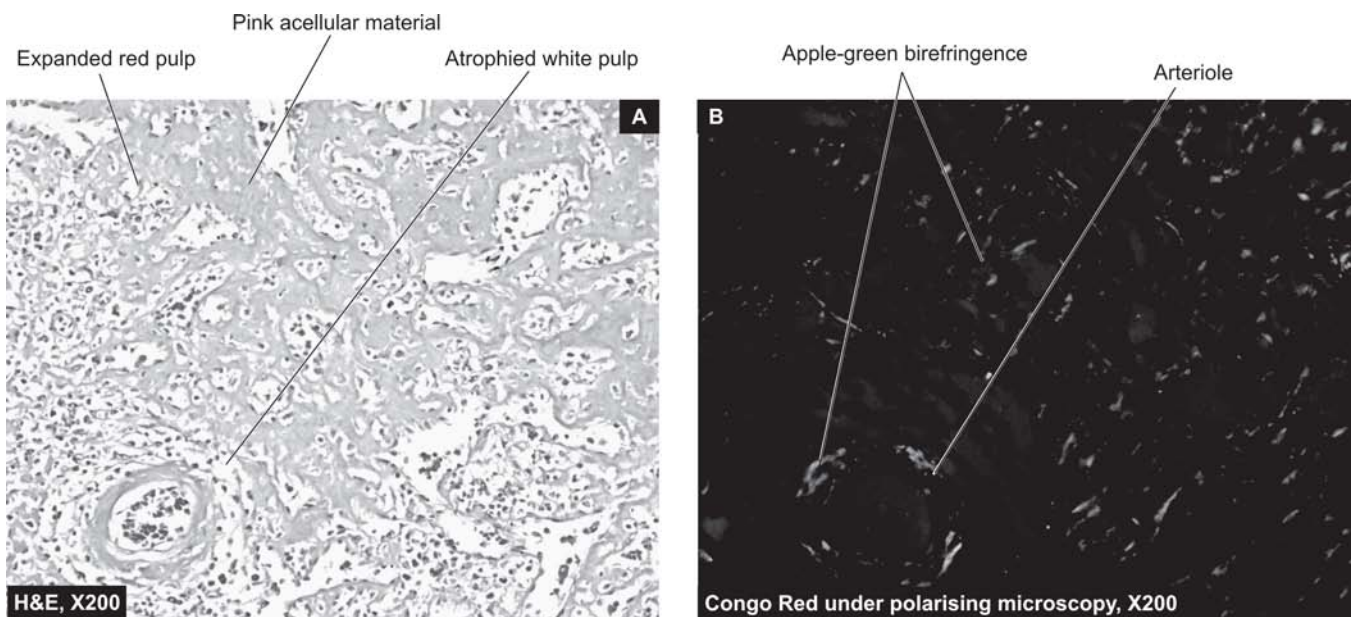


FIGURE 10.5: Amyloidosis spleen. **A**, The pink acellular amyloid material is seen in the red pulp causing atrophy of white pulp. **B**, Congo-Red staining seen under polarising microscopy shows apple-green birefringence in the amyloid containing areas.

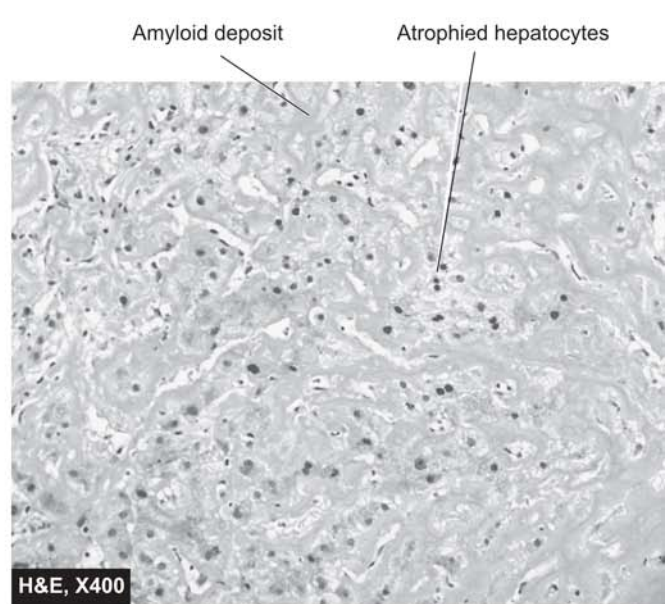
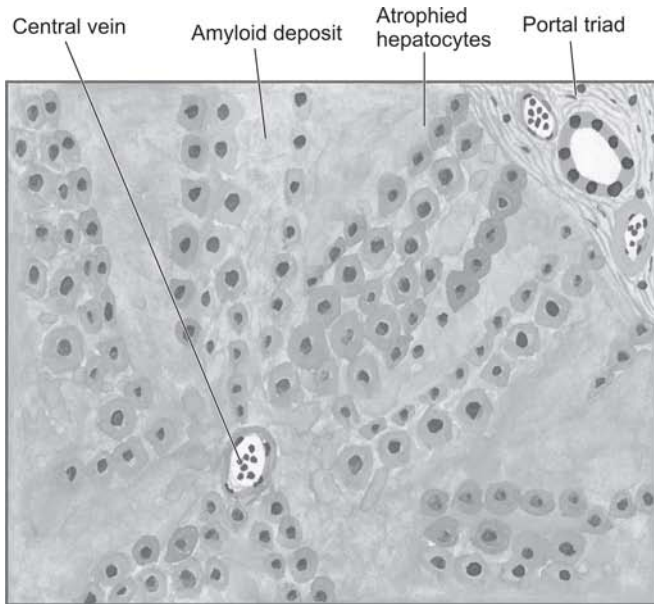


FIGURE 10.6: Amyloidosis of the liver. The deposition is extensive in the space of Disse causing compression and pressure atrophy of hepatocytes.

- iii. *Congo red staining* gives pink or red colour to amyloid by light microscopy and when viewed in polarising light shows green birefringence (Fig. 10.5,B).

AMYLOIDOSIS LIVER

Involvement of the liver is seen in about half the cases of systemic amyloidosis.

G/A Amyloidosis may not be apparent or may cause moderate to marked enlargement. The cut surface is pale, waxy and translucent.

M/E

- i. The amyloid deposits begins in the space of Disse obliterating the space that lies between the hepatocytes and sinusoidal endothelial cells.
- ii. Later, the deposits compress the cords of hepatocytes resulting in atrophy and shrinkage of liver cells and replace the cords of hepatocytes by amyloid (Fig. 10.6).
- iii. Congo red staining demonstrates pink red colour to amyloid by light microscopy and shows green birefringence under viewed under polarising microscopy (Fig. 10.7).

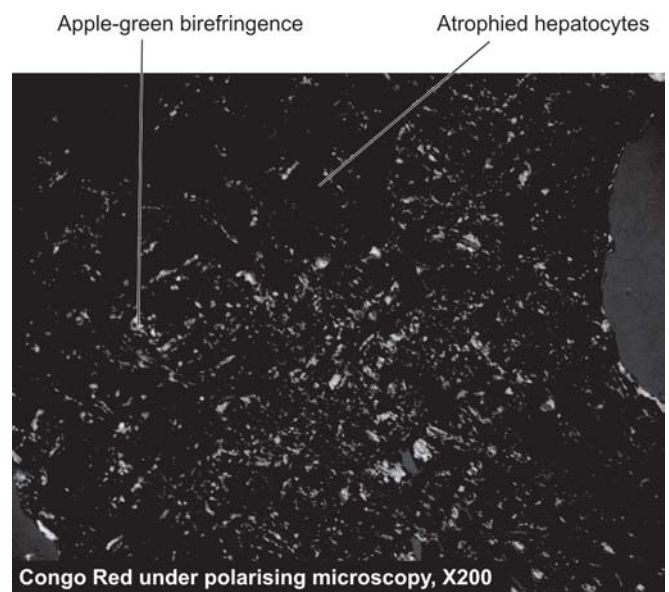


FIGURE 10.7: Amyloidosis of the liver as seen in Congo Red staining after viewing under polarising microscopy showing green birefringence at the amyloid containing areas.

Necrosis

- ◆ Coagulative Necrosis (Infarct) Kidney
- ◆ Liquefactive Necrosis (Infarct) Brain
- ◆ Caseous Necrosis Lymph Node
- ◆ Enzymatic Fat Necrosis Pancreas

Exercise

11

COAGULATIVE NECROSIS (INFARCT) KIDNEY

Coagulative necrosis is the most common type of necrosis caused by irreversible focal cell injury, most often from sudden cessation of blood supply or ischaemia (infarction). The characteristic examples of coagulative necrosis are seen in infarcts of the kidney and spleen, resulting from thromboemboli.

G/A Renal infarcts are often multiple and may be bilateral. Characteristically, they are pale or anaemic and wedge-shaped with base resting under the capsule and apex pointing towards the medulla. Generally, a narrow rim of renal tissue under the capsule is spared because it draws its blood supply from the capsular vessels. The cut surface of renal infarct in the initial 2 to 3 days is red and congested but by 4th day the centre becomes pale yellow. At the end of one week, the infarct is typically anaemic and depressed below the surface of the kidney (Fig. 11.1).

M/E

- The hallmark of coagulative necrosis is that architectural outlines of glomeruli and tubules may be preserved though all cellular details are lost.
- The margin of infarct shows inflammatory reaction, initially by polymorphonuclear cells but later macrophages, lymphocytes and fibrous tissue predominate (Fig. 11.2).

LIQUEFACTIVE NECROSIS (INFARCT) BRAIN

Liquefactive necrosis results commonly due to bacterial infections which constitute powerful stimuli for release of hydrolytic enzymes causing liquefaction. The common example is infarct of the brain.

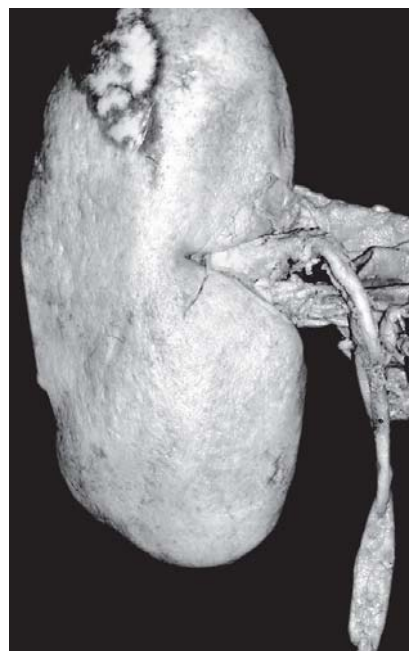


FIGURE 11.1: Infarct kidney, gross appearance. The wedge-shaped infarct is slightly depressed on the surface. The apex lies internally and wide base is on the surface (Photograph courtesy of Dr Amanjit, Dept. of Histopathology, PGI, Chandigarh).

G/A The affected area of the brain is soft with liquefied centre containing necrotic debris. Later, a cyst wall is formed.

M/E

- The cystic space contains necrotic cell debris and macrophages containing phagocytosed material.
- The cyst wall is formed by proliferating capillaries, inflammatory cells and proliferating glial cells (gliosis) (Fig. 11.3).

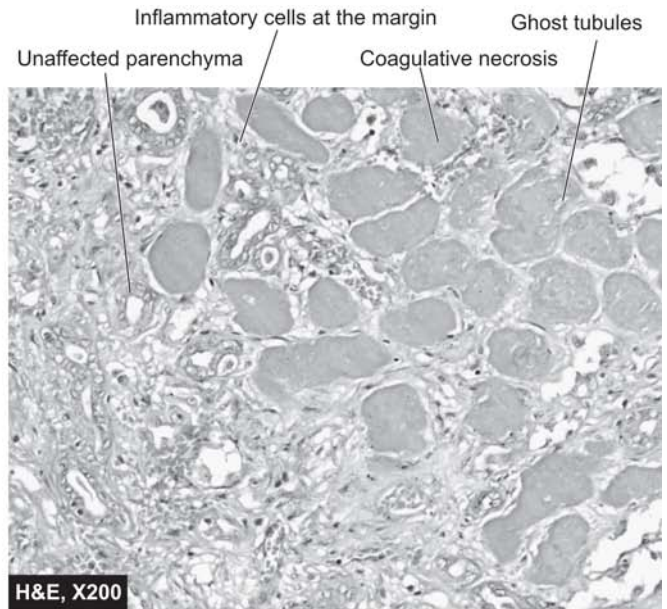
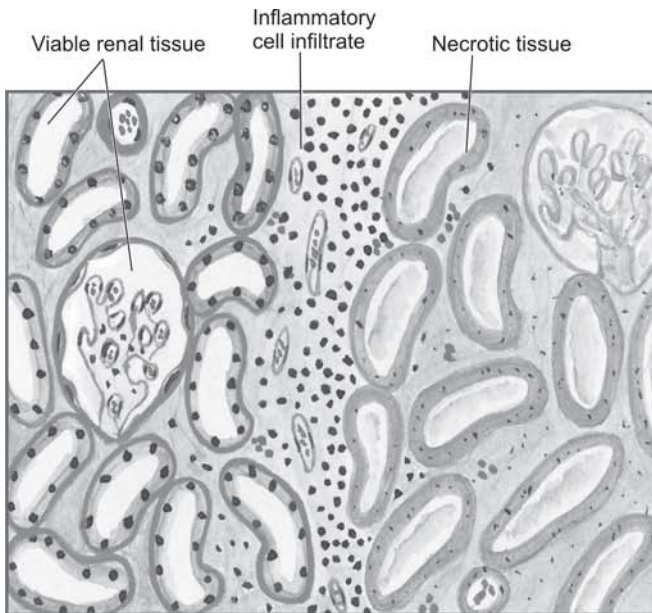


FIGURE 11.2: Microscopic appearance of renal infarct. Renal tubules and glomeruli show typical coagulative necrosis i.e. intact outlines of necrosed cells. There is dense acute inflammatory infiltrate at the periphery of the infarct.

CASEOUS NECROSIS LYMPH NODE

Caseous necrosis is a distinctive form of necrosis encountered in the foci of tuberculous infections. It combines the features of both coagulative and

liquefactive necrosis. Tuberculous lymphadenitis is a common example of caseous necrosis.

G/A The lymph nodes are matted together. The cut surface shows characteristic map-like areas of yellowish,

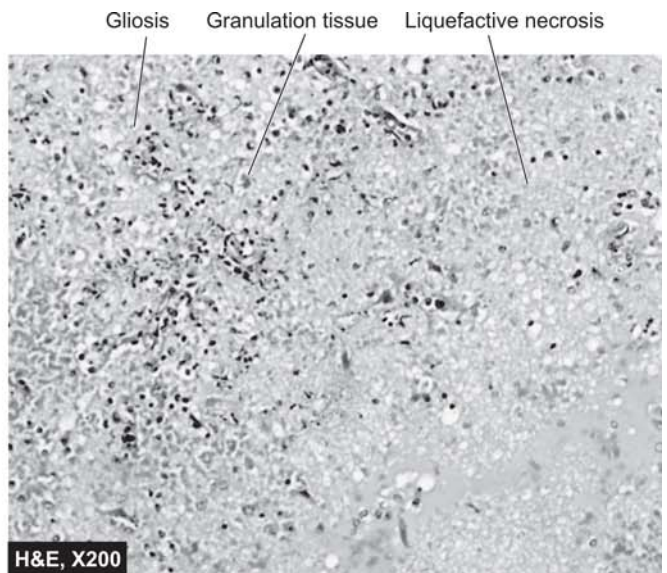
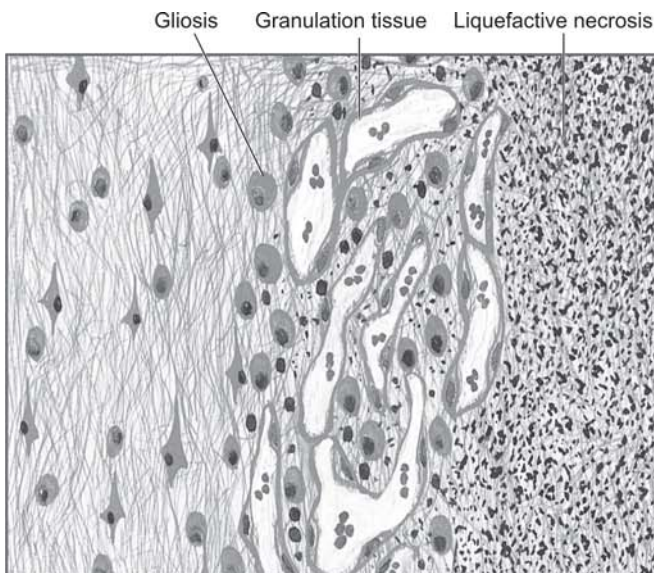


FIGURE 11.3: Liquefactive necrosis in brain. The necrosed area shows liquefied material containing cellular debris surrounded at the periphery by numerous glial cells (gliosis).

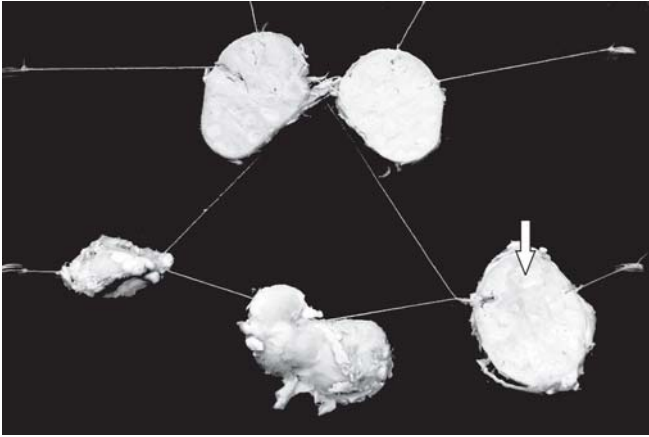


FIGURE 11.4: Tuberculous lymphadenitis. Multiple lymph nodes are matted together and surrounded by fat. Sectioned surface shows merging capsules of adjacent nodes and large areas of yellowish caseation necrosis (arrow).

granular, soft necrotic material resembling dry cheese (Fig. 11.4).

M/E

- i. The necrotic foci are composed of structureless, eosinophilic and granular debris which may contain foci of dystrophic calcification.

- ii. The area surrounding caseous necrosis shows characteristic granulomatous inflammatory reaction consisting of slipper-shaped epithelioid cells with interspersed giant cells of Langhans' type and peripheral mantle of lymphocytes (Fig. 11.5).

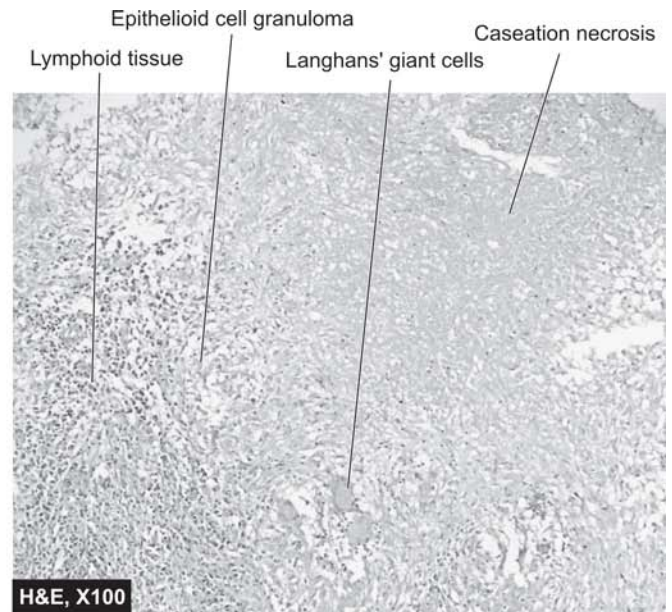
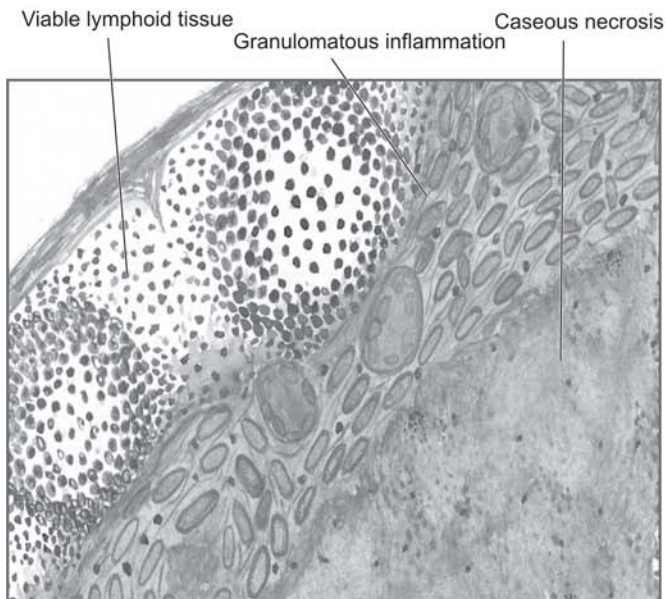


FIGURE 11.5: Tuberculous lymphadenitis. Epithelioid cells with characteristic slipper-shaped nuclei, minute caseation necrosis and Langhans' giant cells.

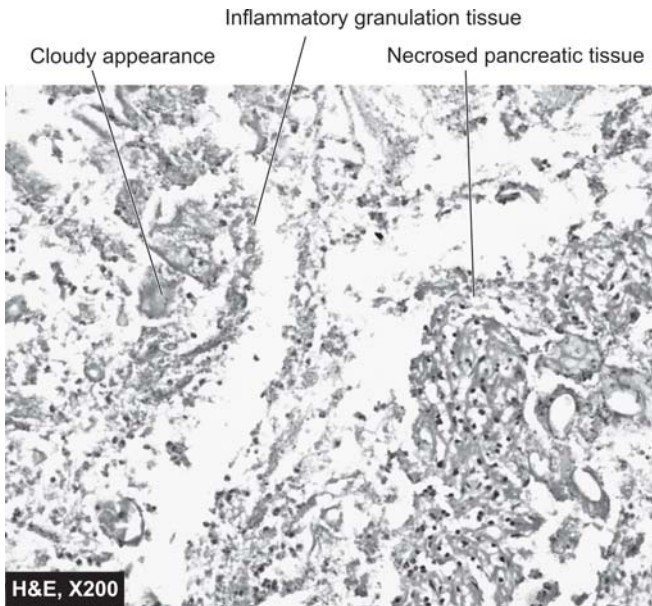


FIGURE 11.6: Fat necrosis of pancreas. Necrosed pancreatic tissue surrounded by inflammatory granulation tissue.

ENZYMATIC FAT NECROSIS PANCREAS

Enzymatic fat necrosis of pancreas is the term used for focal areas of destruction of fat in the peritoneal cavity resulting from liberation of activated pancreatic lipases. This occurs in the fatal condition of acute pancreatic necrosis.

G/A Fat necrosis appears as yellowish-white firm deposits. Formation of calcium soaps imparts the necrosed foci firmer and chalky-white appearance.

M/E

- The necrosed fat cells have cloudy shadowy appearance.
- There are foci of calcium soaps identified in the tissue sections as amorphous, granular and basophilic material.
- There is surrounding zone of inflammatory reaction (Fig. 11.6).



Gangrene and Pathologic Calcification

- ◆ Wet Gangrene Bowel
- ◆ Dry Gangrene Foot
- ◆ Monckeberg's Arteriosclerosis

Exercise

12

WET GANGRENE BOWEL

Wet gangrene occurs in tissues and organs which contain fluid e.g. bowel, lung etc.

G/A The affected zone of the small bowel is suffused with blood causing haemorrhagic infarction which may be due to thrombo-embolic occlusion of the superior mesenteric artery commonly, or less often from thrombosis of the mesenteric veins. In *arterial thrombo-embolic occlusion*, the line of demarcation between gangrenous bowel and normal bowel is usually sharp while *venous occlusion* may lead to a more diffuse appearance. The affected zone is dilated and the external surface may be dark purple, green and black. The mucosa shows haemorrhages and necrosis (Fig. 12.1).

M/E

- There is coagulative and/or liquefactive necrosis affecting the entire thickness of the affected segment of the bowel and exudate on the serosa.
- Bacterial colonies may be identified in different layers of the affected bowel.
- The blood vessels in the bowel wall may show thrombi.
- The line of demarcation between the viable and gangrenous segment shows acute inflammatory cells (Fig. 12.2).

DRY GANGRENE FOOT

The typical form of dry gangrene is seen in most distal part of the foot when the arteries to the foot are obliterated, most often from arteriosclerosis in old age.

G/A The affected part of the foot is dry, shrunken and dark black resembling the foot of mummy. A line of



FIGURE 12.1: Wet gangrene of the small bowel. Affected part is soft, swollen and dark. Line of demarcation between gangrenous segment and the viable bowel is not clear cut (arrow).

separation is seen at the junction of viable tissue with gangrenous part (Fig. 12.3).

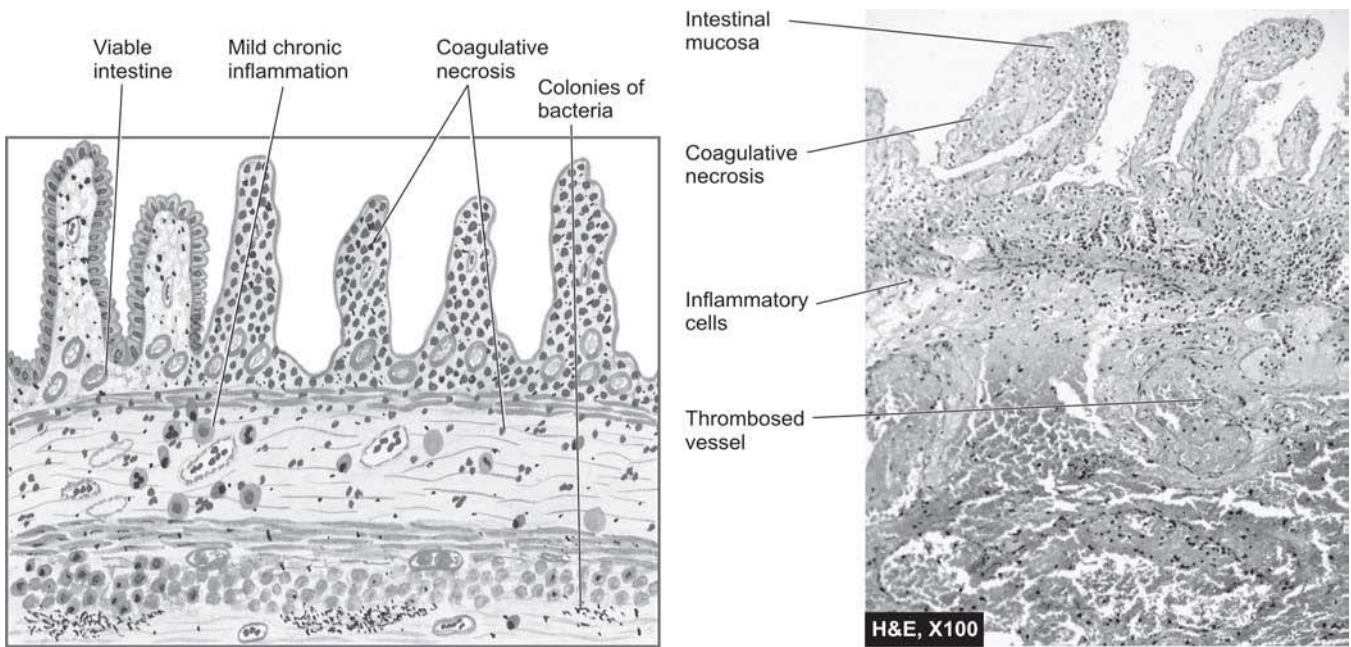


FIGURE 12.2: Gangrene small bowel. Photo micrograph shows non-viable infarcted segment and congested blood vessels.



FIGURE 12.3: Dry gangrene of foot. The affected part is dry, shrunken and dark black. There is a well delineated line of demarcation between the unaffected and affected area (arrow).

M/E

- There is coagulative necrosis of the affected tissue identified by outlines of cells without nuclear and cytoplasmic details.
- In addition, there is blurring and smudging of outlines of cells with disintegration and breaking up.
- The line of separation shows inflammatory granulation tissue (Fig. 12.4).

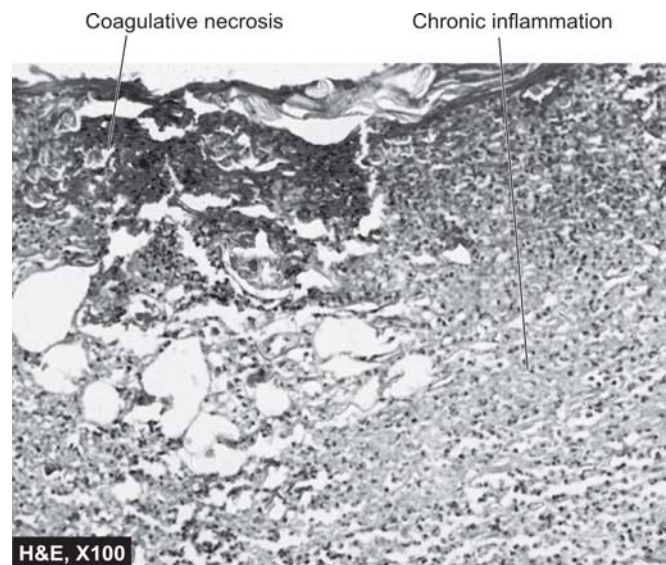


FIGURE 12.4: Dry gangrene foot. There is coagulative necrosis of soft tissues while the margin with viable tissue shows non-specific chronic inflammation.

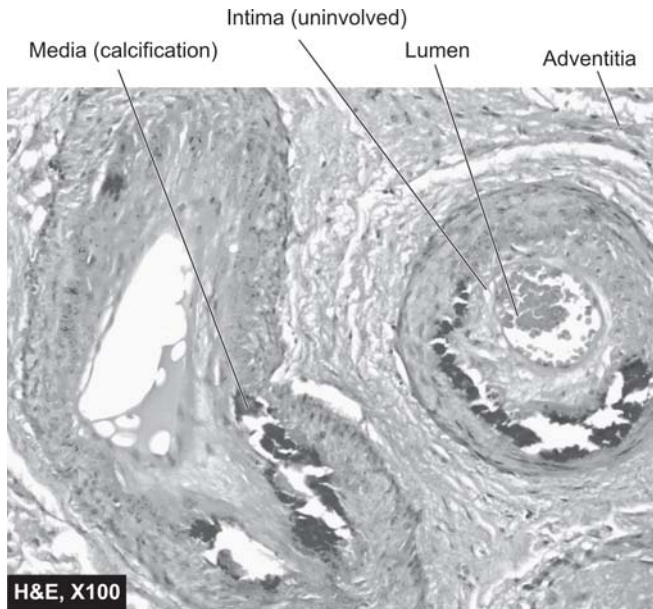


FIGURE 12.5: Monckeberg's arteriosclerosis (Medial calcific sclerosis). There is calcification in the tunica media.

MONCKEBERG'S ARTERIOSCLEROSIS

Monckeberg's arteriosclerosis or medial calcific sclerosis is dystrophic calcification in the degenerated media of large and medium-sized muscular arteries, especially of extremities and of the genital tract in the elderly.

G/A Medial calcification produces pipe-stem-like rigidity of affected artery without significant luminal narrowing. However, coexistent changes of atherosclerosis may be present.

M/E

- Smooth muscle of media is replaced by acellular hyalinised fibrous tissue.
- Foci of dystrophic calcification are seen in the media as basophilic coarse granules (Fig. 12.5).
- There is no significant inflammation.



Derangements of Body Fluids

- ◆ Pulmonary Oedema
- ◆ CVC Lung
- ◆ CVC Liver
- ◆ CVC Spleen

Exercise

13

PULMONARY OEDEMA

Pulmonary oedema is a common clinical and pathologic condition resulting from haemodynamic disturbances or from direct increase in capillary permeability.

G/A The lungs are voluminous, heavy, firm, wet and show marked pitting on pressure. Initially fluid accumulates in the basal region of the lower lobes. Cut surface of lungs permits escape of frothy blood-tinged fluid due to mixture of air and oedema fluid.

M/E

- Initially, alveolar septa are widened due to accumulation of oedema fluid.

- Later, the proteinaceous fluid appears in the alveolar spaces and appears as pink granular material and may have some admixed RBCs and macrophages (Fig. 13.1).

CVC LUNG

Chronic venous congestion of lungs and consequent pulmonary oedema occur in elevated left atrial pressure which raises the pulmonary venous pressure e.g. in mitral stenosis in rheumatic heart disease.

G/A Both lungs are dark brown in colour, heavy and firm. Cut surface shows brown coloration referred to as *brown induration*.

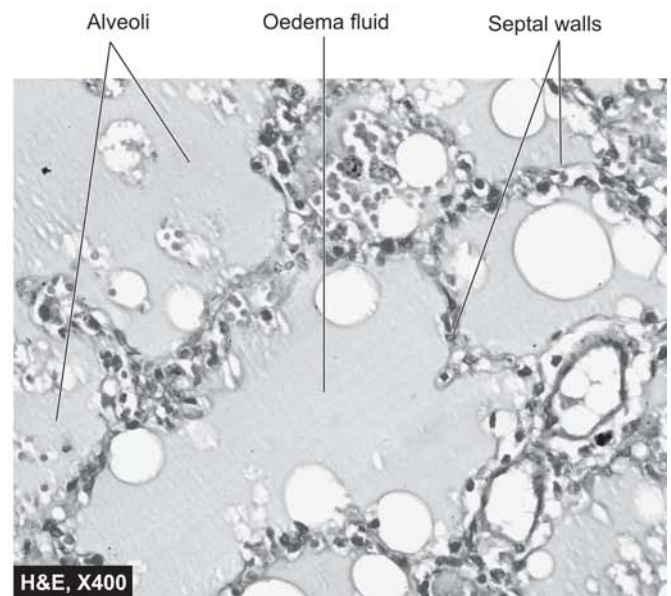
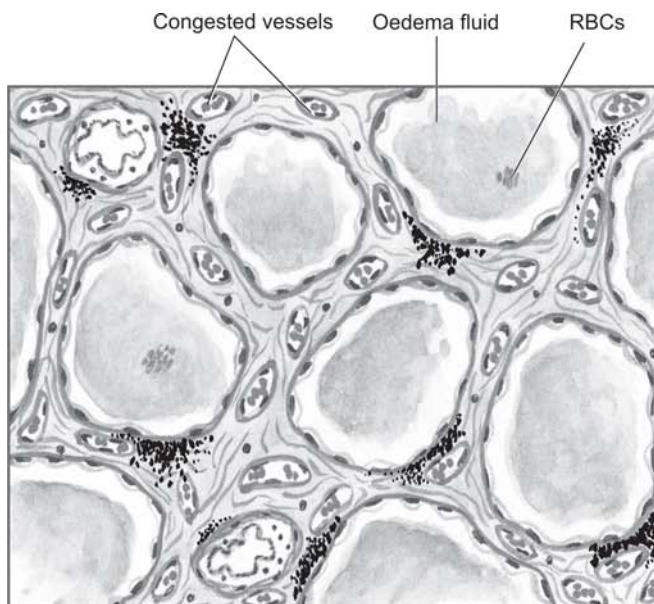


FIGURE 13.1: Pulmonary oedema. The alveolar capillaries are congested. The alveolar spaces as well as interstitium contain eosinophilic, granular, homogeneous and pink proteinaceous oedema fluid alongwith some RBCs and inflammatory cells.

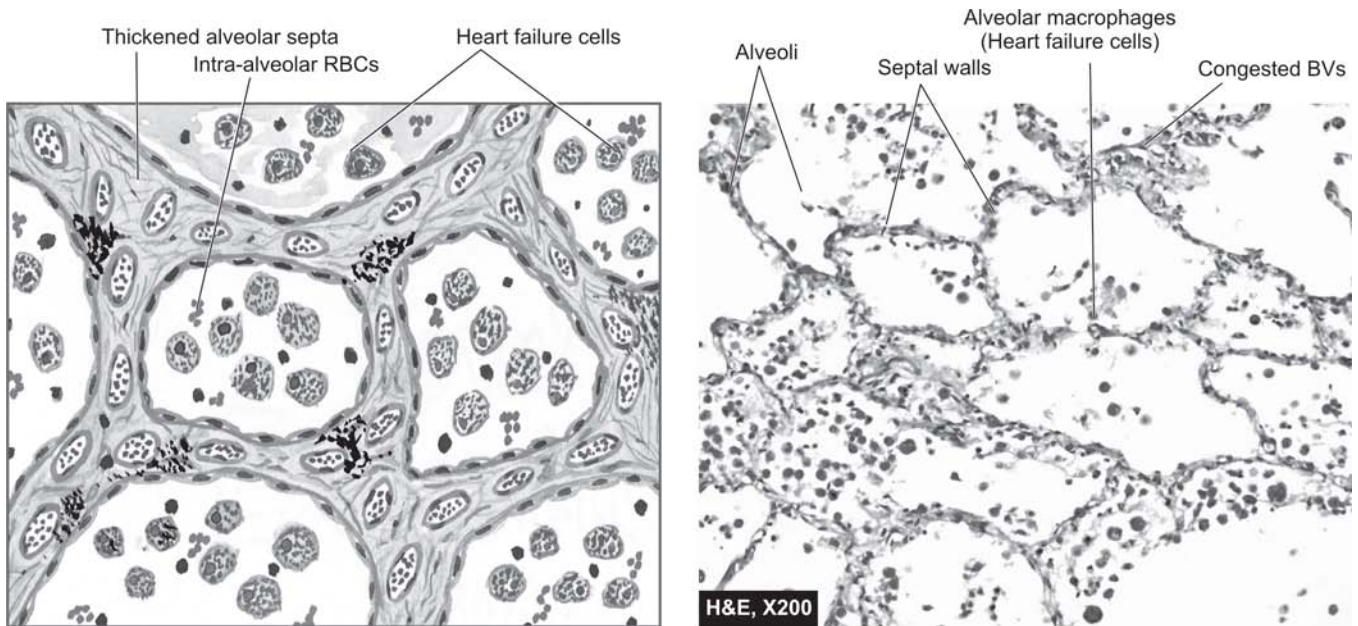


FIGURE 13.2: Histological appearance of CVC lung. The alveolar walls are widened and thickened due to congestion, oedema and mild fibrosis and the alveolar lumina contain heart failure cells.

M/E

- i. Vessels in the alveolar septa are dilated and congested.
- ii. Rupture of dilated and congested capillaries may result in minute intra-alveolar haemorrhages.
- iii. The characteristic finding is the presence of large number of alveolar macrophages filled with yellow-brown haemosiderin pigment, so called *heart failure cells* in the alveoli (Fig. 13.2).
- iv. Pulmonary oedema is a common accompaniment of venous congestion of the lungs.

CVC LIVER

The liver is particularly vulnerable to chronic passive congestion in right heart failure.

G/A The liver is enlarged and tender. The cut surface shows characteristic alternate dark areas representing congested centre of each lobule, and light areas being the fatty peripheral part, so called *nutmeg liver* (Fig. 13.3).

M/E

- i. The central vein and the sinusoids in the centrilobular region are distended with blood.
- ii. The hepatocytes in the centrilobular region undergo degeneration and atrophy, probably as a result of anoxia.

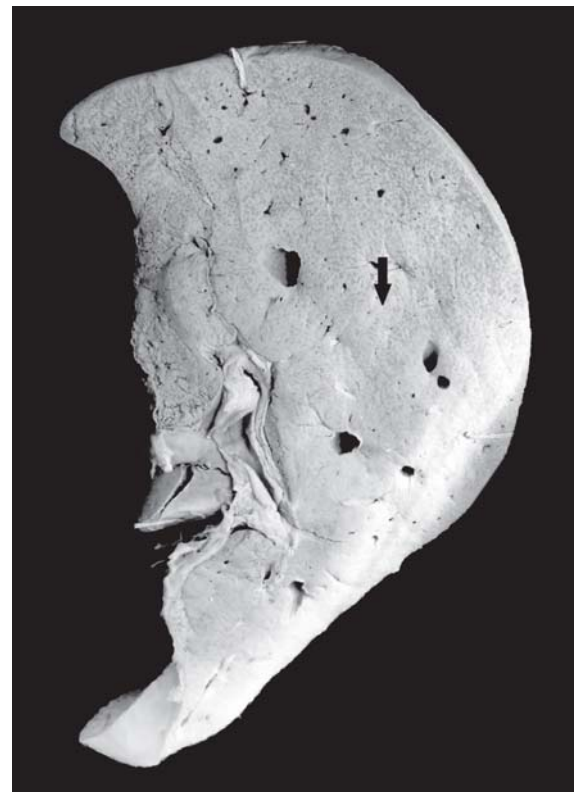


FIGURE 13.3: Nutmeg liver. The cut surface shows mottled appearance—alternate pattern of dark congestion and pale fatty change (arrow).

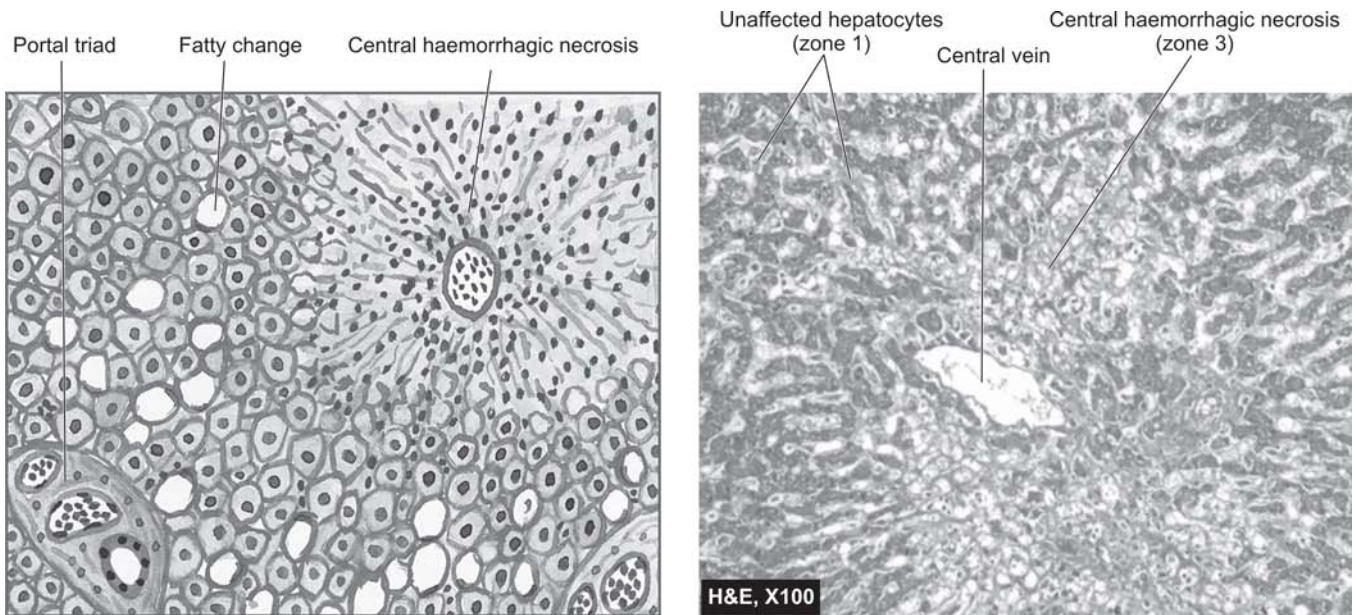


FIGURE 13.4: Histological appearance of CVC liver. The centrilobular zone shows marked degeneration and necrosis of hepatocytes accompanied by haemorrhage while the peripheral zone shows mild fatty change of liver cells.

- iii. Eventually, the centrilobular zone shows central haemorrhagic necrosis (Fig. 13.4).
- iv. The peripheral hepatocytes are either normal or may show fatty change.

CVC SPLEEN

Chronic passive congestion of spleen may result from systemic causes (e.g. in right heart failure) or local causes (e.g. in cirrhosis of liver).

G/A The spleen becomes enlarged, firm and tense. The weight of the organ is increased (250 gm or more). The capsule may get thickened and fibrous. The cut surface has fibrosed and meaty appearance with indistinct malpighian corpuscles (Fig. 13.5).

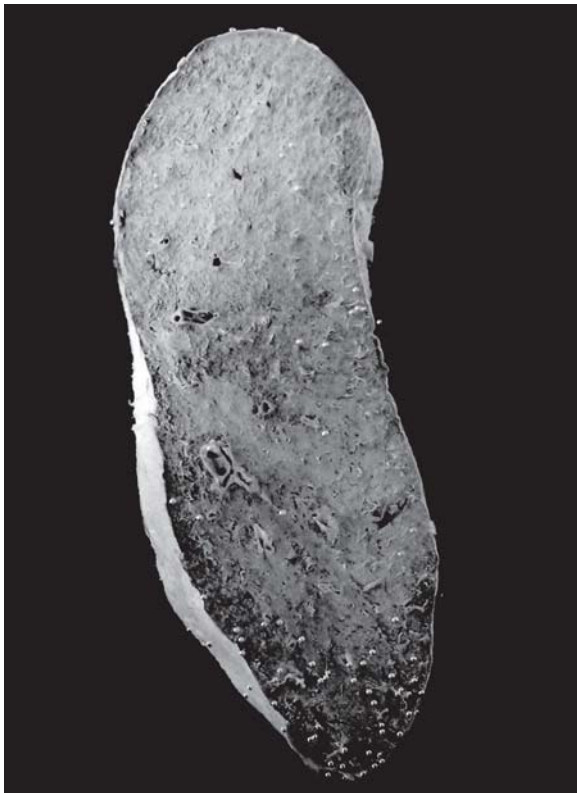


FIGURE 13.5: CVC spleen. The spleen is enlarged and heavy. Cut surface shows grey-tan parenchyma.

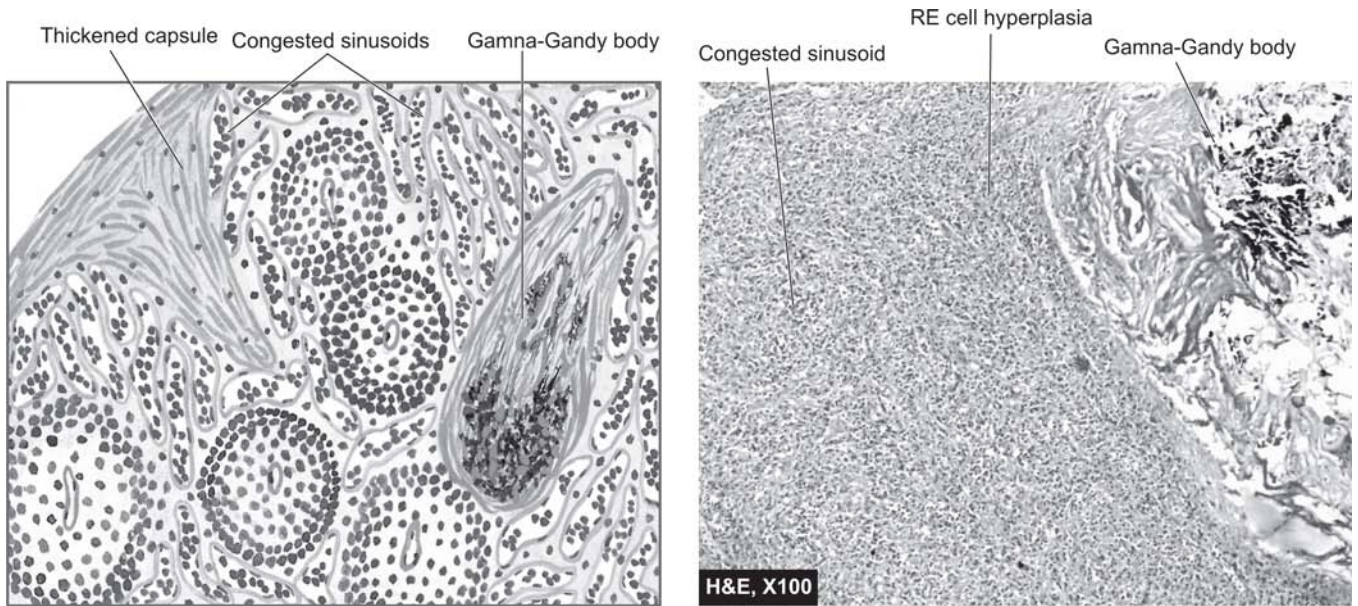


FIGURE 13.6: Histological appearance of CVC spleen. The sinuses are dilated and congested. There is increased fibrosis in the red pulp, capsule and the trabeculae. Gamna-Gandy bodies are also seen.

M/E

- i. The red pulp shows marked congestion and dilatation of sinusoids with areas of recent and old haemorrhages.
- ii. The haemorrhages may get organised and show diffuse fibrosis together with iron pigment and

calcium deposits, so called siderotic nodules or Gamna-Gandy bodies.

- iii. In the late stages, there is hyperplasia of pigment-laden macrophages and thickening of fibrous framework of the organ, termed fibrocongestive splenomegaly (Fig. 13.6).



Obstructive Circulatory Disturbances

- ◆ Thrombus Artery
- ◆ Pale Infarct Spleen
- ◆ Haemorrhagic Infarct Lung

Exercise

14

THROMBUS ARTERY

Thrombi may occur anywhere in the arteries but the most common site is in the coronary arteries.

G/A Coronary arterial thrombi are generally firmly attached to the vessel wall (mural) and occlude the lumen (occlusive). The arterial thrombus invariably overlies an atherosclerotic lesion. A slit-like lumen may be formed on contraction of freshly-formed thrombus restoring some flow. Arterial thrombus is grey-white and friable. The cut surface shows laminations called the lines of Zahn.

M/E

- The internal elastic lamina is degenerated and disrupted at the site of attachment of thrombus to the vessel wall.
- The residual lumen of the original artery is slit-like and shows flowing blood (Fig. 14.1).
- The structure of thrombus shows lines of Zahn composed of layers of light-staining fibrin strands and platelets enmeshed in dark-staining red cells.
- The underlying atheromatous plaque may be seen.
- Organised thrombus shows in-growth of granulation tissue at the base having spindle cells and capillary channels.

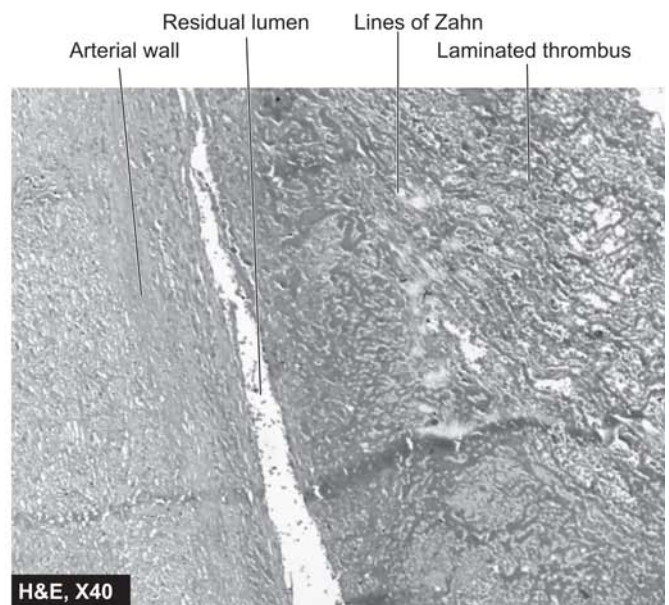
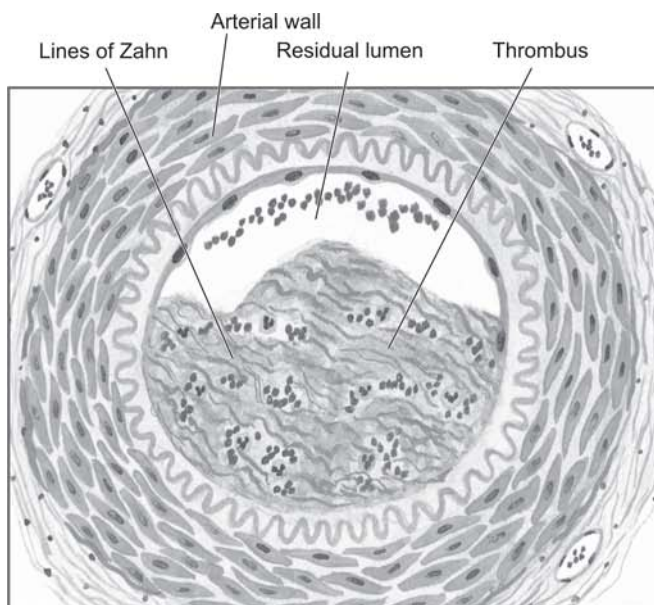


FIGURE 14.1: Thrombus in an artery. The thrombus is adherent to the arterial wall and is seen occluding most of the lumen. It shows lines of Zahn composed of granular-looking platelets and fibrin meshwork with entangled red cells and leucocytes.

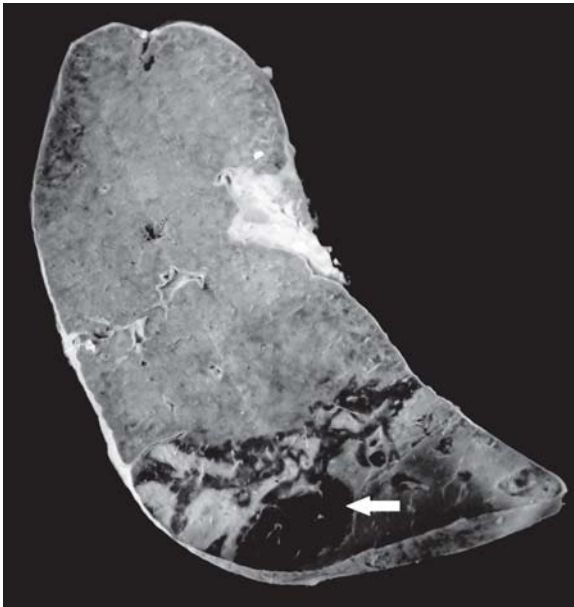


FIGURE 14.2: Pale infarct spleen. A wedge-shaped area of pale colour with base resting under the capsule is seen while the margin is congested tan (arrow).

PALE INFARCT SPLEEN

Splenic infarction is one of the common types resulting from occlusion of the splenic artery or its branches.

G/A Infarcts of spleen are often multiple and pale or anaemic. They are usually wedge-shaped with their base lying on the splenic capsule and apex pointing inwards (Fig. 14.2).

M/E

- i. There is coagulative necrosis of the cells in the affected area i.e. outlines of cells and structures are identified but without intact nuclei and cytoplasmic details.
- ii. Margin of the infarct with the viable tissue shows zone of inflammatory reaction, initially acute and later chronic (Fig. 14.3).

HAEMORRHAGIC INFARCT LUNG

Embolism of pulmonary arteries may produce pulmonary infarction, though not always.

G/A Pulmonary infarcts are variable in size and are always red or haemorrhagic because the organ has dual blood supply. The infarct appears as firm, bright red, wedge-shaped area, with the base of the wedge at the surface and covered by fibrinous pleural exudate. The

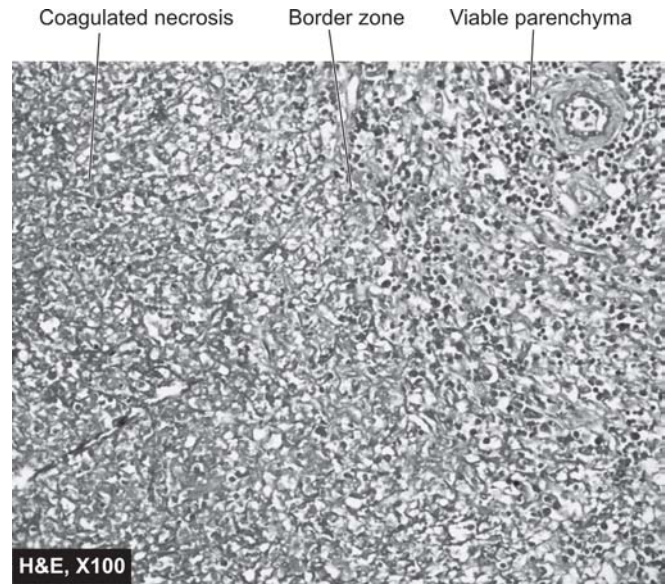


FIGURE 14.3: Pale infarct spleen showing outlines of cells in infarcted area on left.

infarcted area is slightly raised above the adjacent area. The cut surface is dark red (Fig. 14.4).

M/E

- i. There is ischaemic necrosis of the lung parenchyma in the affected area of haemorrhage i.e. the alveolar

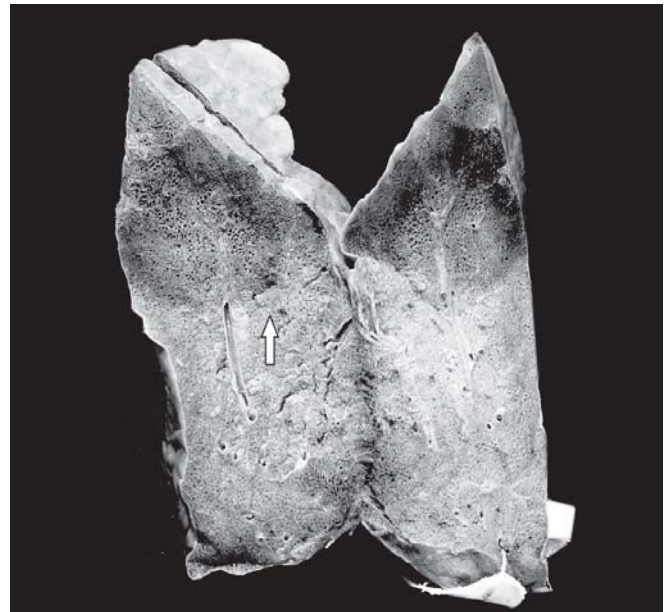


FIGURE 14.4: Haemorrhagic infarct lung. The sectioned surface shows dark tan firm area (arrow) with base on the pleura.

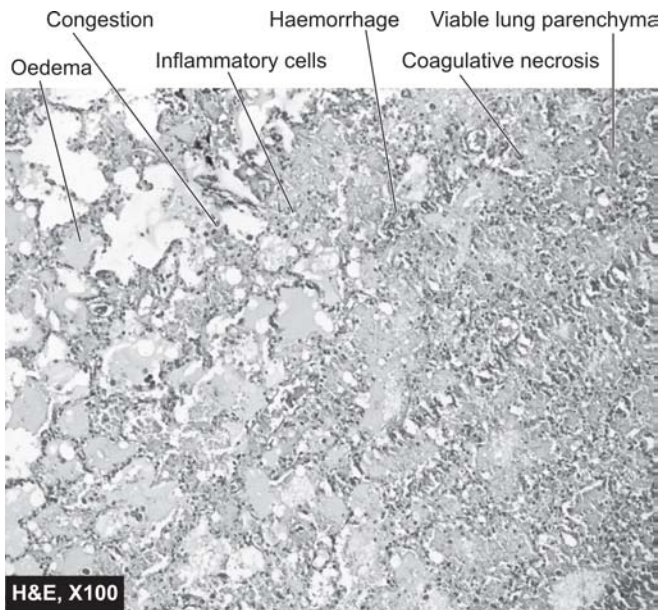


FIGURE 14.5: Haemorrhagic infarct lung. Infarcted area shows ghost alveoli filled with blood.

walls, bronchioles and vessels in the infarcted zone show outlines but loss of nuclear and cytoplasmic details (Fig. 14.5).

- ii. Margin of the infarct shows inflammatory infiltrate, initially by neutrophils and later their place is taken by macrophages and haemosiderin.
- iii. If the infarct is caused by an infected embolus, the infarct shows more intense neutrophilic infiltration.



Inflammation

- ◆ Abscess Lung
- ◆ Chronic Inflammatory Granulation Tissue
- ◆ Tuberculous Lymphadenitis

Exercise

15

ABSCESS LUNG

Abscess is formation of cavity as a result of extensive tissue necrosis following pyogenic bacterial infection accompanied by intense neutrophilic infiltration. Abscess of the lung may occur due to inhalation, embolic phenomena, and pneumonia.

G/A Abscess is more common in the right lung but may occur in upper or lower lobe. Size of the cavity may vary from small to fairly large. The wall is ragged and necrotic but advanced lesions may show fibrous and smooth wall (Fig. 15.1). The inhalation abscess may communicate with the bronchus.

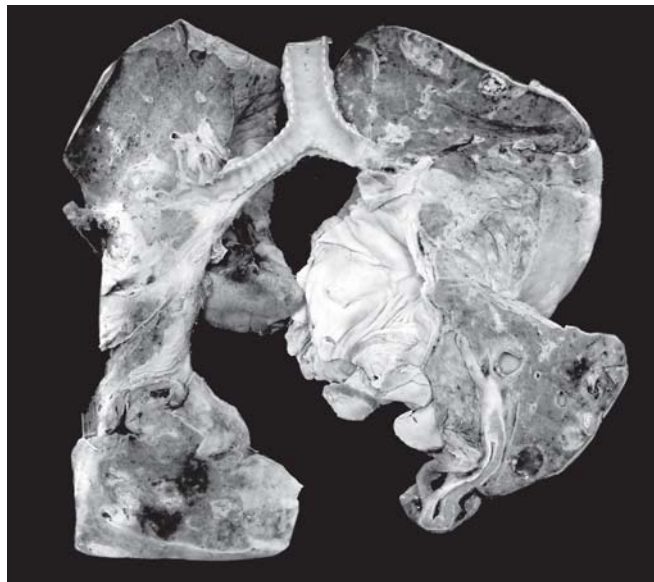


FIGURE 15.1: Lung abscess. The lung shows thickened pleura. Cut surface shows multiple cavities 1-4 cm in diameter, having irregular and ragged inner wall (arrows). The lumen contains necrotic debris. The surrounding lung parenchyma shows consolidation.

M/E

- The wall of the abscess shows dense infiltration by polymorphonuclear leucocytes and varying number of macrophages.
- More chronic cases show fibroblasts at the periphery.
- Alveolar walls in the affected area are destroyed.
- Lumen of the abscess contains pus consisting of purulent exudate, some red cells, fragments of tissue debris and fibrin (Fig. 15.2).

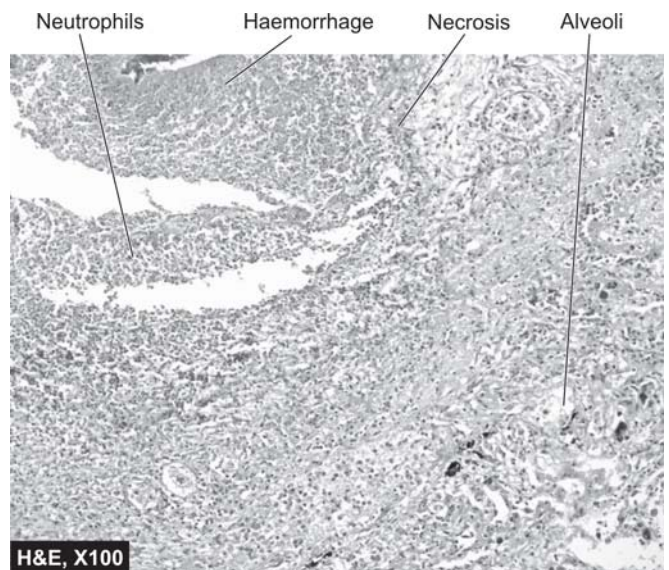


FIGURE 15.2: Abscess lung. The left half of field shows abscess formed by necrosed alveoli and dense acute and chronic inflammatory cells.

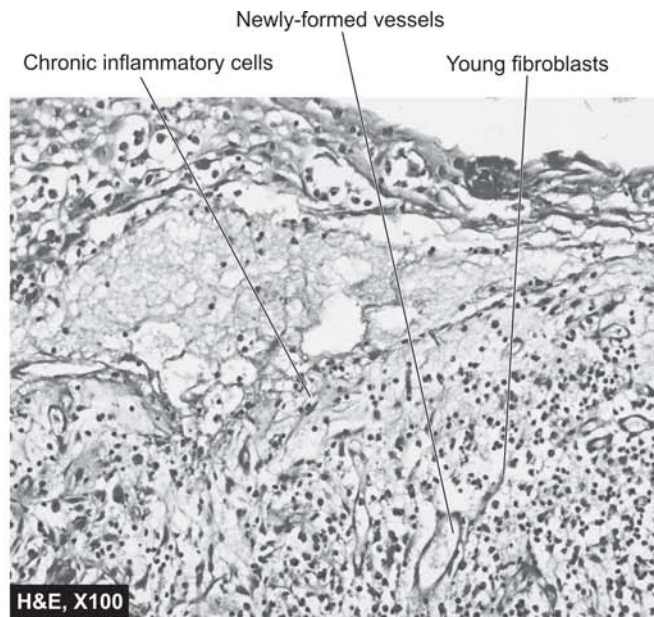
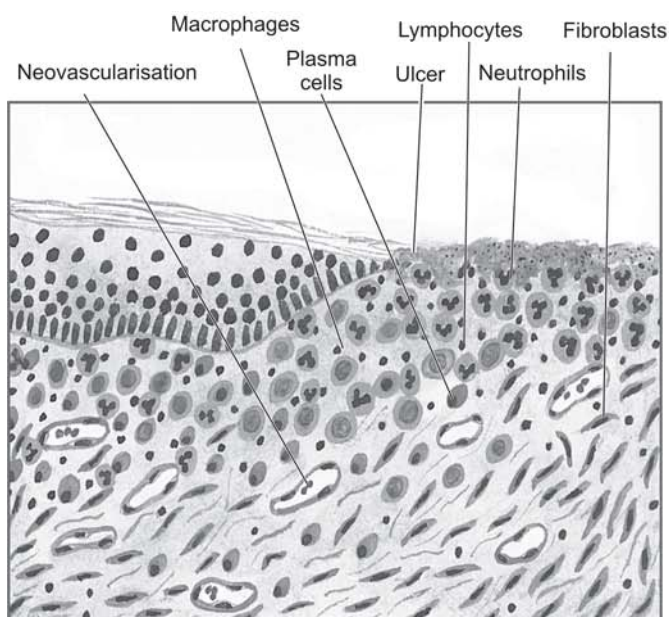


FIGURE 15.3: Chronic granulation tissue. The surface is ulcerated while the underlying tissues show new blood vessels, inflammatory cells and young fibroblasts.

CHRONIC INFLAMMATORY GRANULATION TISSUE

Granulation tissue is the granular and pink appearance of the tissue in a healing ulcer and in secondary union of wounds.

G/A Floor of the lesion contains pink granulations composed of the vascular connective tissue, while the edges are sloping and bluish-white.

M/E

- Surface of the ulcer contains mixture of blood, fibrin and inflammatory exudate.
- The zone underneath contains granulation tissue composed of proliferating fibroblasts, newly-formed small blood vessels and varying number of inflammatory cells which are initially polymorphs but in the later stages macrophages and lymphocytes predominate.
- The epithelium grows from the edge of the wound as spurs.
- Granulation tissue matures from below upwards and late stage shows dense collagen, scanty vascularity and fewer inflammatory cells (Fig. 15.3).

TUBERCULOUS LYMPHADENITIS

Tuberculosis of the lymph nodes is always secondary to tuberculosis elsewhere.

G/A The lymph nodes are enlarged and are matted together due to periaadenitis. The cut surface in the tuberculous areas is yellow, cheesy, opaque and caseous while elsewhere is grey brown (Fig. 15.4).

M/E

- The caseous areas show debris composed of fragmented coagulated cells.
- The periphery of caseous foci shows granulomatous inflammation consisting of epithelioid cells (identi-

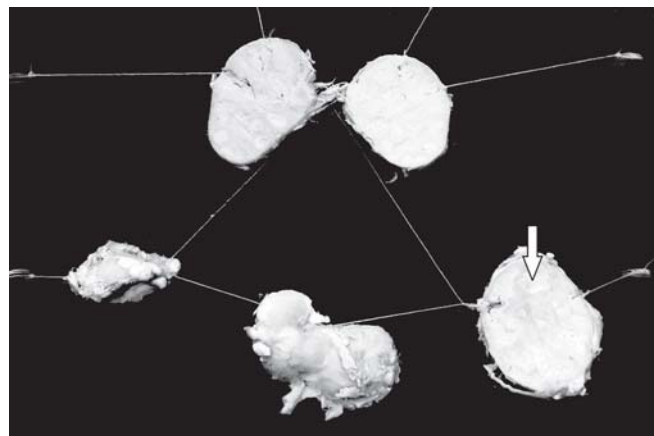


FIGURE 15.4: Tuberculous lymphadenitis. Multiple lymph nodes are matted together and surrounded by fat. Sectioned surface shows merging capsules of adjacent nodes and large areas of yellowish caseation necrosis.

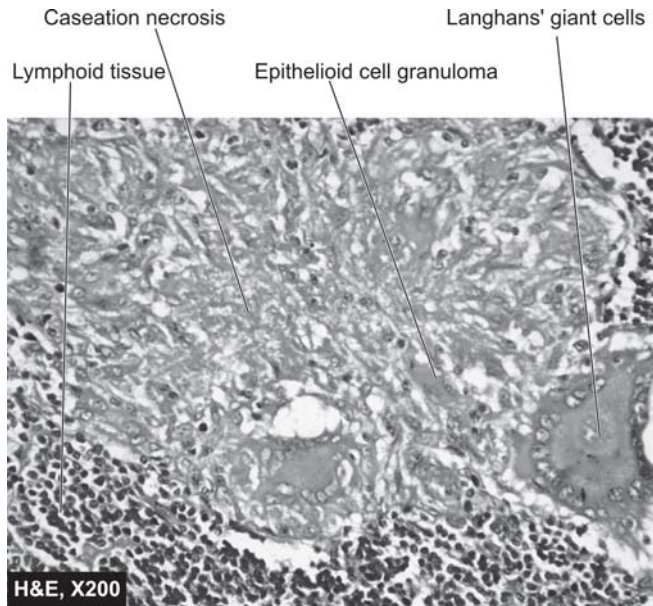


FIGURE 15.5: Tuberculous lymphadenitis. Characteristic sliper-shaped epithelioid cells, minute caseation necrosis and a Langhans' giant cell.

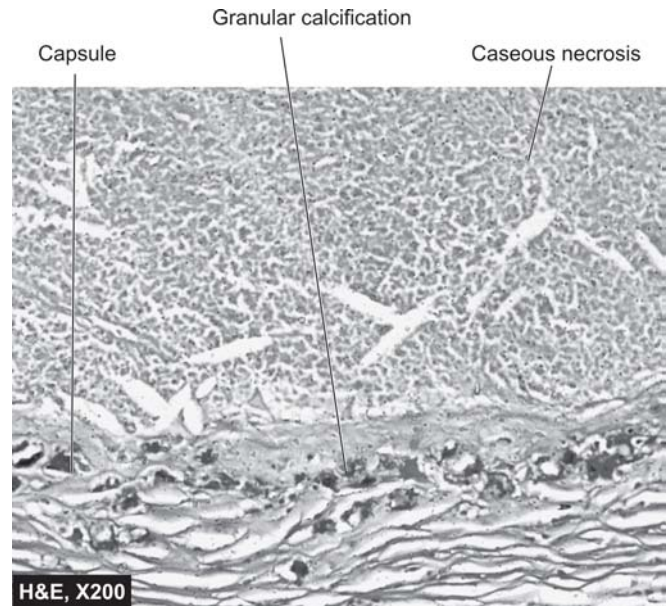


FIGURE 15.6: Tuberculous lymphadenitis. Large areas of caseation necrosis with dystrophic calcification, surrounded by epithelioid cells.

- fied by large size, epithelium-like appearance, abundant pale cytoplasm and oval vesicular nuclei), surrounded by lymphocytes and some plasma cells. Epithelioid cells may fuse to form giant cells in the granuloma and may have nuclear arrangement at the periphery of the cell (Langhans' giant cells) or the nuclei may be distributed haphazardly (foreign body giant cells) (Fig. 15.5).
- iii. Depending upon the age of granuloma, fibroblasts may surround the granulomas.
 - iv. It is not uncommon to find areas of dystrophic calcification appearing as bluish granularity in the caseous areas (Fig. 15.6).
 - v. Ziehl-Neelsen staining may demonstrate presence of acid fast bacilli (Fig. 15.7)
 - v. Normal nodal architecture may be seen at the periphery only.

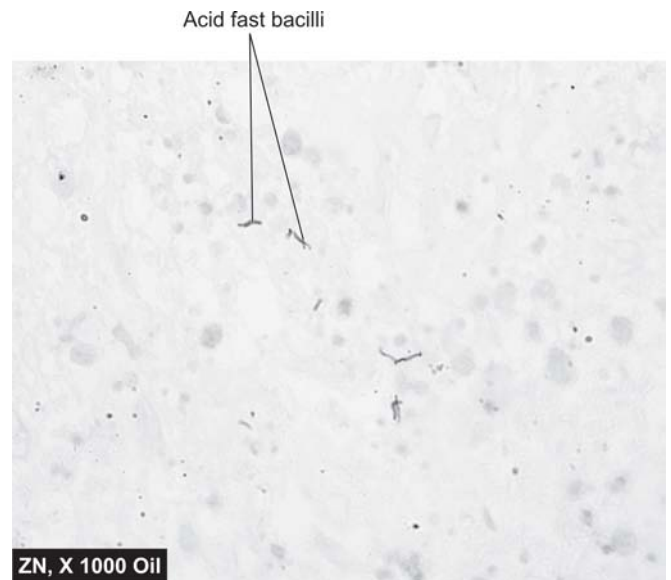


FIGURE 15.7: Tuberculous lymphadenitis. Ziehl-Neelsen staining shows the presence of acid fast bacilli.



Tuberculous Granulomatous Inflammation

- ◆ Fibrocaseous Tuberculosis Lung
- ◆ Tuberculosis Intestine

- ◆ Miliary Tuberculosis Lung
- ◆ Miliary Tuberculosis Spleen

Exercise

16

FIBROCASEOUS TUBERCULOSIS LUNG

The breakdown of caseous tissue in the lung in chronic cases results in fibrocaseous tuberculosis.

G/A The cavity is seen most commonly at the apex and is fairly large and may communicate through the bronchial wall ('open tuberculosis'). In progressive form of the disease, however, cavities may be formed in the lower lobe too. Wall of the cavity is smooth (unlike ragged lining of the pyogenic abscess), fibrous and may be traversed by bronchi and blood vessels (Fig. 16.1).

M/E

- Basic lesion is the *tubercle*, consisting of epithelioid cells, lymphocytes and giant cells, and central area of caseation necrosis. The tubercles coalesce to form confluent areas.

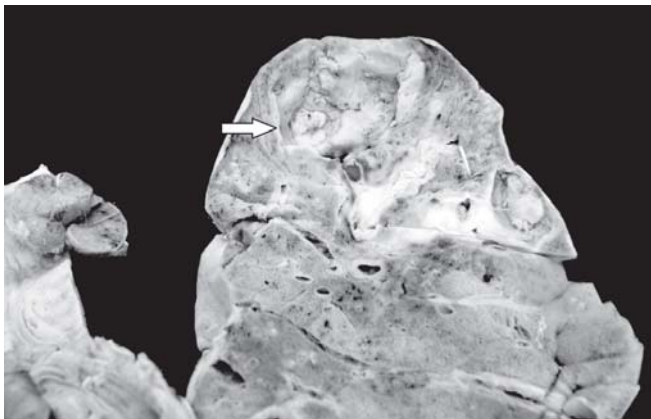


FIGURE 16.1: Chronic fibrocaseous tuberculosis lung. Sectioned surface of the lung shows a cavity in the apex of the lung (arrow). The cavity is lined by yellowish caseous necrotic material. The lung tissue around the cavity is consolidated.

- Periphery of the lesion shows proliferating fibroblasts and fibrosis (Fig. 16.2).
- The arteries may show endarteritis obliterans closing the lumen.
- The surrounding alveoli may contain cellular exudate.

TUBERCULOSIS INTESTINE

Intestinal tuberculosis occurs in 3 forms: primary, secondary and hyperplastic. Secondary tuberculosis of small intestine is most common.

G/A The intestine shows large ulcers which are transverse to the long axis of the bowel. These ulcers may be coated with caseous material. Advanced cases show transverse fibrous strictures and intestinal obstruction (Fig. 16.3).

M/E

- Presence of caseating tubercles in all the layers of intestine (Fig. 16.4).
- Ulceration of mucosa with slough on the surface.
- Variable fibrosis in the muscular layer.

MILIARY TUBERCULOSIS LUNG

If the caseous pulmonary tuberculous lesion discharges its contents into a blood vessel or lymphatic, it results in lympho-haematogenous dissemination of tuberculosis which may be confined to the lungs or may involve other organs.

G/A The lungs show intense congestion and minute millet seed-sized (1 to a few mm in diameter) yellowish-white firm lesions in which caseous centre may not be visible grossly (Fig. 16.5).

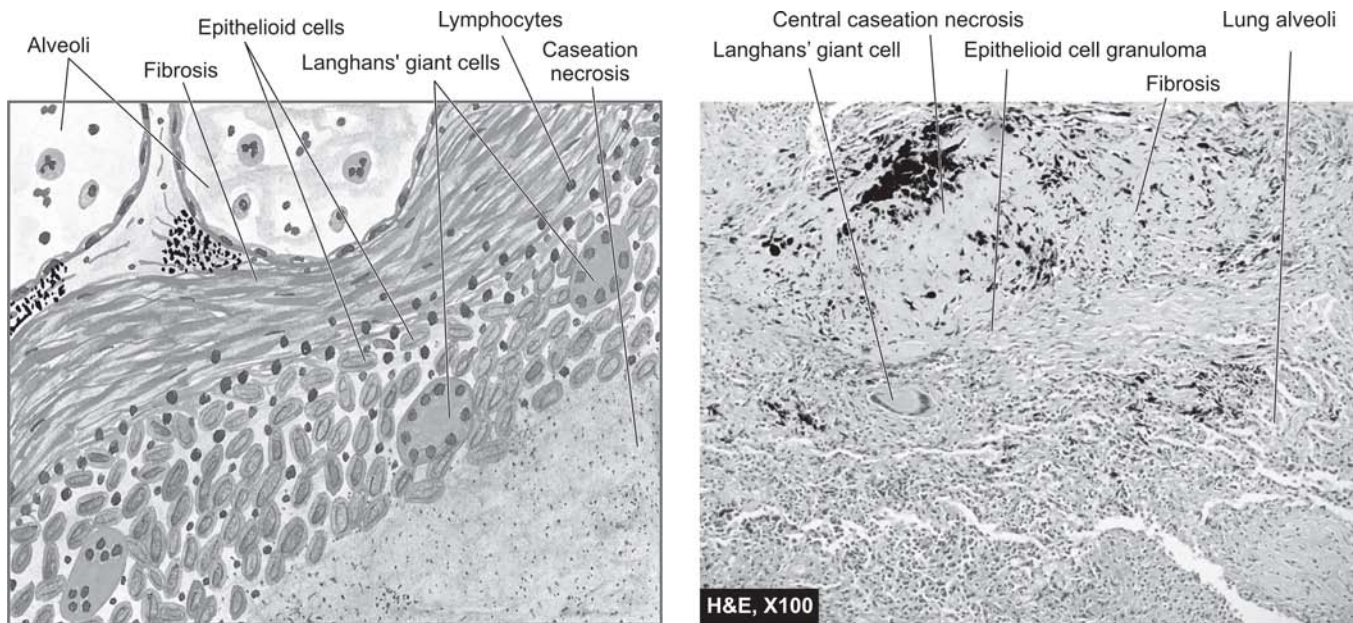


FIGURE 16.2: Fibrocaceous tuberculosis lung. The cavity shows caseation necrosis while the wall shows epithelioid cells admixed with lymphocytes and some Langhans' giant cells and surrounded at the periphery by fibrosclerosis.

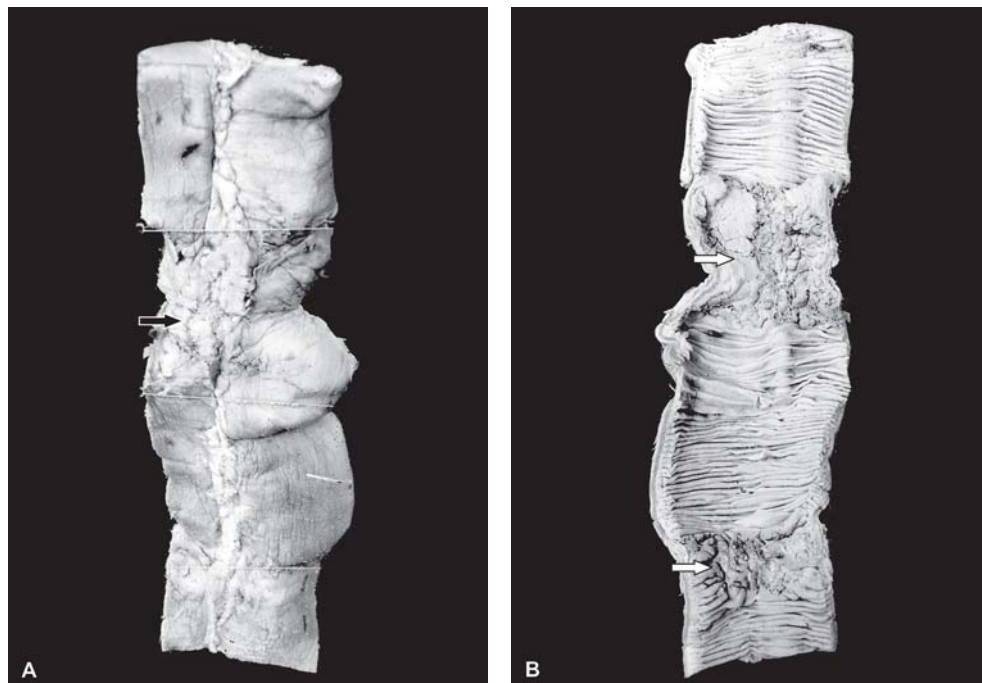


FIGURE 16.3: Intestinal tuberculosis. **A**, The external surface shows strictures and cut section of lymph node showing caseation necrosis (black arrow). **B**, The lumen shows transverse ulcers and strictures (transverse to the long axis of intestine) (arrows). The intestinal wall in the strictured areas is thickened and grey-white and mucosa over it is ulcerated.

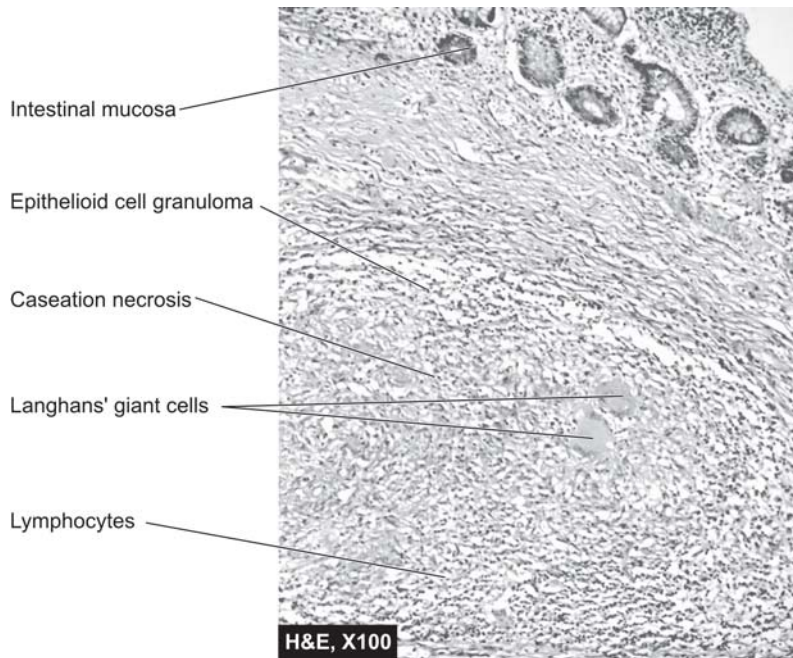


FIGURE 16.4: Tuberculosis small intestine. The wall of intestine shows caseating epithelioid cell granulomas.

M/E

- The tubercles (as described above) are seen in the fibrous stromal framework of the lung and contain minute areas of caseation necrosis in the centre.
- The intervening lung alveoli are either empty or may contain cellular exudate (Fig. 16.6).

MILIARY TUBERCULOSIS SPLEEN

Lympho-haematogenous spread of chronic pulmonary tuberculosis may result in acute miliary tuberculosis of the spleen.

G/A The miliary tubercles are scattered throughout the liver. They appear as yellowish-white firm lesions of a few millimeter in diameter (Fig. 16.7).

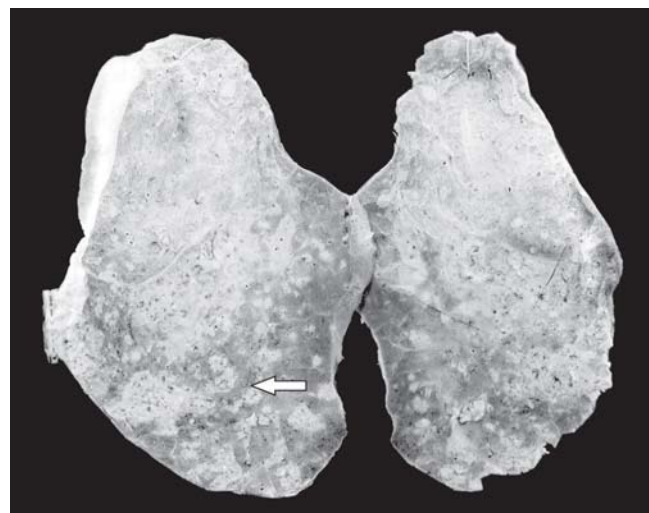


FIGURE 16.5: Miliary tuberculosis lung. The pleural as well as sectioned surface shows presence of minute (about pinhead sized) yellowish nodules with central necrosis called miliary tubercles (arrow).

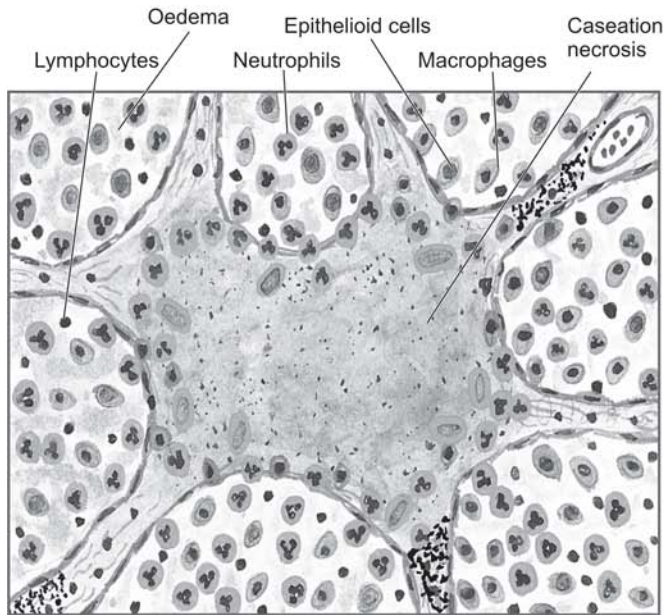
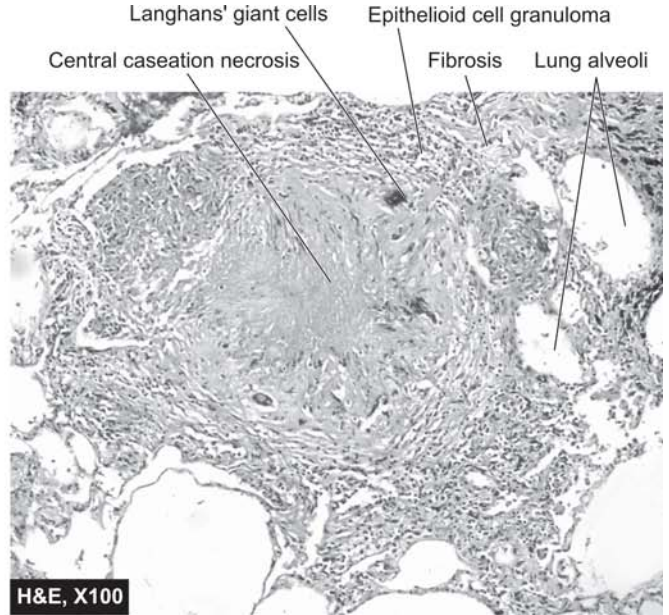


FIGURE 16.6: Miliary tuberculosis lung. Miliary tubercle with minute area of caseation necrosis surrounded by epithelioid cells, mantle of lymphocytes and peripheral fibroblasts.



M/E

- Tubercles with minute areas of central caseation necrosis are seen scattered in the splenic parenchyma.
- The neighbouring splenic parenchyma may show congestion (Fig. 16.8).

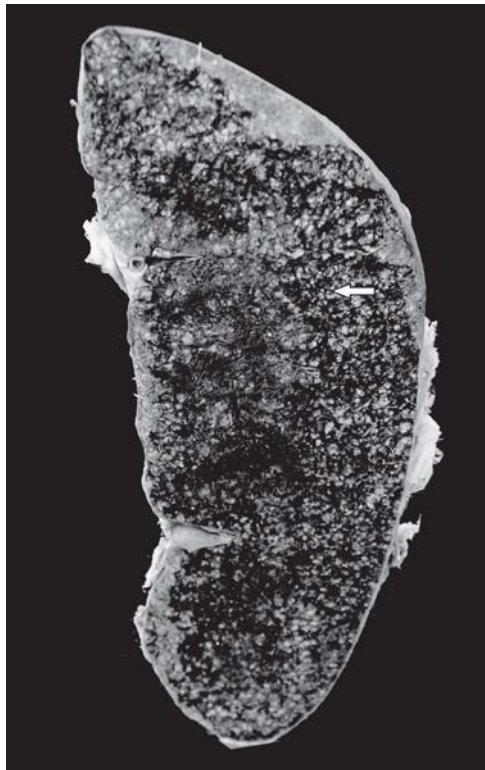


FIGURE 16.7: Miliary tuberculosis spleen. The capsule as well as sectioned surface shows presence of minute (about pinhead sized) yellowish nodules with central necrosis called tubercles (arrow).

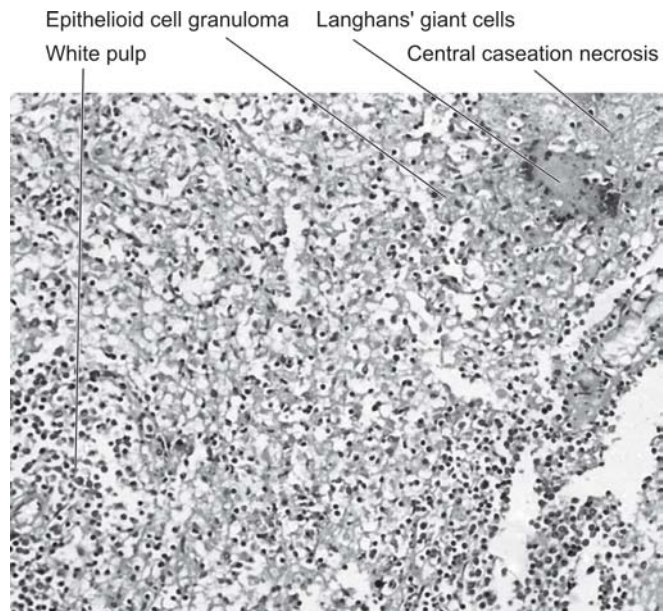


FIGURE 16.8: Miliary tuberculosis spleen. Small caseating granuloma is seen in the splenic tissue.

Other Granulomatous Inflammations

- ◆ Lepromatous Leprosy
- ◆ Tuberculoid Leprosy
- ◆ Sarcoidosis Lung

Exercise

17

LEPROMATOUS LEPROSY

Leprosy or Hansen's disease caused by *Mycobacterium leprae* affects the skin and peripheral nerves. The causative organism is less acid-fast than the tubercle bacillus. The polar form of lepromatous leprosy lacks cell-mediated immunity.

G/A The lepromatous lesions are characterised by development of nodules or masses formed in the skin, particularly on the face, hands and feet.

M/E

- i. The dermis contains large aggregates of lipid-laden foamy macrophages known as *lepra cells*.

- ii. The dermal infiltrate of lepra cells characteristically does not encroach upon the basal layer of epidermis leaving uninvolved ('clear zone') subepidermal dermis.
- iii. The overlying epidermis is *thin and flat* (Fig. 17.1).
- iv. With Fite-Faraco stain, lepra cells are crowded with acid-fast *lepra bacilli* (Fig. 17.2).

TUBERCULOID LEPROSY

The polar form of tuberculoid leprosy represents high resistance type (good cell-mediated immune response).

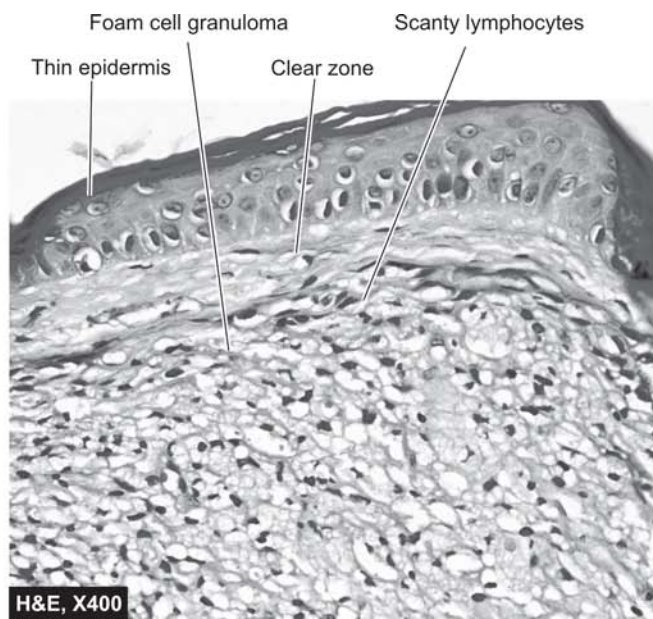
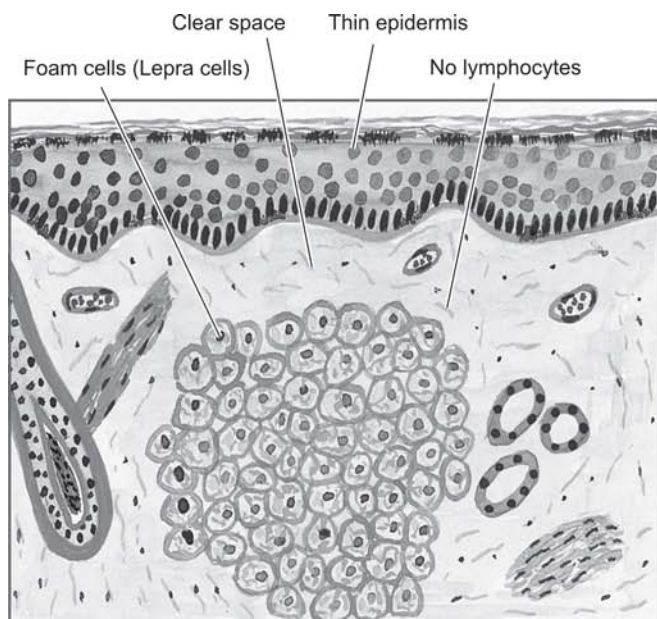


FIGURE 17.1: Lepromatous leprosy (LL). There is a clear subepidermal zone and underlying collection of foamy macrophages or lepra cells.

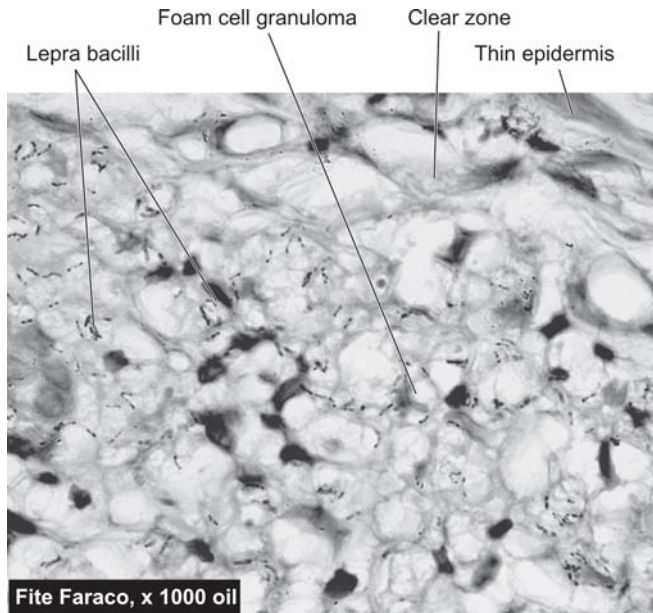
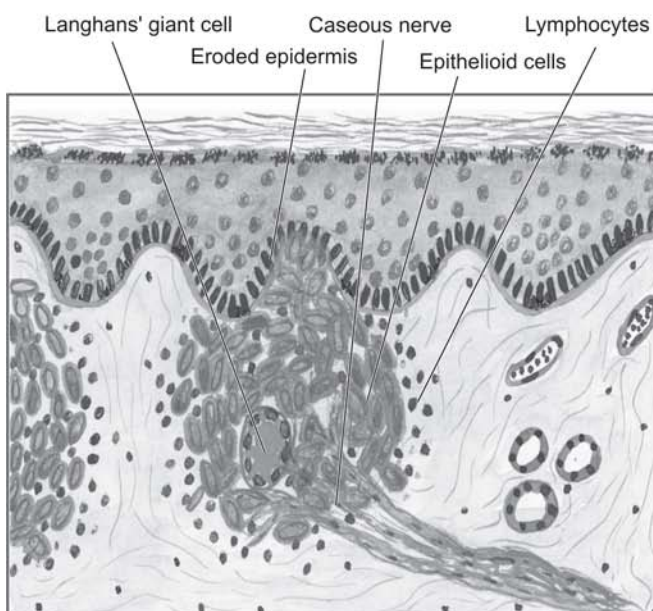


FIGURE 17.2: Lepra bacilli in LL. Numerous globi and cigarettes-in-pack appearance of lepra bacilli inside foamy macrophages.

G/A The skin lesions have hyperpigmented margins and pale centre. Neuronal involvement predominates in tuberculoid leprosy and small nerves may be destroyed.

M/E

- i. The dermis shows granulomas which closely resemble *hard tubercles* i.e. they consist of epithelioid



cells, Langhans' giant cells and a few lymphocytes at the periphery but no caseation necrosis. Lepra bacilli are almost never found.

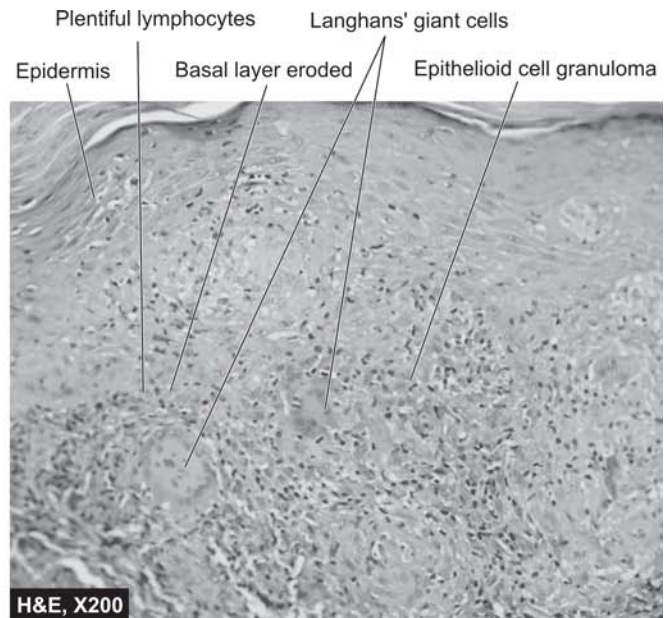
- ii. The granulomas erode the basal layer of the epidermis i.e. there is *no clear zone*.
- iii. Lesions of tuberculoid leprosy have predilection for *dermal nerves* which may be destroyed and infiltrated by epithelioid cells and lymphocytes (Fig. 17.3).

SARCOIDOSIS LUNG

The lesions in sarcoidosis are generalized and may affect various organs and tissues but the brunt of the disease is borne by the lungs and lymph nodes.

M/E

- i. There are non-caseating epithelioid cell granulomas having Langhans' and foreign body giant cells.
- ii. Typically, sarcoid granulomas are devoid of lymphocytes i.e. they are "naked" granulomas (Fig. 17.4,A)
- iii. Old granulomas may show condensation of fibrosis at the periphery.
- iv. Sometimes, giant cells may show various types of cytoplasmic inclusions e.g. asteroid bodies, Schaumann's bodies, conchoid bodies (Fig. 17.4,B) etc.



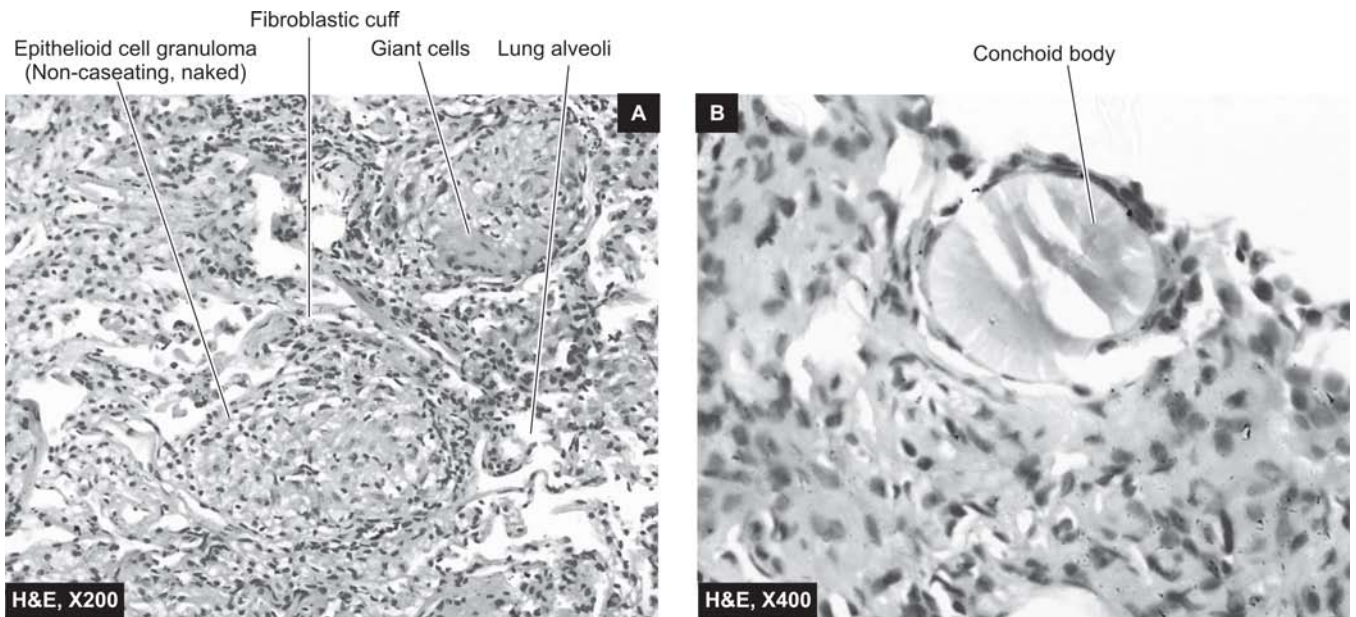


FIGURE 17.4: Sarcoidosis lung. **A**, Characteristic non-caseating epithelioid cell granulomas with a few giant cells and paucity of lymphocytes. **B**, Higher magnification showing characteristic cytoplasmic inclusions.



Specific Infections and Infestations I

- ◆ Actinomycosis Skin
- ◆ Madura Foot
- ◆ Aspergillosis Lung

Exercise

18

ACTINOMYCOSIS SKIN

Actinomycosis is a chronic suppurative disease caused by anaerobic bacteria, *Actinomyces israelii*. Head and neck region is the most common location of the lesion.

G/A There is a firm swelling in the region of the lower jaw initially but later sinuses and abscesses are formed. The pus contains characteristic yellow *sulphur granules* (Fig. 18.1).

M/E

- The inflammatory reaction is a granuloma with central suppuration. Centre of the lesion shows abscess and at the periphery are seen chronic inflammatory cells, giant cells and fibroblasts.

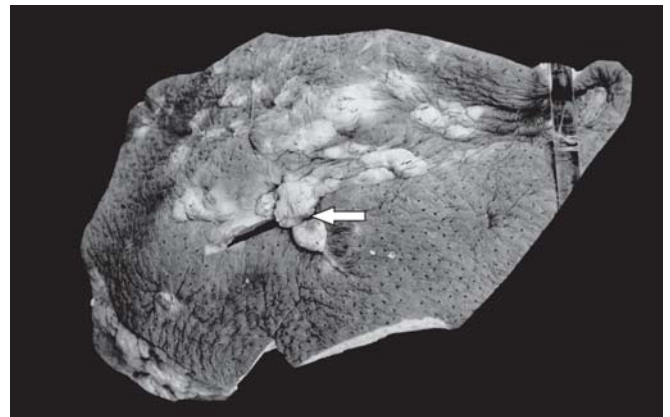


FIGURE 18.1: Skin surface shows multiple draining sinuses with blackish grains (arrow). These sinuses extend into underlying tissues as well.

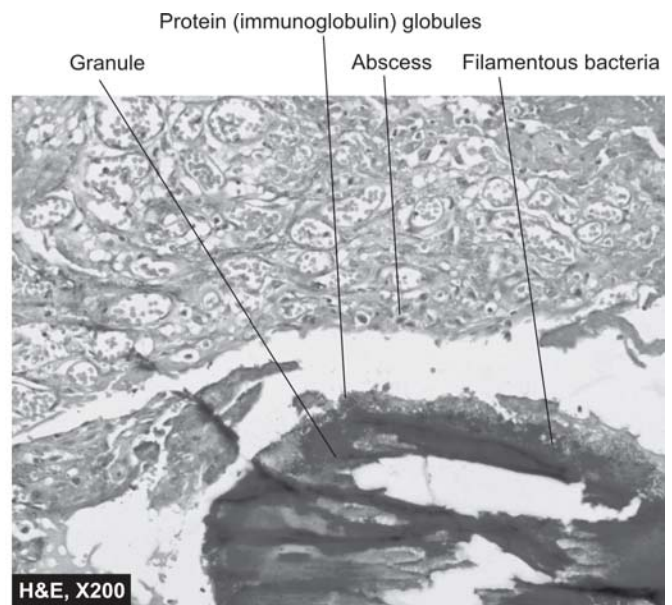
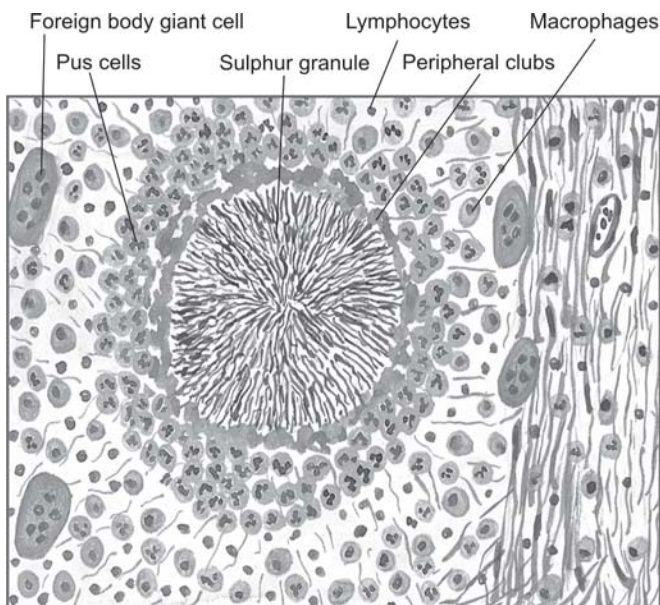


FIGURE 18.2: Actinomycosis. A characteristic 'sulphur granule' surrounded by neutrophils.

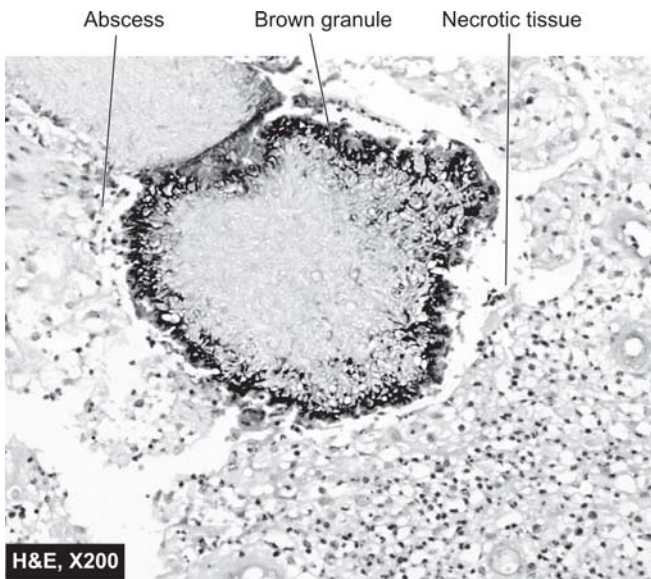


FIGURE 18.3: Madura foot. Discharging sinus containing brown grain lying in necrotic tissue.

- ii. The bacterial colony, sulphur granule, characterised by basophilic radiating filaments with hyaline, eosinophilic, club-like ends is seen in the centre of suppuration (Fig. 18.2).

MADURA FOOT

Eumycetoma is a chronic suppurative infection caused by true fungi, most commonly by *Madurella mycetomatis* or *Madurella grisea*. Foot is the most common location of the lesion.

G/A The affected site in chronic lesions produces swelling and black granules from discharging sinuses.

M/E

- i. The lesion shows the sinus tract discharging purulent material and grains.
- ii. The grains of brown colonies of the fungus are seen in the purulent foci (Fig. 18.3).
- iii. The surrounding tissue shows mononuclear inflammatory cell reaction and fibrosis.

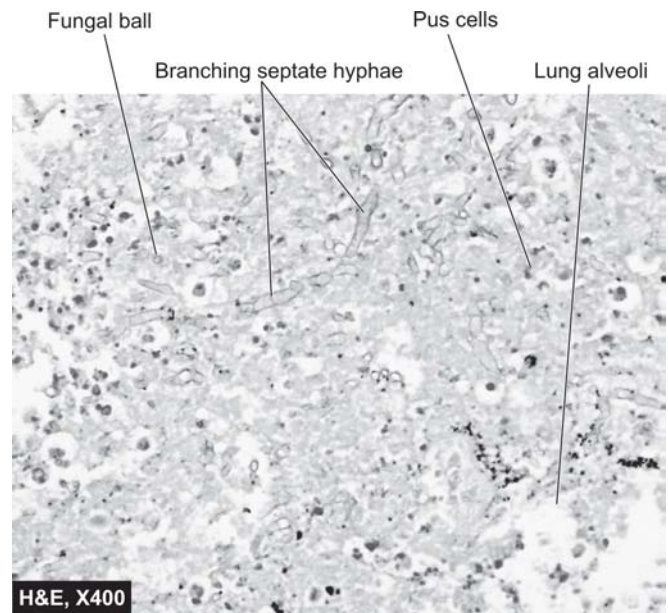


FIGURE 18.4: Aspergillosis lung. Acutely branching hyphae surrounded by mononuclear cells in the lung.

ASPERGILLOSIS LUNG

Aspergillosis is the most common opportunistic fungal infection, usually involving the lungs. The most common human pathogen is *Aspergillus fumigatus*. It occurs in 3 forms—allergic broncho-pulmonary aspergillosis, aspergilloma and invasive aspergillosis.

G/A Aspergilloma occurs in pulmonary cavities or in bronchiectasis as fungal ball.

M/E

- i. There is a mass of tangled hyphae lying within a cavity with fibrous wall.
- ii. The organism has characteristic septate hyphae (2-7 μ m in diameter) and has multiple dichotomous branching at acute angles.
- iii. The wall of cavity shows chronic inflammatory cells (Fig. 18.4).



Specific Infections and Infestations II

- ◆ Rhinosporidiosis Nose
- ◆ Cysticercosis Soft Tissue
- ◆ Hydatid Cyst Liver

Exercise

19

RHINOSPORIDIOSIS NOSE

Rhinosporidiosis of the nose is caused by the fungus, *Rhinosporidium seeberi*.

G/A Rhinosporidiosis occurs in a nasal polyp typically. The polypoid mass is gelatinous with smooth and shining surface.

M/E

- Structure of nasal polyp of inflammatory or allergic type is seen i.e. subepithelial loose oedematous connective tissue containing mucous glands and varying number of inflammatory cells like lymphocytes, plasma cells and eosinophils. The

surface of the polyp is covered by respiratory epithelium which may show squamous metaplasia.

- Large number of organisms of the size of erythrocytes with chitinous wall are seen in the thick-walled sporangia. Spores are also seen in the submucosa and on the surface of the mucosa (Fig. 19.1).

CYSTICERCOSIS SOFT TISSUE

Cysticercus cellulosae is caused by the larval stage of pork tapeworm, *Taenia solium*. The most common sites in the body are skeletal muscle, brain (Fig. 19.2), skin and heart.

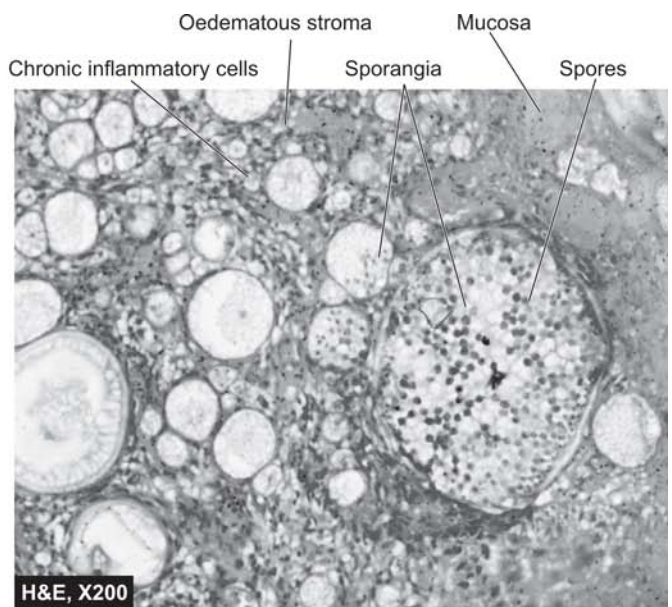
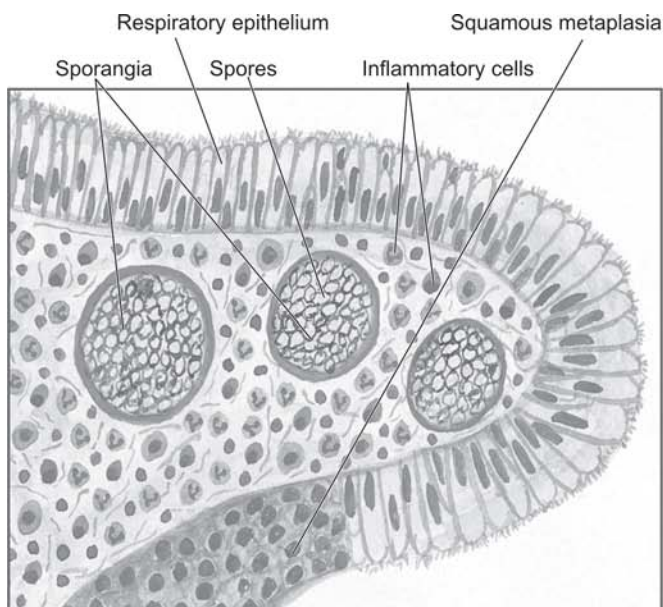


FIGURE 19.1: Rhinosporidiosis in a nasal polyp. The spores are present in sporangia under the nasal mucosa as well as are intermingled in the inflammatory cell infiltrate.

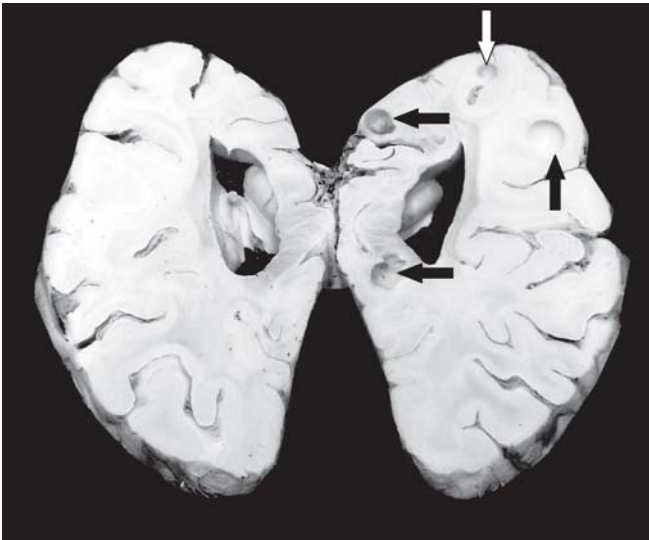


FIGURE 19.2: Cysticercosis brain. The sliced surface of the cerebral hemispheres of the brain shows many whitish nodules and cysts, about 1 cm in diameter (arrows).

G/A The lesions may be solitary or multiple and appear as round to oval white cyst, about 1 cm in diameter (Fig. 19.2). The cyst contains milky fluid.

M/E

- i. The cysticercus cellulosae lying in the cyst shows continuity of epithelium on surface and that lining the body canal. Sometimes the parasite is degenerated or even calcified.
- ii. The dead and degenerated forms incite intense tissue reaction. The cyst wall is lined by palisades of histiocytes. It is surrounded by inflammatory cell reaction consisting of mixed infiltrate including prominence of eosinophils (Fig. 19.3).

HYDATID CYST LIVER

Hydatid disease occurs as a result of infection by the larval stage of the tapeworm, *Echinococcus granulosus*. The liver is a common site for development of hydatid cyst.

G/A The cyst may vary in size and may attain the size over 10 cm in diameter. The cyst wall has laminated membrane and the lumen contains clear fluid (Fig. 19.4).

M/E The cyst wall is composed of 3 distinguishable zones (Fig. 19.5):

- i. Outer *Pericyst* is the inflammatory reaction by the host consisting of mononuclear cells, eosinophils,

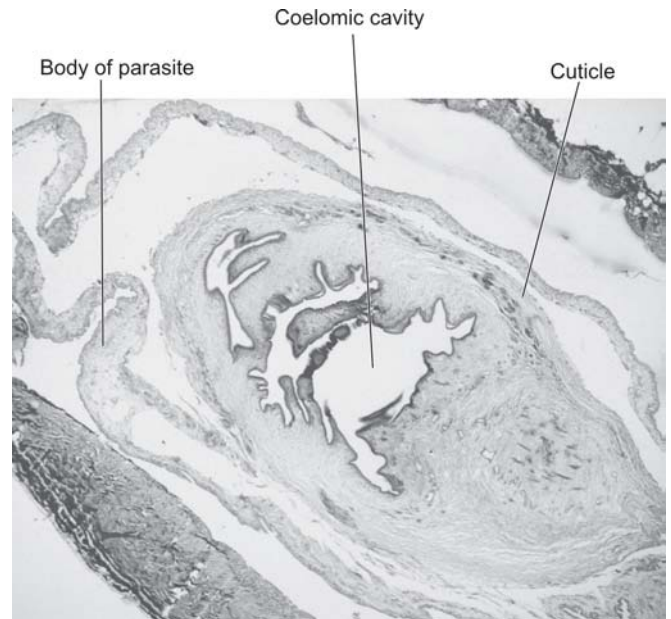


FIGURE 19.3: Cysticercosis muscle. The worm is seen in the cyst while the cyst wall below contains palisaded histiocytes.

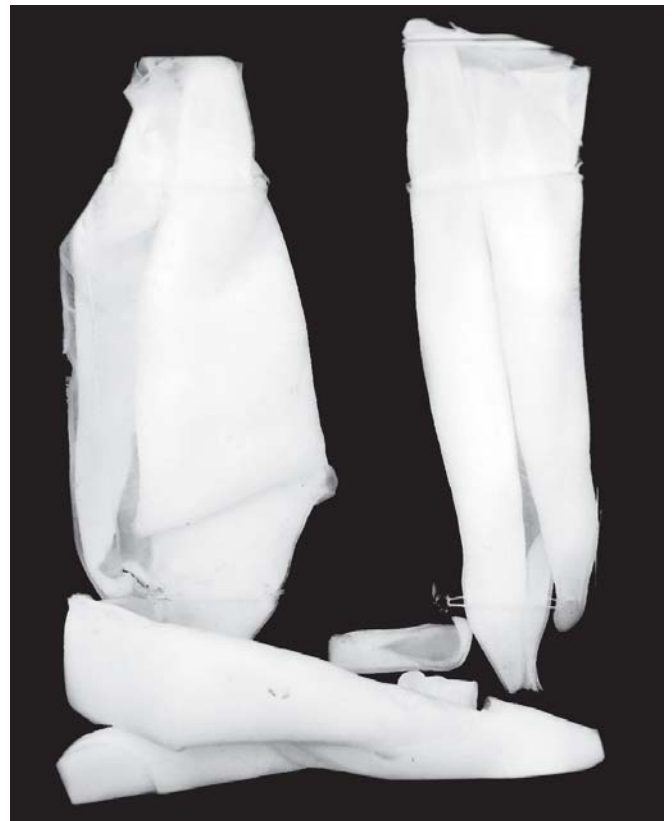


FIGURE 19.4: Hydatid cyst in the liver. It shows a cyst wall composed of curled up whitish membranous layer resembling the membrane of a hard-boiled egg.

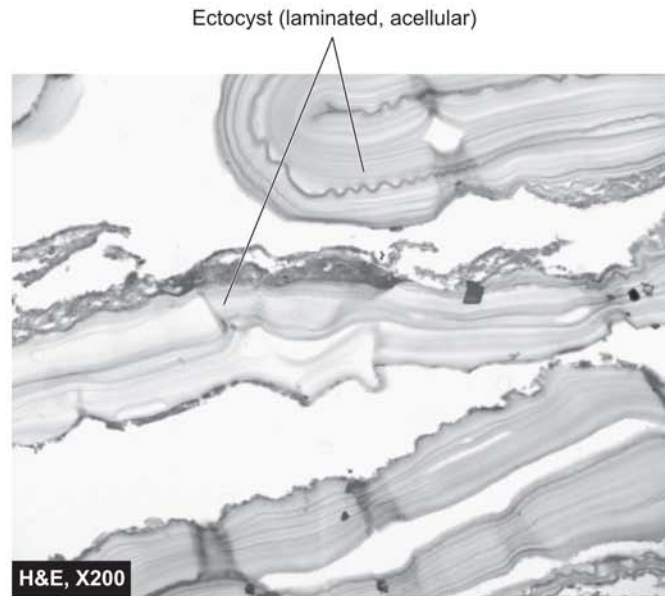
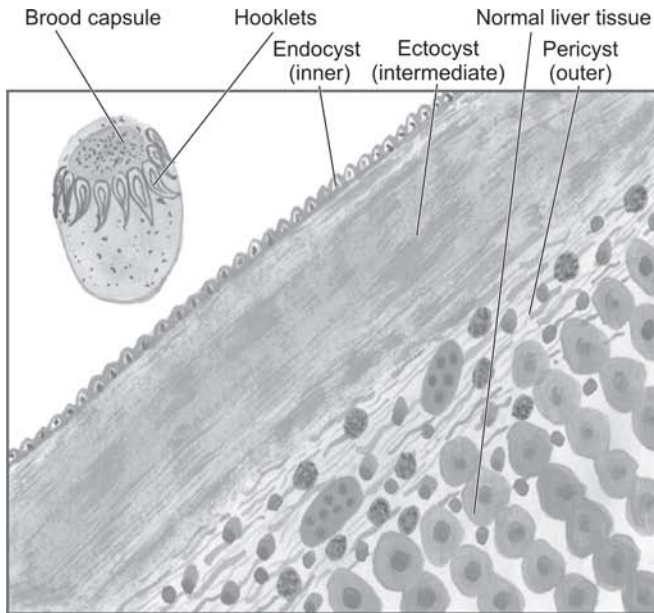


FIGURE 19.5: Hydatid cyst. Microscopy shows three layers in the wall of hydatid cyst—endocyst, ectocyst and pericyst.

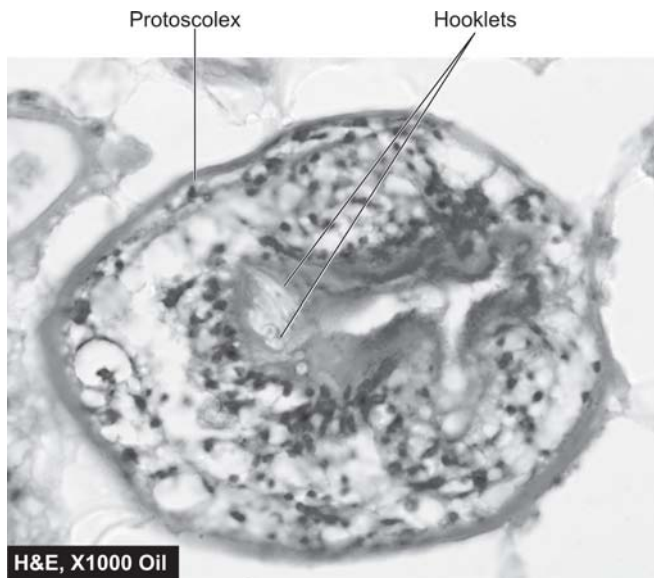


FIGURE 19.6: Hydatid cyst. Close up of scolex.

some giant cells and surrounded peripherally by fibroblasts.

- ii. *Ectocyst* is the characteristic intermediate layer composed of acellular, chitinous laminated, hyaline material.
- iii. *Endocyst* is the inner germinal layer bearing the daughter cysts and scolices projecting into the lumen (Fig. 19.6).



Growth Disorders

- ◆ Testicular Atrophy
- ◆ Cardiac Hypertrophy
- ◆ Reactive Hyperplasia Lymph Node
- ◆ Squamous Metaplasia Cervix

Exercise

20

TESTICULAR ATROPHY

Atrophy means reduction in number of cells which were once normal. Testicular atrophy may occur from various causes; senile atrophy is one common cause.

G/A The testis is small in size, firm and fibrotic.

M/E

- Seminiferous tubules:** There is progressive depletion of germ cell elements. The tubular basement membrane is thickened and there is peritubular fibrosis. Some tubules may show hyalinisation.

- Interstitial stroma:** There is increase in interstitial fibrovascular stroma in which Leydig cells are prominent, seen singly or as clusters (Fig. 20.1).

CARDIAC HYPERTROPHY

Hypertrophy is an increase in the size of individual cells resulting in enlargement of the organ. Hypertrophy of the cardiac muscle occurs commonly due to aortic stenosis and systemic hypertension.

G/A The heart is enlarged and heavy and may weigh as much as 700-800 g as compared to average normal

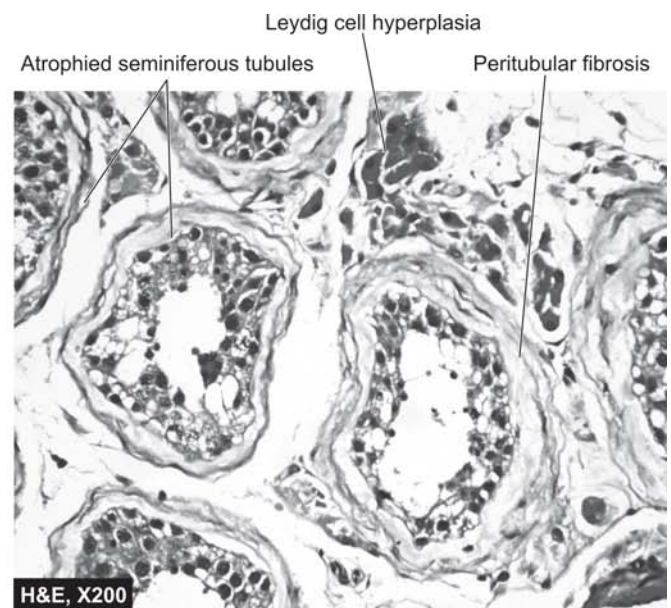
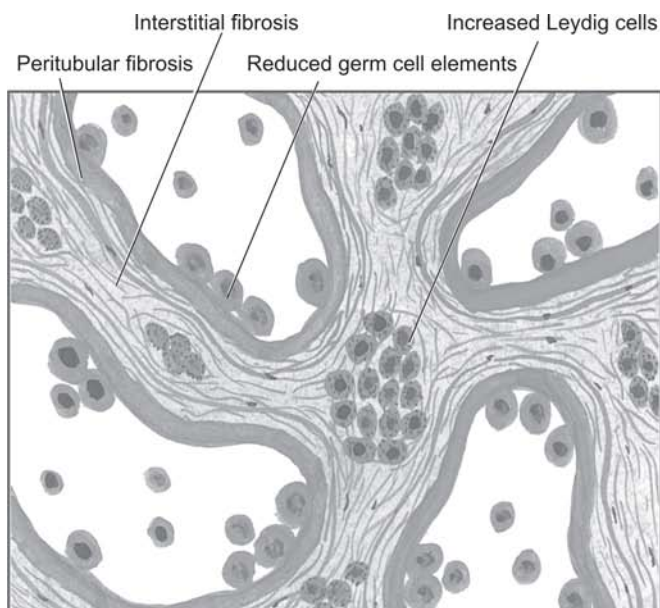


FIGURE 20.1: Microscopic appearance of testicular atrophy. Some of the seminiferous tubules show hyalinisation while others show peritubular fibrosis. There is prominence of Leydig cells in the interstitium.

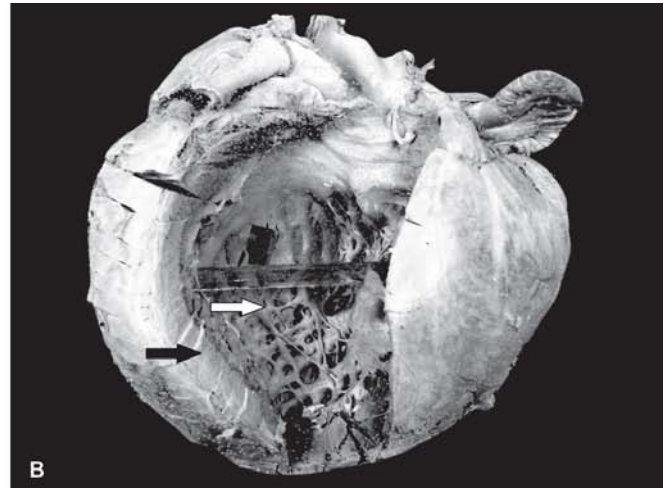
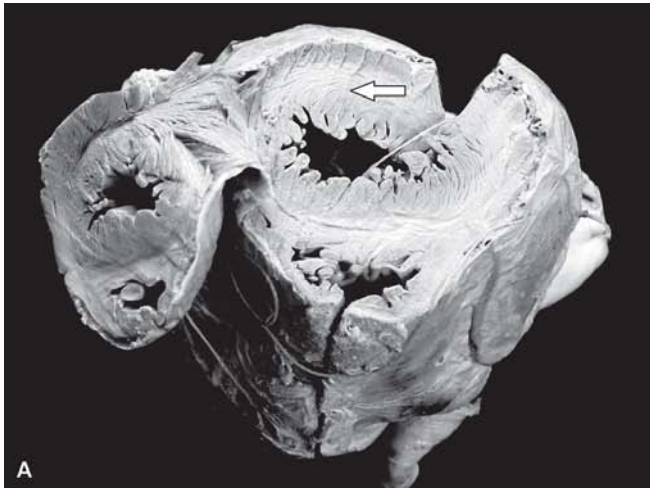


FIGURE 20.2: **A**, Concentric cardiac hypertrophy. The weight of the heart is increased. A slice of the heart is cut off at the apex revealing lumina and walls of both the ventricles. The left ventricular wall is thickened concentrically around the chamber (arrow) while the left ventricular lumen is obliterated.

B, Eccentric cardiac hypertrophy. The weight of the heart is increased. The free left ventricular wall is thickened (black arrow) while the left ventricular lumen is dilated (white arrow). The right ventricular lumen and wall are unaffected.

weight of 350 g. Cross section of the heart at the apex shows left ventricular hypertrophy. Thickness of the left ventricular wall is over 2 cm compared to normal upto 1.5 cm. Cardiac hypertrophy without dilatation of the chamber is termed *concentric* and when associated with dilatation is termed *eccentric* (Fig. 20.2).

M/E

- There is an increase in the size of individual muscle fibres with prominent nuclei.
- Foci of degenerative changes and necrosis in the hypertrophied myocardium may be seen (Fig. 20.3).

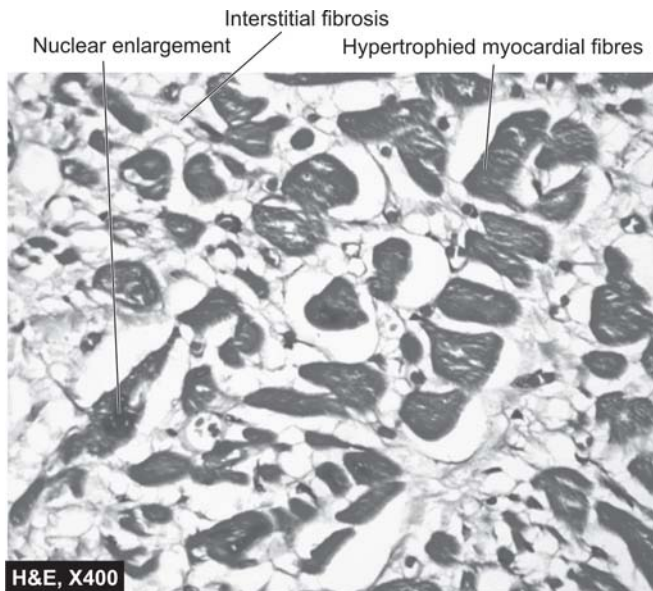


FIGURE 20.3: Cardiac hypertrophy. Individual myocardial fibres are thick and have prominent vesicular nuclei.

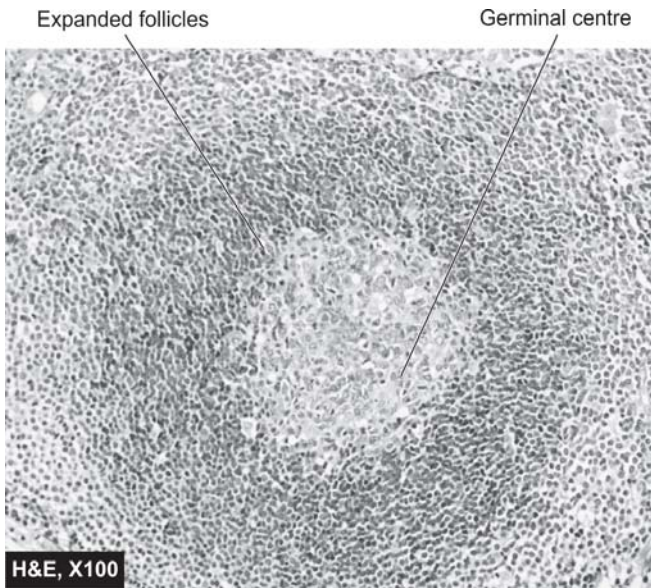


FIGURE 20.4: Reactive hyperplasia lymph node, follicular type.

REACTIVE HYPERPLASIA LYMPH NODE

Hyperplasia means an increase in the number of the cells of a part. Lymphoid tissue readily undergoes reactive hyperplasia in response to local irritation.

G/A The affected lymph nodes are enlarged, firm and non-tender.

M/E Reactive hyperplasia of lymph nodes in chronic nonspecific lymphadenitis shows one of the following three patterns:

- i. *Follicular hyperplasia* is most common and is characterised by enlargement and prominence of germinal centres of lymphoid follicles (Fig. 20.4).
- ii. *Paracortical lymphoid hyperplasia* is due to hyperplasia and enlargement of paracortex so that lymphoid follicles are effaced.

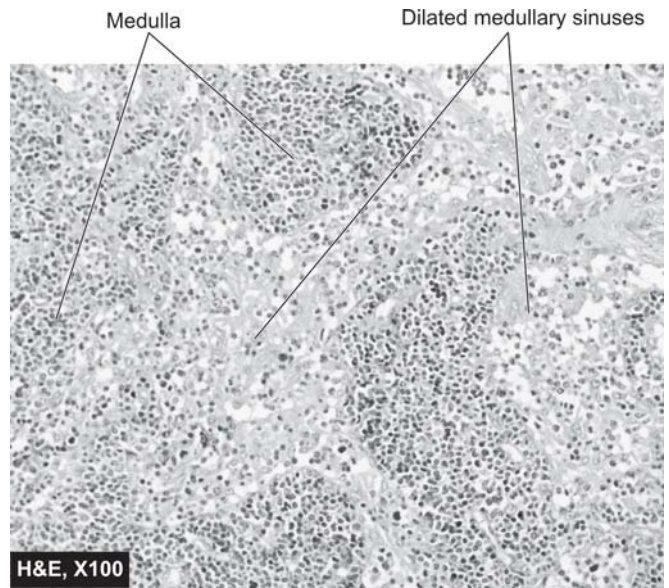


FIGURE 20.5: Reactive hyperplasia lymph node, sinus histiocytosis type.

- iii. *Sinus histiocytosis* refers to distention and engorgement of the sinusoids of the lymph node with histiocytes and endothelial cells (Fig. 20.5).

SQUAMOUS METAPLASIA CERVIX

Squamous metaplasia is defined as a reversible change of one type of epithelium to the squamous type which is a less well-specialised epithelium.

G/A Squamous metaplasia of the cervix is commonly encountered in prolapsed uterus. The cervix is pearly white and isthmus is elongated.

M/E

- i. Columnar lined mucosa as well as cervical glands show foci of squamous cell change.
- ii. Surface of the cervix may show keratinisation and hyperkeratosis.
- iii. There is generally some degree of subepithelial chronic inflammatory cell infiltrate (Fig. 20.6).

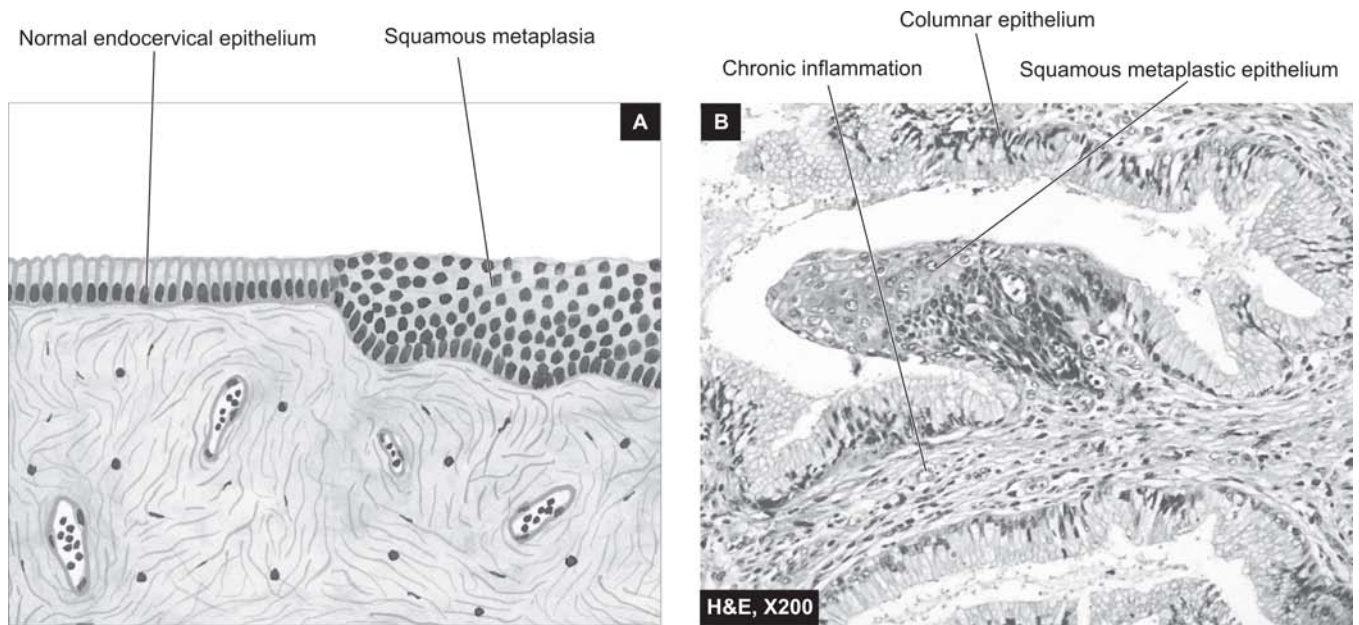


FIGURE 20.6: **A**, Schematic diagram showing change in uterine cervix from normal mucus-secreting endocervical epithelium to squamous metaplastic epithelium. **B**, Squamous metaplasia cervix. Part of some of the glands are lined by columnar epithelium while other parts show change to squamous lining.



Neoplasia I

- ◆ Squamous Cell Papilloma
- ◆ Squamous Cell Carcinoma
- ◆ Malignant Melanoma
- ◆ Basal Cell Carcinoma

Exercise

21

SQUAMOUS CELL PAPILLOMA

Squamous cell papilloma is a common benign epithelial tumour of the skin.

G/A Surface of the tumour shows finger-like processes (Fig. 21.1).

M/E

- The epidermis is thickened but orderly and is thrown into finger-like processes or papillae.

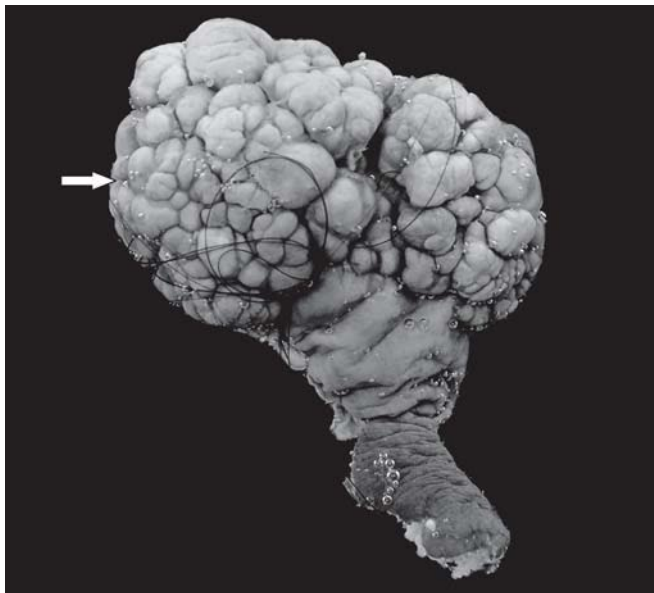


FIGURE 21.1: Squamous cell papilloma skin. The skin surface shows a papillary growth on the surface (arrow) having a pedicle. It is elevated above the adjoining normal skin without any invasion.

- The central core of the papillae is composed of loose fibrovascular tissue (Fig. 21.2).

SQUAMOUS CELL CARCINOMA

Squamous cell or epidermoid carcinoma occurs most commonly in the skin, oral cavity, oesophagus, uterine cervix and at the edge of chronic ulcers.

G/A The tumour is either in the form of nodular and ulcerative growth, or fungating and polypoid mass without ulceration. The margin of the growth is elevated and indurated. Cut section of the growth shows grey-white endophytic as well as exophytic tumour (Fig. 21.3).

M/E

- The tumour is characterised by malignant cells which may show variable degree of differentiation.
- The masses of tumour cells invade through the basement membrane into dermis.
- In better differentiated tumours, the cells are arranged in concentric layers called epithelial pearls and contain keratin material in the centre of the cell masses.
- The masses of tumour cells are separated by lymphocytes (Figs 21.4).

MALIGNANT MELANOMA

Malignant melanoma or melanocarcinoma is the malignant counterpart of naevus and is the most rapidly spreading malignant tumour of the skin.

G/A Malignant melanoma may appear as flat, macular or slightly elevated, nodular lesion. The lesion exhibits variation in pigmentation appearing in shades of black, brown, grey, blue or red. The borders are irregular (Fig. 21.5).

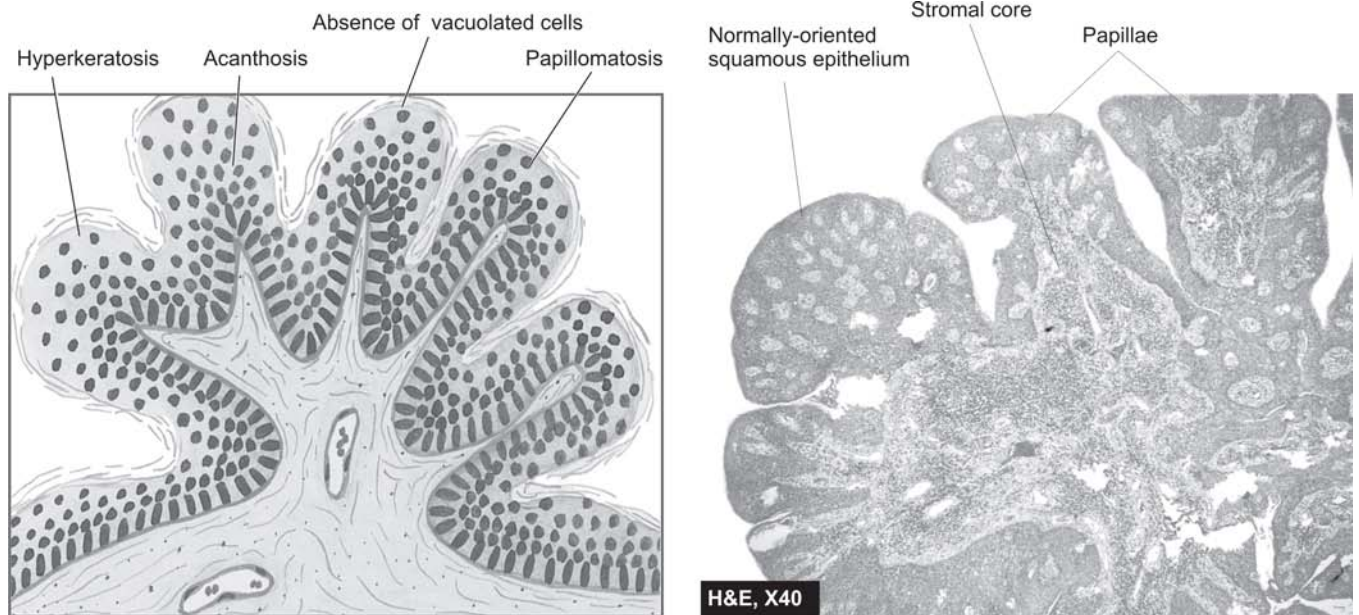


FIGURE 21.2: Finger-like projections are covered by normally-oriented squamous epithelium while the stromal core contains fibrovascular tissue.

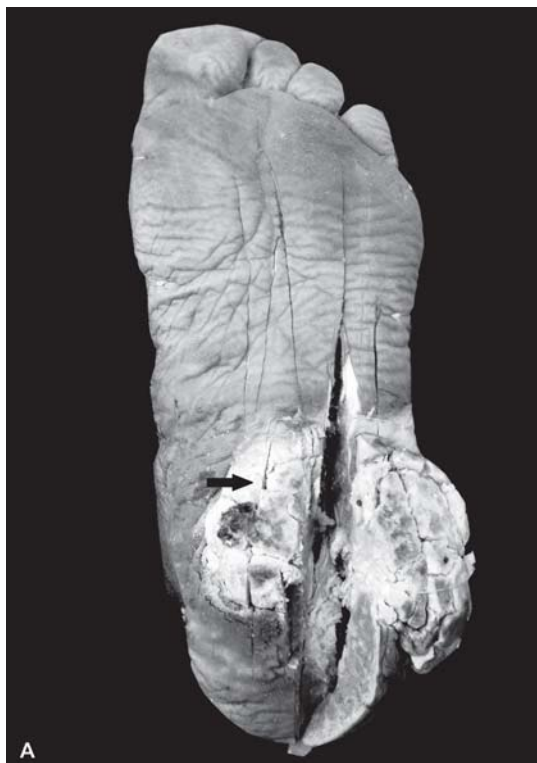


FIGURE 21.3: Squamous cell carcinoma. **A**, The skin surface on the sole of the foot shows a fungating and ulcerated growth (arrow). **B**, Carcinoma oesophagus showing narrowing of the lumen and thickening of the wall (arrow).

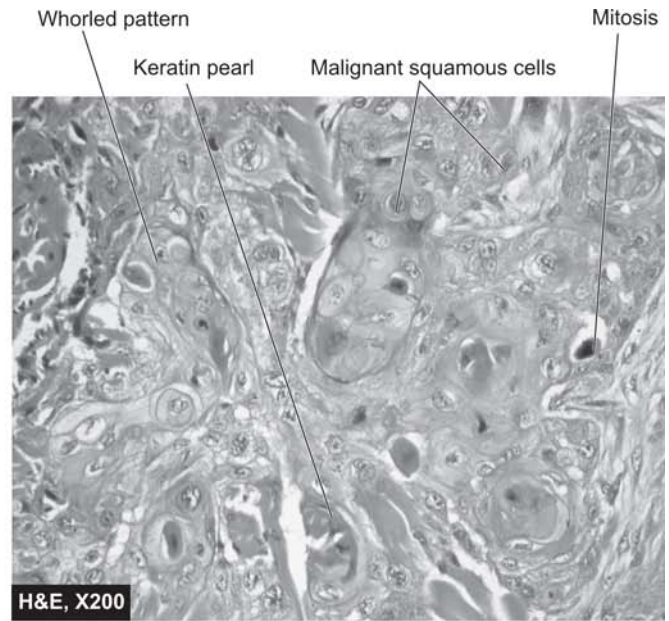
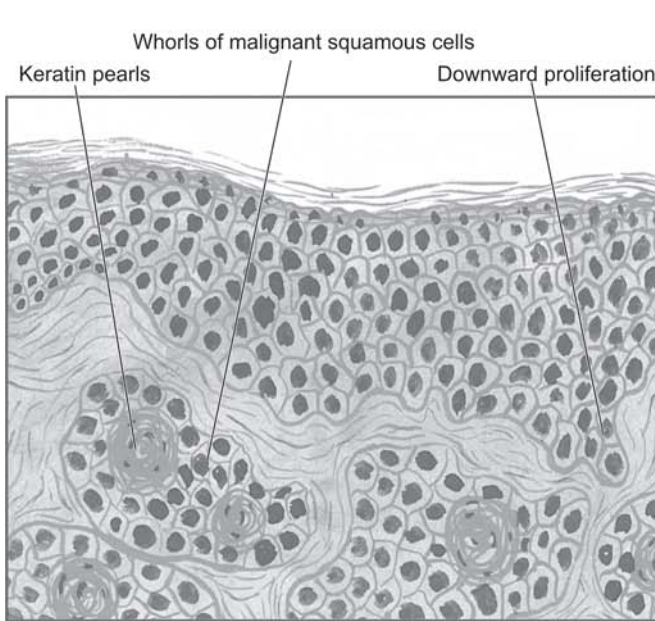


FIGURE 21.4: Squamous cell carcinoma, well-differentiated type. The dermis is invaded by downward proliferating epidermal masses of cells which show atypical features. A few horn pearls with central laminated keratin are present. There is marked inflammatory reaction in the dermis between the masses of tumour cells.

M/E

- The tumour has marked junctional activity at the epidermo-dermal junction and grows horizontally as well as downwards into the dermis.
- The tumour cells are arranged in a variety of patterns—solid masses, sheets, islands etc.



FIGURE 21.5: Malignant melanoma oral cavity. In this hemimaxillectomy specimen, whitish oral mucosa shows an elevated blackish area with ulceration. Cut surface shows blackish tumour with irregular outlines (arrow).

- The individual tumour cell are usually larger than the naevus cells, contain large vesicular nuclei with peripherally condensed chromatin and having prominent eosinophilic nucleoli. The cytoplasm is amphophilic (Fig. 21.6).
- Melanin pigment is present in the cytoplasm in the form of uniform fine granules (unlike cells at the periphery of the lesion).

BASAL CELL CARCINOMA

Basal cell carcinoma or rodent ulcer is a locally invasive slow-growing tumour of the skin of face in the middle-aged that rarely metastasises.

G/A The tumour is commonly a nodular growth with central ulceration (nodulo-ulcerative). The margins of the tumour are pearly white and rolled while the base shows ulceration and destruction of underlying tissues like a rodent.

M/E

- The tumour cells resemble normal basal cell layer of the skin and grow downwards from the epidermis in a variety of patterns—solid masses, nests, islands, strands, keratotic masses, adenoid etc.
- All patterns of tumour cells have one common characteristic feature—the cells forming the

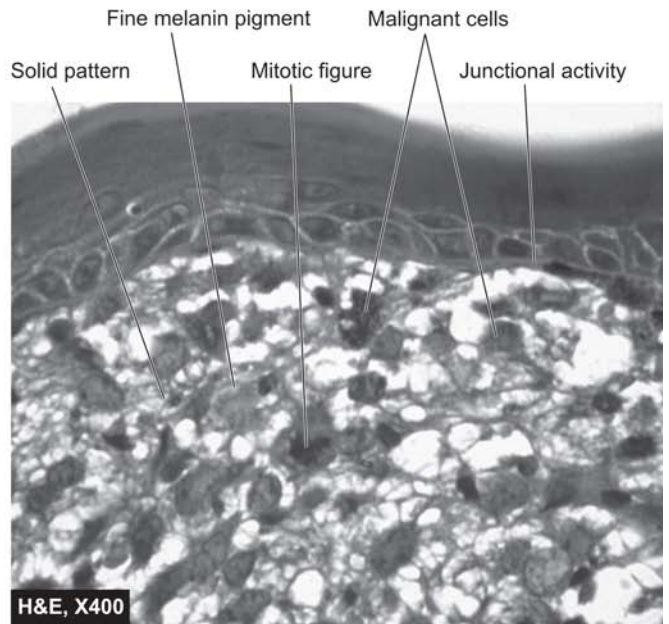
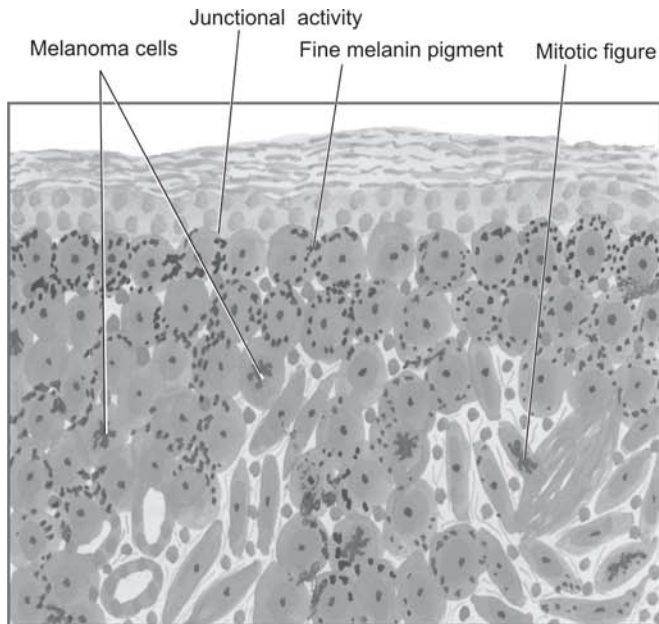


FIGURE 21.6: Malignant melanoma. There is marked junctional activity at the dermal-epidermal junction. Tumour cells resembling epithelioid cells with pleomorphic nuclei and prominent nucleoli are seen as solid masses in the dermis. Many of the tumour cells contain fine granular melanin pigment.

- periphery of tumour have parallel alignment or show palisading (basaloid cells).
- iii. The tumour cells are basophilic with hyperchromatic nuclei (Fig. 21.7).

- iv. Stroma shrinks away from epithelial tumour nests, creating clefts which help in differentiating it from the adnexal tumours.

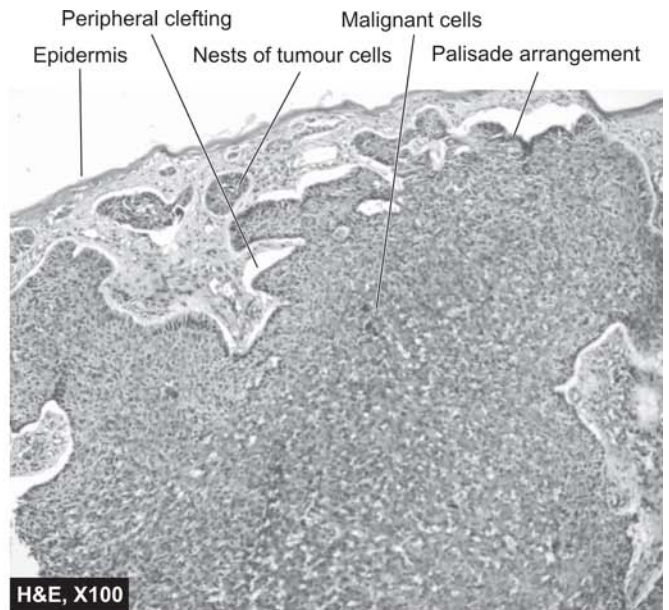
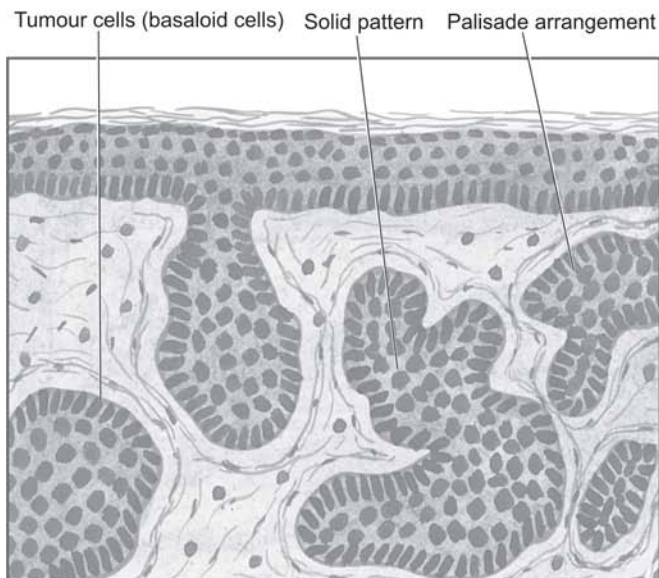


FIGURE 21.7: Solid basal cell carcinoma. The dermis is invaded by irregular masses of basaloid cells with characteristic peripheral palisaded appearance.

Neoplasia II

- ◆ Lipoma
- ◆ Pleomorphic Rhabdomyosarcoma
- ◆ Metastatic Carcinoma Lymph Node
- ◆ Metastatic Sarcoma Lung

Exercise

22

LIPOMA

Lipoma is a common benign tumour occurring in the subcutaneous tissues.

G/A The tumour is small, encapsulated, round to oval. The cut surface is soft, lobulated, yellowish and greasy (Fig. 22.1).

M/E

- A thin fibrous capsule surrounds the periphery.
- The tumour is composed of lobules of mature adipose cells separated by thin fibrous septa (Fig. 22.2).

PLEOMORPHIC RHABDOMYOSARCOMA

Malignant tumour of skeletal muscle origin is called rhabdomyosarcoma. Out of the various growth patterns and histology, pleomorphic rhabdomyosarcoma occurs

in older adults, commonly in the extremities, and is a highly malignant tumour.

G/A The tumour forms a well-circumscribed, soft, whitish mass with areas of haemorrhages and necrosis (Fig. 22.3).

M/E

- The tumour is composed of highly anaplastic cells having bizarre appearance and many multinucleate giant cells (Fig. 22.4).
- The tumour cells may have variety of shapes—racquet shape, tadpole appearance, large strap cells, ribbon shape etc.
- Cross striations may be demonstrable in the cytoplasm of the tumour cells in routine staining or by special stain, PTAH.

METASTATIC CARCINOMA LYMPH NODE

The regional lymph nodes may show metastatic deposits, most commonly from carcinomas but sometimes sarcomas may also metastasise to the regional lymph nodes.

G/A The affected lymph nodes are enlarged and matted. Cut surface shows homogeneous, grey-white deposits with areas of necrosis (Fig. 22.5).

M/E The features of metastatic carcinoma reproduce the picture of primary tumour. In a metastatic carcinoma from infiltrating duct carcinoma breast, the features are as under:

- The nodal architecture is replaced by masses of malignant cells forming solid nests, cords and poorly-formed glandular structures.
- Part of cortex and capsule of the lymph node are intact (Fig. 22.6).



FIGURE 22.1: Lipoma. The tumour shows a thin outer capsule. Cut surface is soft, lobulated, yellowish and greasy.

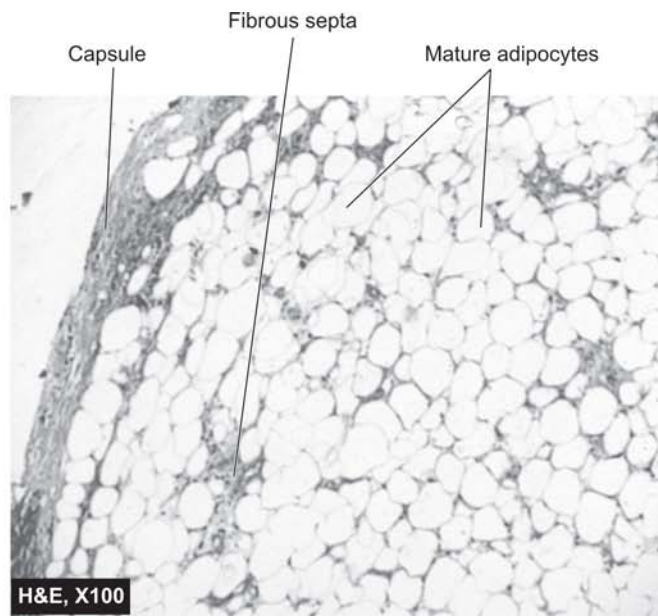
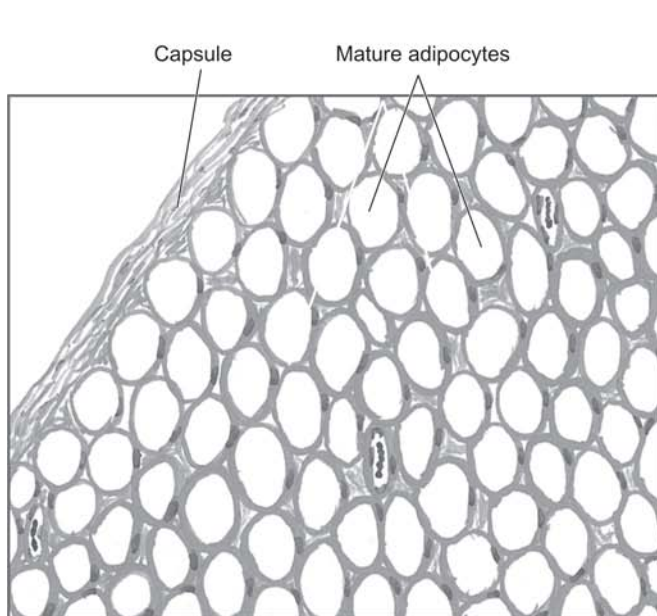


FIGURE 22.2: Lipoma. The tumour composed of mature fat cells is enclosed by thin fibrous capsule.

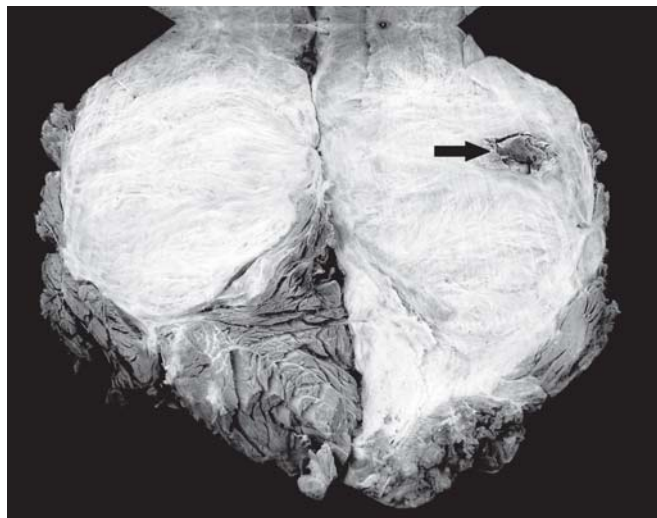


FIGURE 22.3: Pleomorphic rhabdomyosarcoma. Sectioned surface shows an irregular and unencapsulated tumour invading muscle and has multiple nodularity and lobulations. Cut surface is grey-white fleshy with areas of haemorrhage (arrow) and necrosis.

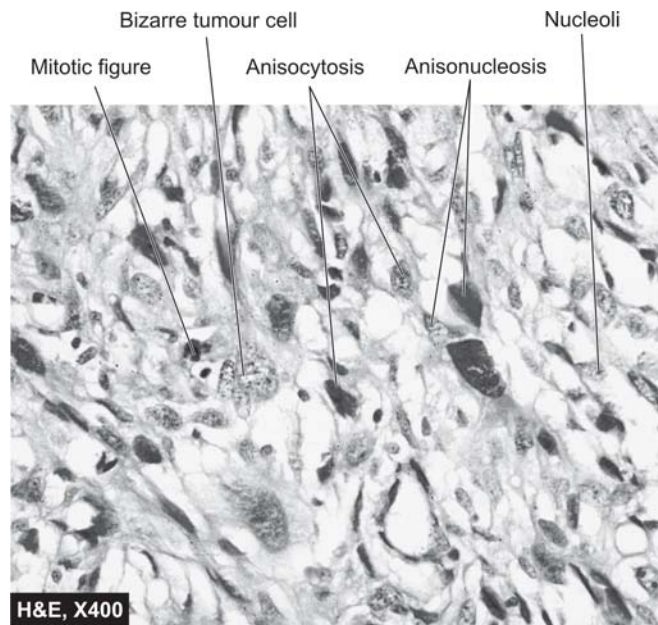


FIGURE 22.4: Pleomorphic rhabdomyosarcoma. Marked pleomorphism, anisonucleosis, hyperchromatic nuclei, prominent nucleoli, mitotic figures and bizarre tumour cells.

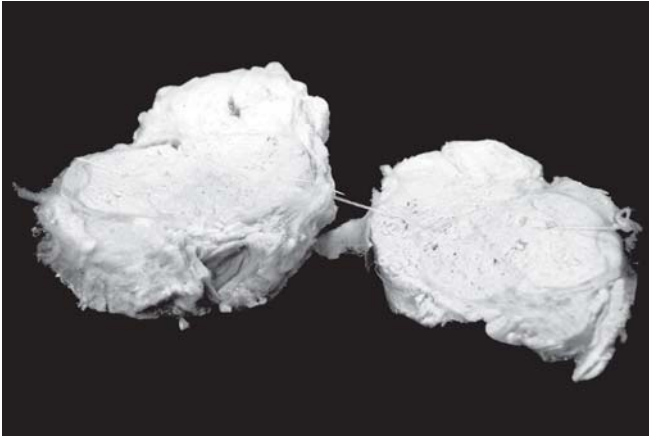


FIGURE 22.5: Metastatic carcinoma in lymph nodes. Matted mass of lymph nodes is surrounded by fat. Cut surface shows large irregular areas of grey-white colour replacing grey-brown nodal tissue.

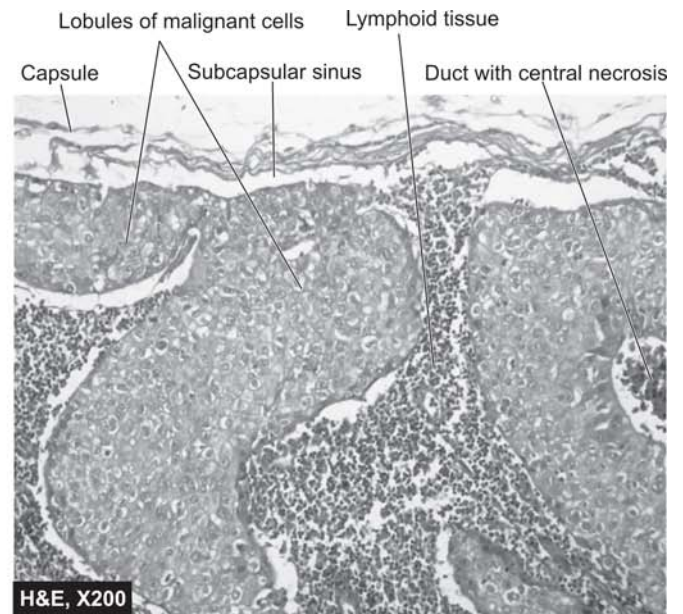
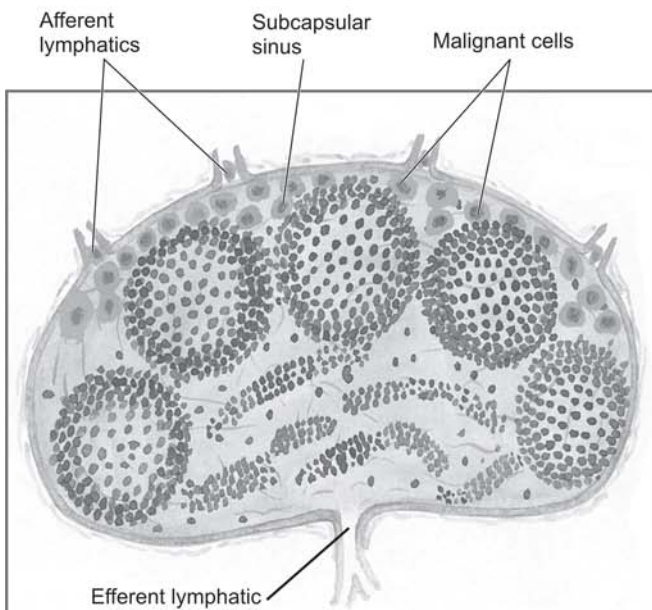


FIGURE 22.6: Metastatic carcinoma in lymph node. Lymphatic spread beginning by lodgement of tumour cells in subcapsular sinus via afferent lymphatics entering at the convex surface of the lymph node.

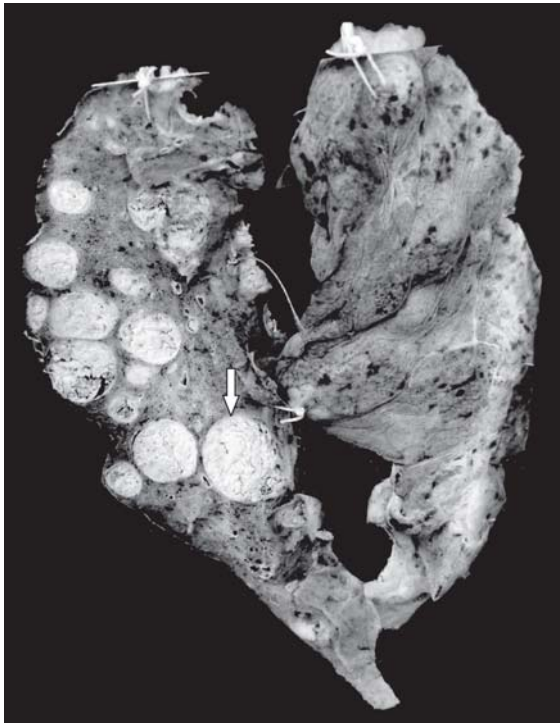


FIGURE 22.7: Metastatic sarcoma lung. Cut surface of the lung shows replacement of spongy parenchyma by multiple, variable-sized, circumscribed nodular masses (arrow). These masses are grey-white in colour and some show areas of haemorrhage and necrosis.

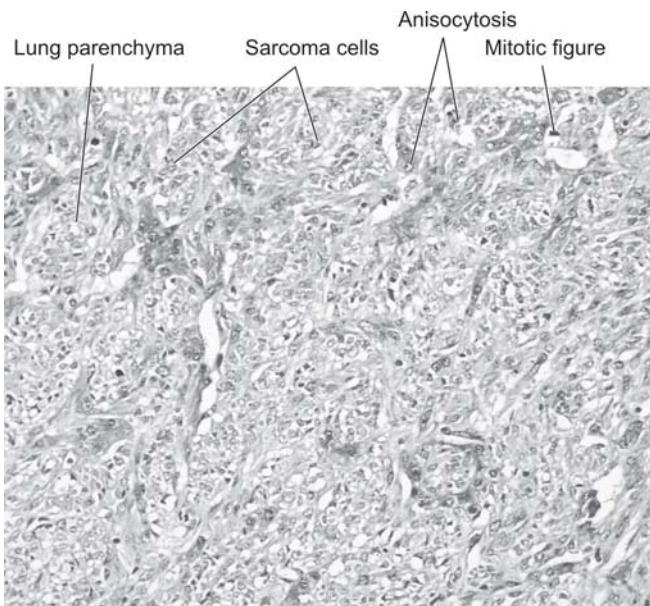


FIGURE 22.8: Metastatic sarcoma lung. Large mass of highly pleomorphic mesenchymal cells has replaced lung tissue on right.

METASTATIC SARCOMA LUNG

Sarcomas commonly metastasise through haematogenous route to lungs, liver, bones, kidneys etc. Some carcinomas, however, too spread by haematogenous route.

G/A The metastatic nodules are scattered throughout all lobes. The tumour nodules are circumscribed, soft and fleshy (Fig. 22.7).

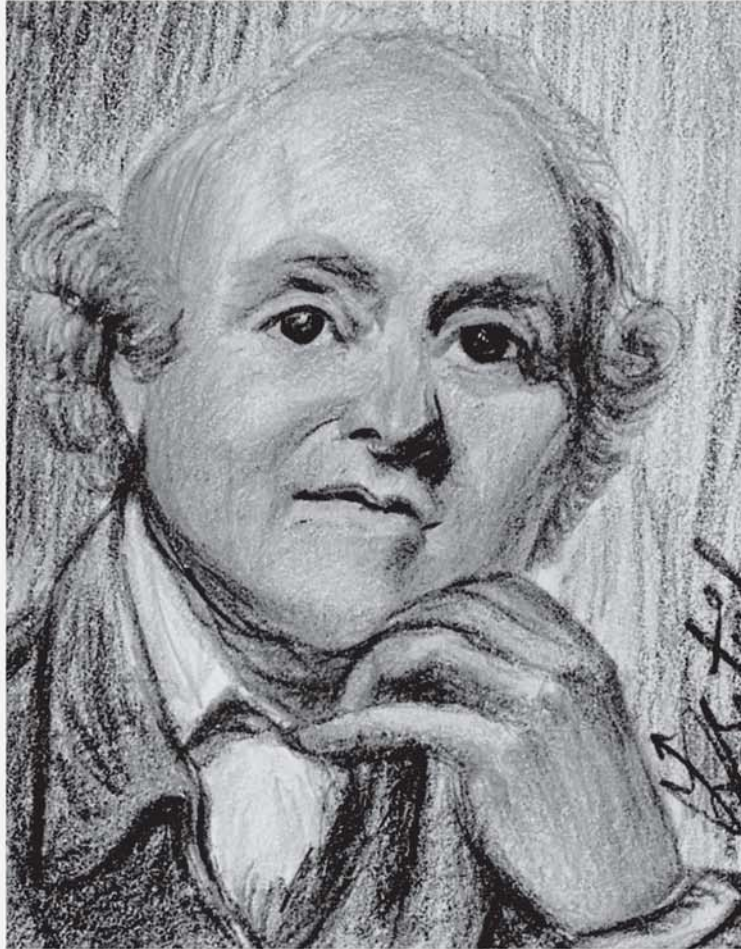
M/E The metastatic tumour reproduces the picture of primary sarcoma. In metastatic deposits from malignant fibrous histiocytoma, the features are as under:

- i. The tumour cells are pleomorphic and are oval to spindle-shaped.
- ii. Multinucleate tumour giant cells are seen.
- iii. The background may show myxoid material and areas of necrosis.
- iv. There is generally rich vascularity (Fig. 22.8).



SECTION FOUR

SYSTEMIC PATHOLOGY



JOHN HUNTER (1728-1793)

‘FOUNDER OF MEDICAL MUSEUM TECHNIQUES’

Scottish surgeon regarded as the greatest surgeon-anatomist of all times. He established unique collection of pathological specimens that later resulted in Hunterian Museum of the Royal College of Surgeons, London.

He described syphilitic chancre (Hunterian chancre) and adductor canal (Hunterian canal). His brother William Hunter was famous anatomist of his time.

SECTION IV: SYSTEMIC PATHOLOGY

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- ★ Learn to describe and identify museum specimens of common conditions pertaining to various organ systems in pathology encountered by an undergraduate student.
- ★ Learn to describe, draw and identify common conditions in microscopic study of systemic pathology.

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Blood Vessels and Lymphatics

- ◆ Atheroma Coronary Artery
- ◆ Capillary Haemangioma Skin
- ◆ Cavernous Haemangioma Liver
- ◆ Lymphangioma Tongue

Exercise

23

ATHEROMA CORONARY ARTERY

A fully-developed atherosclerotic lesion is called atheromatous plaque or atheroma. It is located most commonly in the aorta (Fig. 23.1) and major branches of the aorta including coronaries.

G/A The atheromatous plaque in the coronary is eccentrically located bulging into the lumen from one side. The plaque lesion is white to yellowish-white and may have ulcerated surface. Cut section shows firm *fibrous cap* and central yellowish-white soft porridge-like *core*. Frequently, there is grittiness owing to calcification in the lesion.

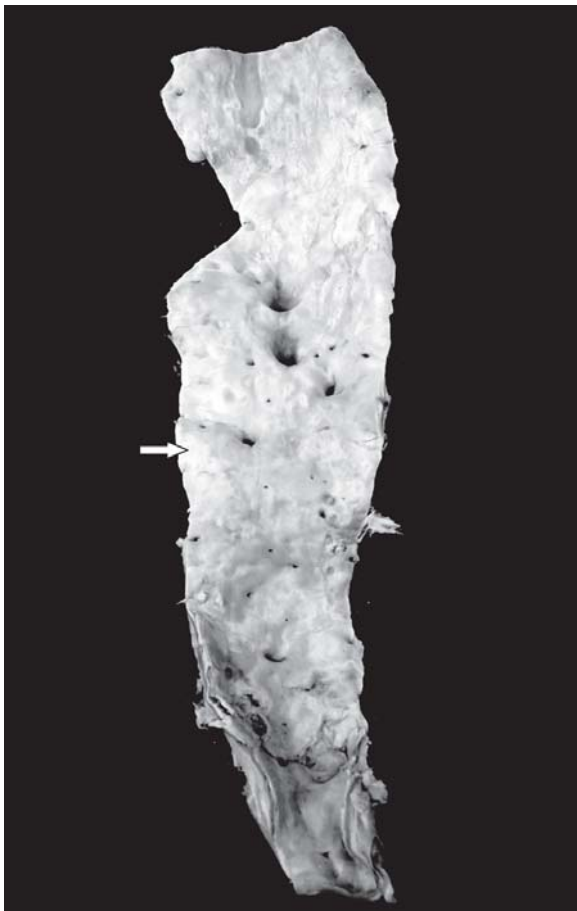


FIGURE 23.1: Fully-developed atheroma. The opened up aorta shows arterial branches coming out. The intimal surface shows yellowish-white lesions, slightly raised above the surface (arrow). A few have ulcerated surface. Many of these lesions are located near the ostial openings on the intima, thus partly occluding them.

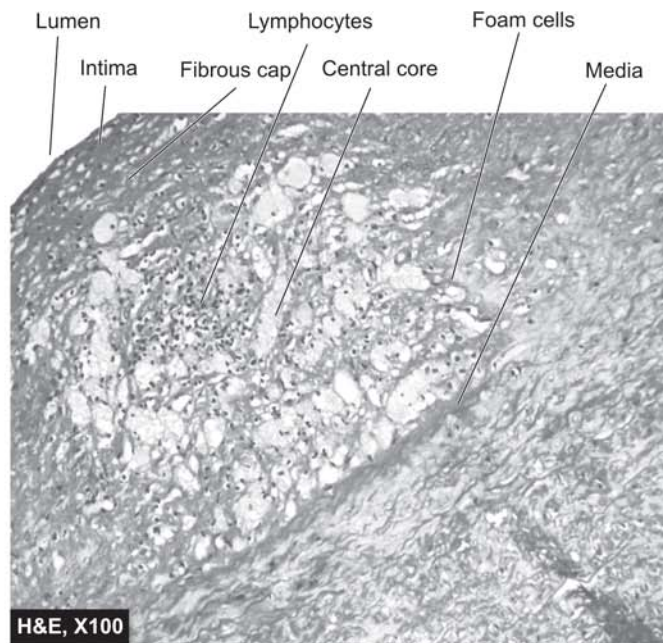
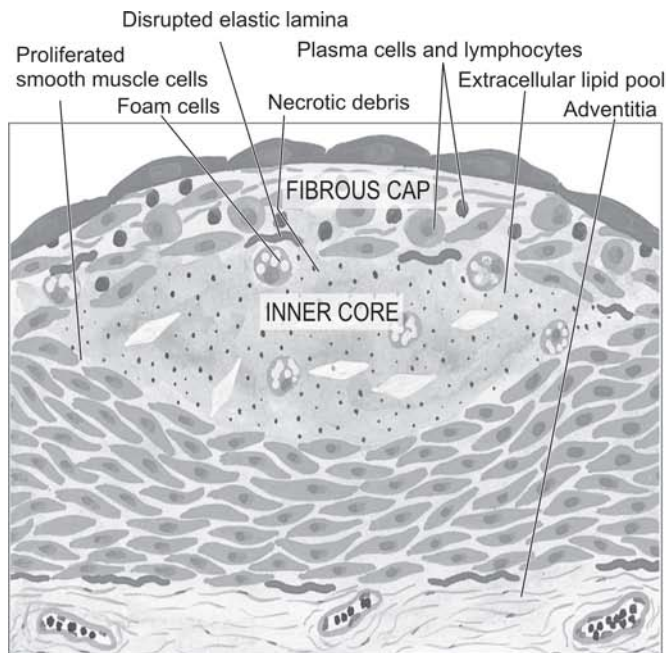


FIGURE 23.2: **A**, Diagrammatic view of the histologic appearance of a fully-developed atheroma. **B**, Atheromatous plaque showing fibrous cap and central core.

M/E The appearance of plaque varies depending upon the age of lesion. However, the following features are invariably present:

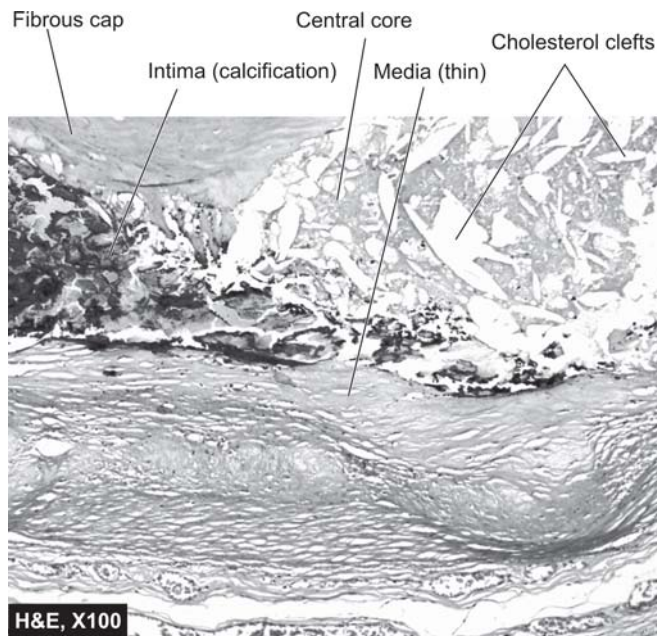


FIGURE 23.3: Complicated plaque lesion. There is critical narrowing of the coronary due to atheromatous plaque having dystrophic calcification.

- The superficial luminal part of *fibrous cap* is covered by endothelium and is composed of smooth muscle cells, dense connective tissue and extracellular matrix.
- The cellular area under the fibrous cap is composed of macrophages, foam cells and lymphocytes.
- The deeper central soft *core* consists of extracellular lipid material, cholesterol clefts, necrotic debris and lipid-laden foam cells (Fig. 23.2).
- Calcium salts are deposited in the vicinity of necrotic area and in the lipid pool deep in the thickened intima (Fig. 23.3).

CAPILLARY HAEMANGIOMA SKIN

Haemangiomas are common lesions on the skin in infancy and childhood.

G/A Haemangioma is a small or large, flat or slightly elevated, red to purple, soft and lobulated lesion varying in size from a few millimeters to a few centimeters in diameter.

M/E The lesion is well-defined but in the form of unencapsulated lobules.

- The lobules are composed of capillary-sized, thin-walled, blood-filled vessels.

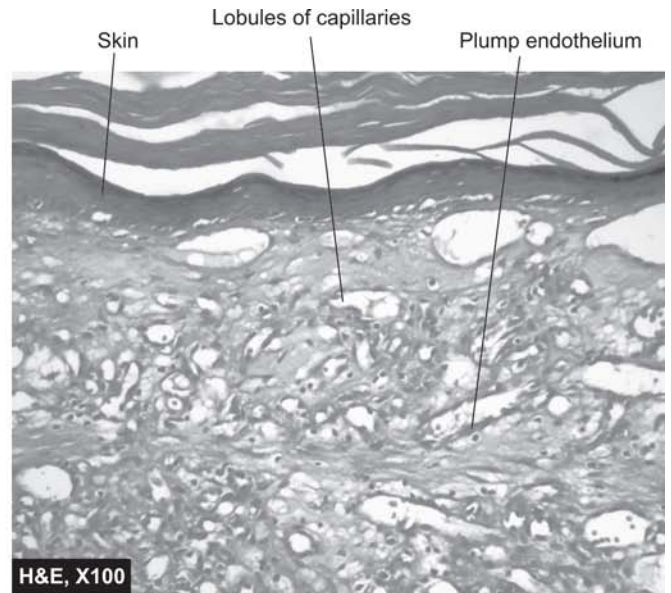
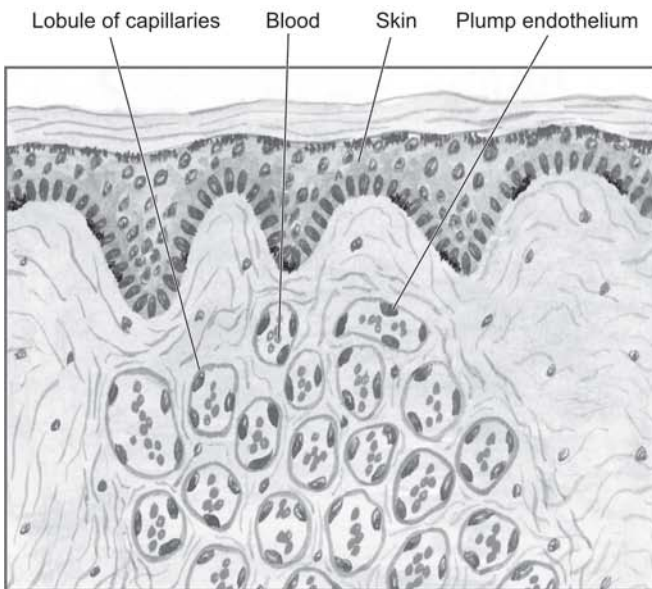


FIGURE 23.4: Capillary haemangioma of the skin. Lobules of capillary-sized vessels lined by plump endothelial cells and containing blood are lying in the dermis.

- ii. The vessels are lined by single layer of plump endothelial cells surrounded by a layer of pericytes.
- iii. Some stromal connective tissue separates lobules of blood vessels (Fig. 23.4).

CAVERNOUS HAEMANGIOMA LIVER

Cavernous haemangioma is a single or multiple, discrete or diffuse, soft and spongy mass.

G/A Cavernous haemangioma varies from 1 to 2 cm in diameter and is located in the organ in the form of red to blue, soft and spongy mass.

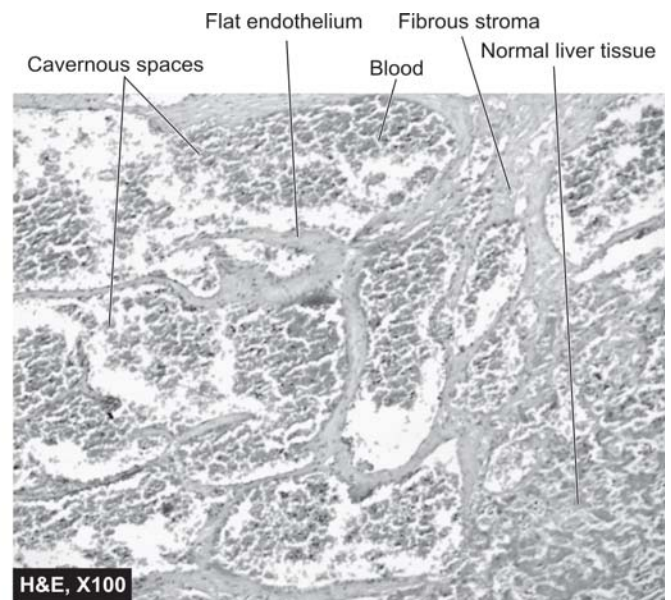
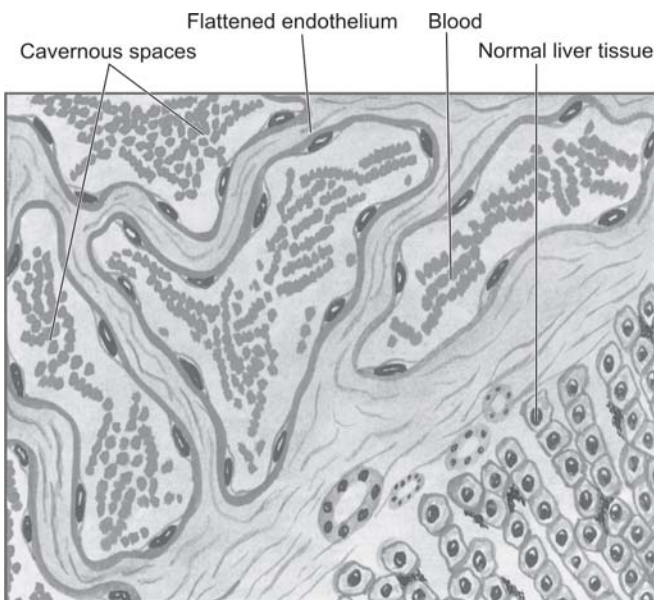


FIGURE 23.5: Cavernous haemangioma of the liver. Large cavernous spaces containing blood are seen in the liver tissue. Scanty connective tissue stroma is seen between the cavernous spaces.

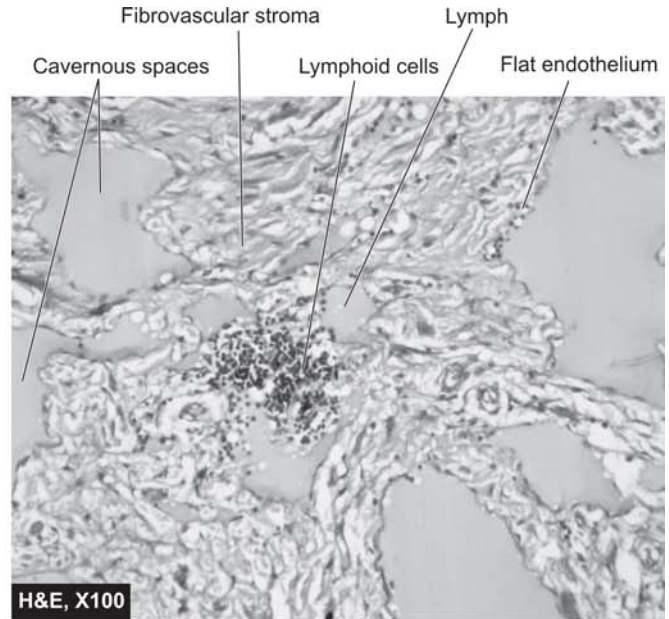
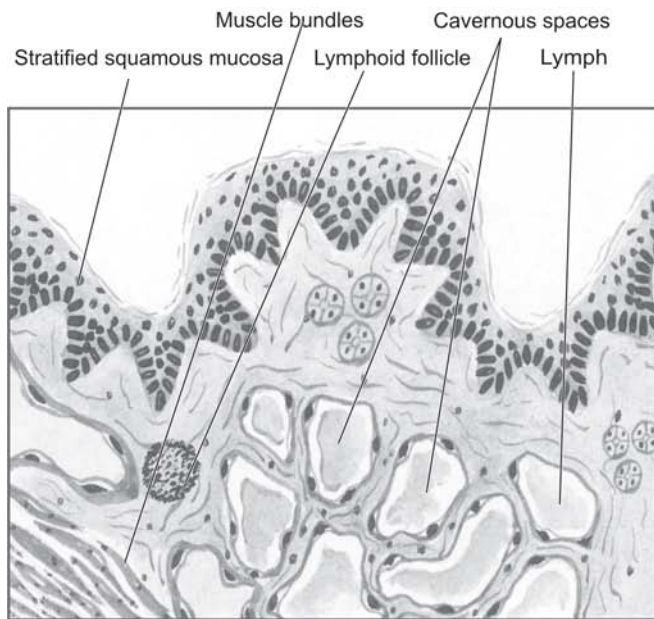


FIGURE 23.6: Cavernous lymphangioma of the tongue. Large cystic spaces lined by the flattened endothelial cells and containing lymph are present. Stroma shows scattered collection of lymphocytes.

M/E

- The lesion is composed of thin-walled cavernous vascular spaces, filled partly or completely with blood.
- The vascular spaces are lined by flattened endothelial cells.
- The intervening stroma consists of scanty connective tissue (Fig. 23.5).

LYMPHANGIOMA TONGUE

Lymphangiomas are lymphatic counterparts of haemangioma and may be capillary or cavernous type, the latter being more common.

G/A Lymphangioma is a spongy mass which infiltrates the adjacent soft tissue diffusely.

M/E

- There are large dilated lymphatic spaces containing homogeneous pink lymph fluid.
- These spaces are lined by flattened endothelial cells.
- The intervening stromal tissue consists of connective tissue and lymphoid infiltrate, sometimes lymphoid follicles.
- Skeletal muscle bundles are present in the intervening stroma showing infiltration of the lesion into the muscle (Fig. 23.6).



Heart

- ◆ Bacterial Endocarditis
- ◆ Healed Myocardial Infarct
- ◆ Chronic IHD
- ◆ Fibrinous Pericarditis

Exercise

24

BACTERIAL ENDOCARDITIS

Bacterial endocarditis (BE) is a serious bacterial infection of the valvular and mural endocardium, more often on pre-diseased heart, and is characterised by typical infected and friable vegetations. The disease exists in 2 forms—acute (ABE) and subacute (SABE) forms, the latter being more common.

G/A The vegetations are mainly found on the valves of left heart, most frequently on the atrial surface of mitral valve, ventricular surface of aortic valve, and combined mitral and aortic valvular involvement. The vegetations are variable in size, grey to greenish, irregular, and typically friable (Fig. 24.1). They may be flat, filiform, fungating or polypoid.



FIGURE 24.1: Vegetations on valves in bacterial endocarditis. The chambers and valves of the left heart are opened up. The mitral valve on its atrial (superior) surface show irregular, soft, elevated, greyish areas of varying size (white arrow).

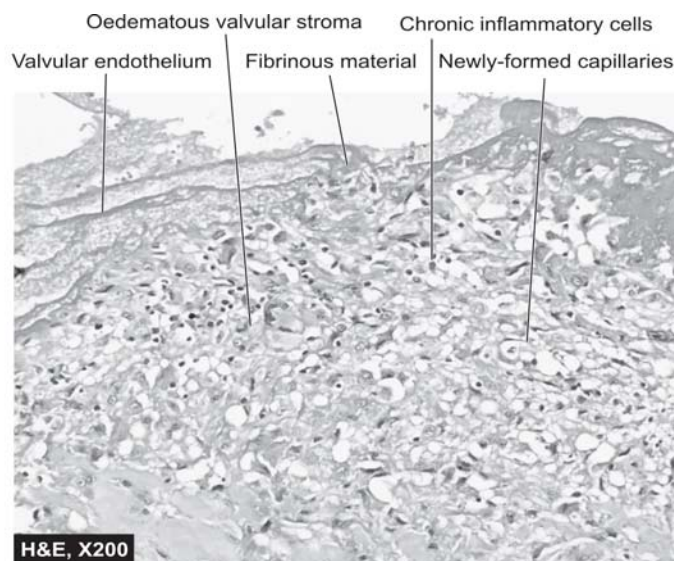
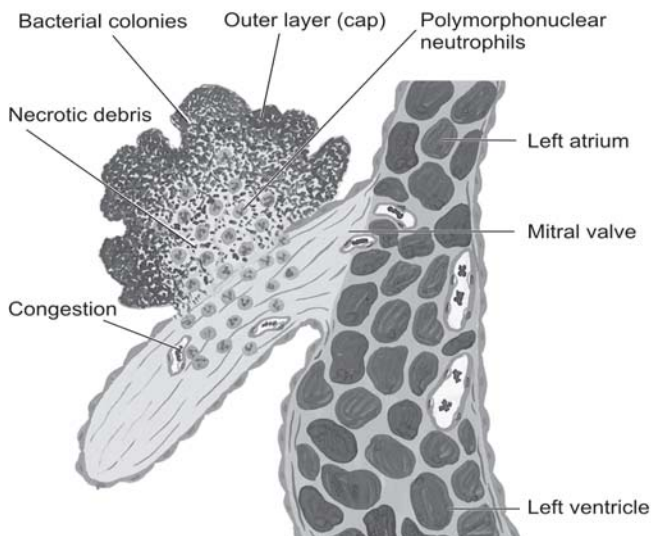


FIGURE 24.2: Bacterial endocarditis: The vegetation on the mitral valve is composed of 3 zones—cap, basophilic bacterial layer and deeper zone of inflammatory reaction.

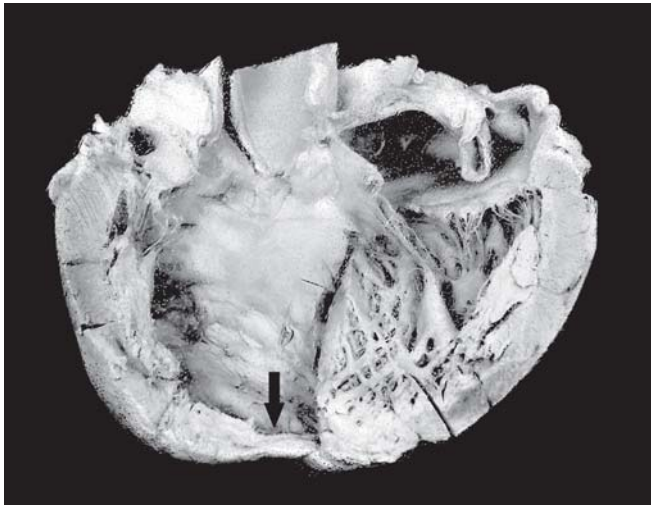
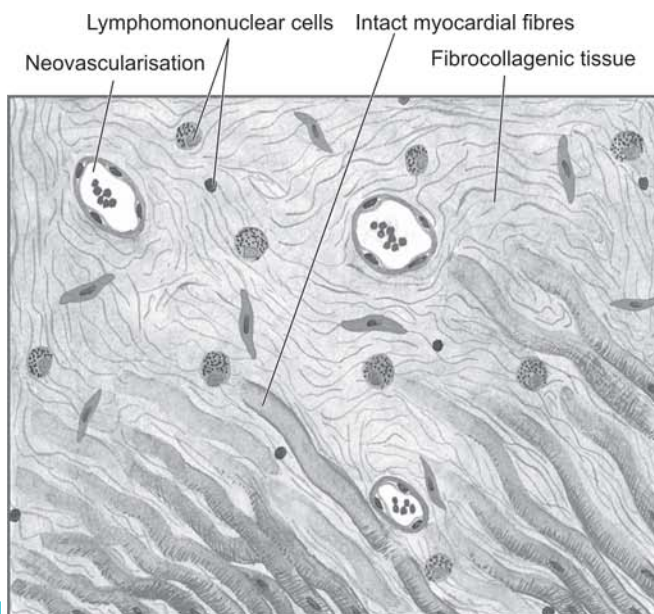


FIGURE 24.3: Myocardial infarction, healed. The left side of the heart has been opened. The left ventricular wall shows a grey-white and firm area of scarring near the apex where the wall is thinned (arrow).

M/E The vegetations of BE consist of 3 zones:

- Outer layer or cap* composed of eosinophilic material of fibrin and platelets.
- Underneath is the *basophilic zone* containing colonies of bacteria in untreated cases.
- The *deeper zone* consists of nonspecific inflammatory reaction in the cusp (Fig. 24.2).



HEALED MYOCARDIAL INFARCT

The myocardial infarct undergoes healing in about 6 weeks.

G/A The infarcted area is replaced by a thin, grey white, hard, shrunken fibrous scar (Fig. 24.3).

M/E

- There is replacement of myocardium by dense fibrocollagenous tissue with entrapment of groups of myocardial fibres.
- The infiltrate consists of some pigmented macrophages, lymphocytes and plasma cells.
- A few blood vessels are seen at the periphery (Fig. 24.4).

CHRONIC ISCHAEMIC HEART DISEASE

Chronic IHD is found in elderly patients of progressive IHD who have had repeated episodes of angina.

G/A The heart may be normal sized or hypertrophied. The left ventricular wall shows foci of grey white fibrosis.

M/E

- Scattered areas of myocardial fibrosis, especially around blood vessels in the interstitial tissue of the myocardium.
- Intervening myocardial fibres show variation in fibre size (Fig. 24.5).
- Areas of brown atrophy are seen.

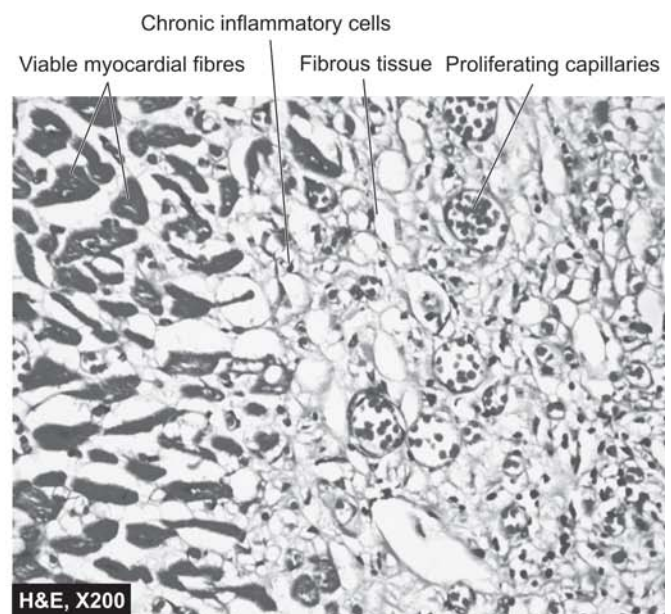


FIGURE 24.4: Myocardial infarction (old). The infarcted area on right shows ingrowth of granulation tissue.

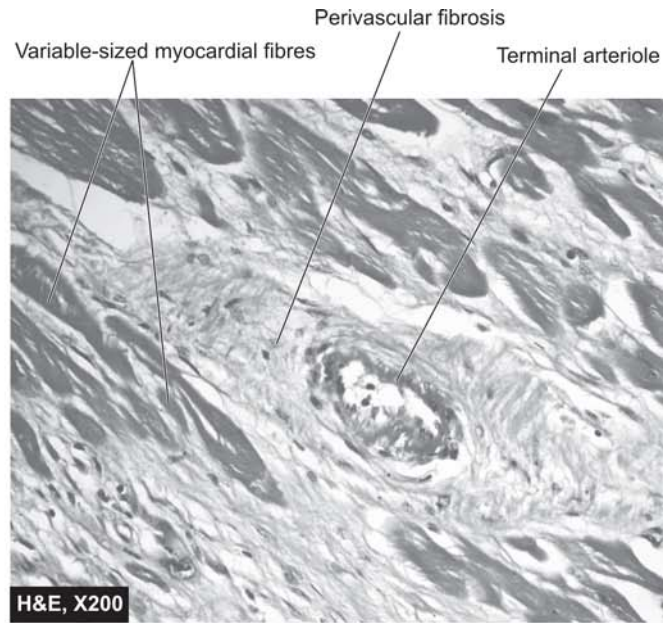
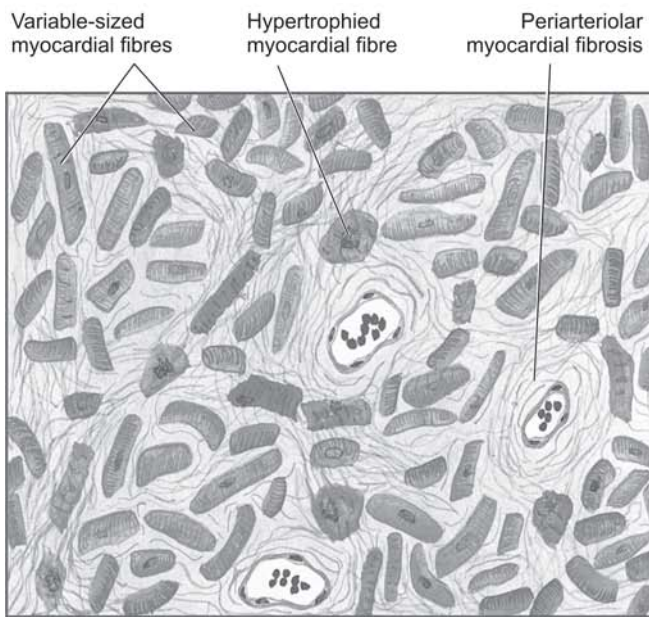


FIGURE 24.5: Chronic ischaemic heart disease. There is patchy myocardial fibrosis, especially around small blood vessels in the interstitium. The intervening single cells and groups of myocardial cells show myocytolysis.

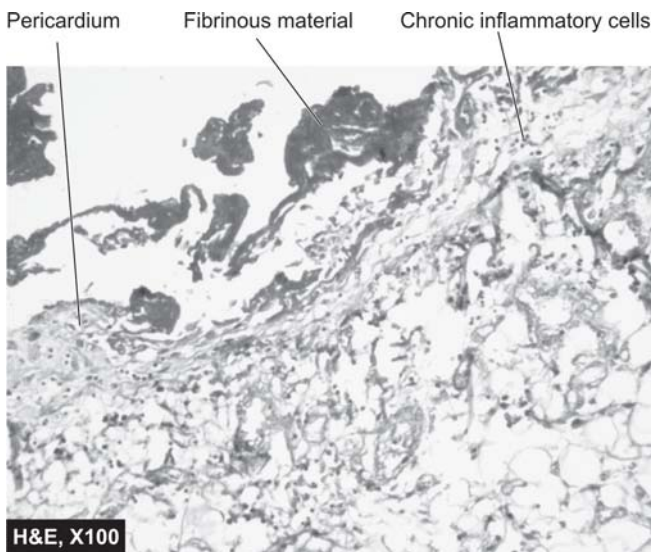


FIGURE 24.6: Serofibrinous pericarditis. There is pink fibrinous exudate on the pericardial surface while space between the two layers of pericardium shows inflammatory cells.

FIBRINOUS PERICARDITIS

This is the most common form of pericarditis.

G/A The pericardial cavity contains admixture of fibrinous exudate with serous fluid. When two layers of pericardium are pulled apart, '*bread and butter*' appearance is produced. Advanced cases may show healing by organisation.

M/E

- Pericardial surface contains pink fibrinous exudate.
- The pericardium contains some nonspecific chronic inflammatory cells, chiefly lymphocytes, plasma cells and macrophages (Fig. 24.6).



Respiratory System I

- ◆ Lobar Pneumonia—Acute Congestion Stage
- ◆ Lobar Pneumonia—Red Hepatisation Stage
- ◆ Lobar Pneumonia—Grey Hepatisation Stage
- ◆ Bronchopneumonia

Exercise

25

LOBAR PNEUMONIA—ACUTE CONGESTION STAGE

Lobar pneumonia is an acute bacterial infection of a large portion of a lobe/lobes of one or both the lungs.

This initial stage of lobar pneumonia represents the early acute inflammatory response to bacterial infection that lasts for 1 to 2 days.

G/A The affected lobe is enlarged, heavy, dark red and congested. Cut surface exudes blood-stained frothy fluid.

M/E

- Dilatation and congestion of capillaries in the alveolar walls.

- Pale eosinophilic oedema fluid in the air spaces.
- A few red cells and neutrophils in the intra-alveolar fluid (Fig. 25.1).
- Bacteria may be demonstrable by Gram's staining.

LOBAR PNEUMONIA—RED HEPATISATION STAGE

This phase lasts for 2 to 4 days. The term *hepatisation* in pneumonia refers to liver-like consistency of the affected lobe on cut section.

G/A The affected lobe is red, firm and consolidated. Cut surface of the involved lobe is airless, red-pink, dry, granular and has liver-like consistency.

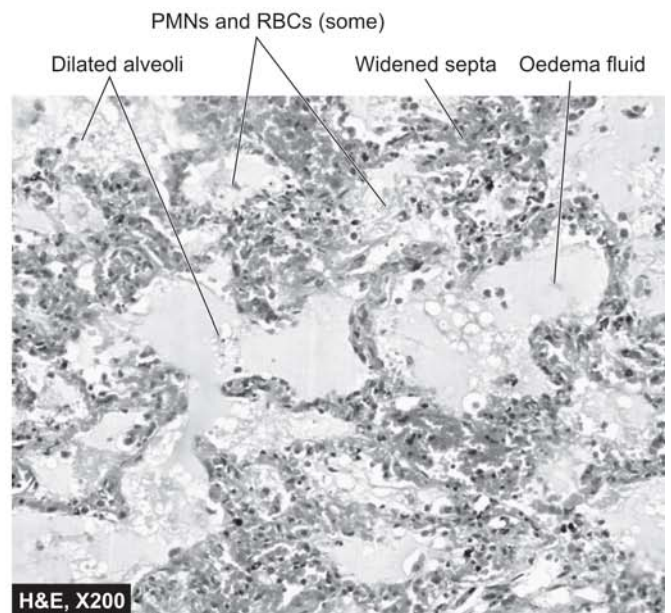
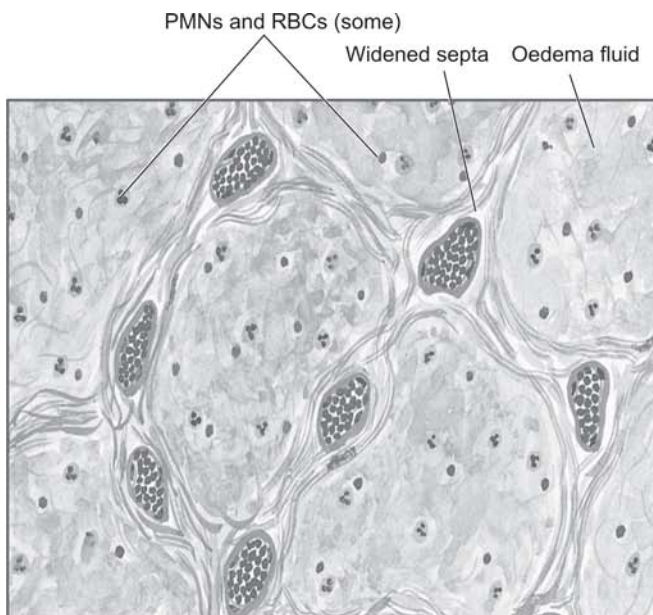


FIGURE 25.1: Lobar pneumonia, acute congestion. There is congestion of septal walls while the air spaces contain pale oedema fluid and a few red cells.

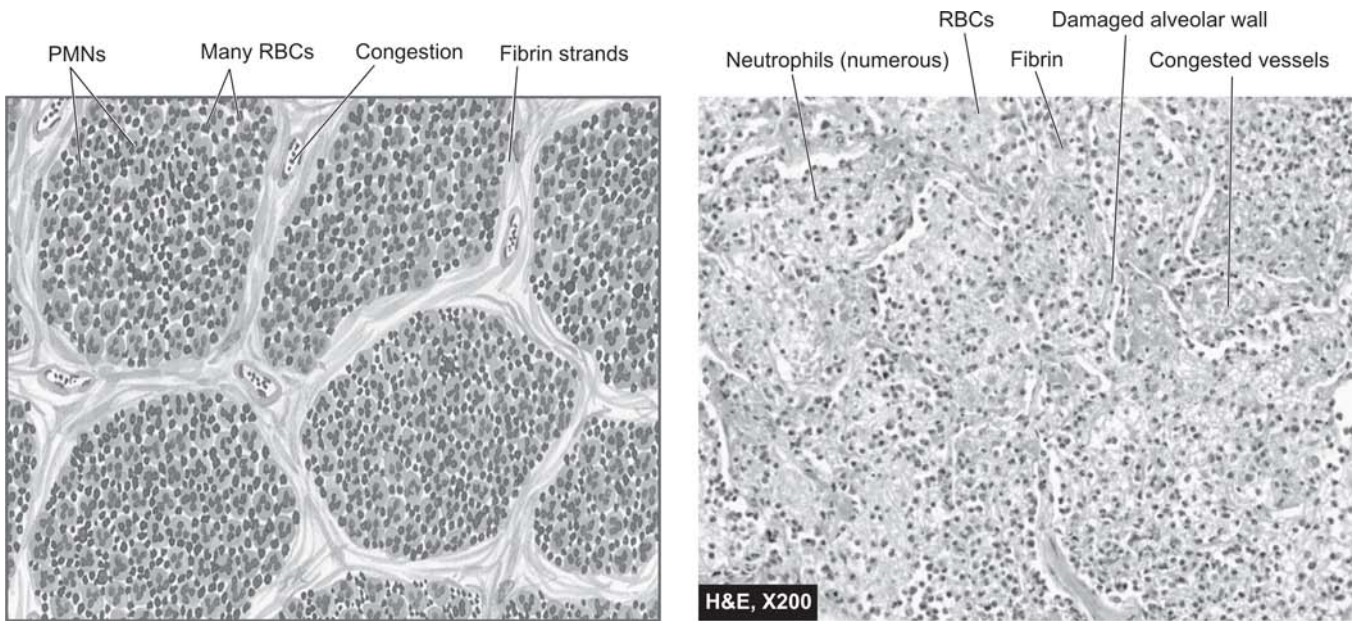


FIGURE 25.2: Lobar pneumonia, red hepatisation. The alveoli are filled with cellular exudate of neutrophils and some red cells.

M/E

- i. Air spaces contain strands of fibrin.
- ii. There is marked cellular exudate of neutrophils and extravasation of red cells (Fig. 25.2).
- iii. Neutrophils may show ingested bacteria.

LOBAR PNEUMONIA—GREY HEPATISATION STAGE

This phase lasts for 4-8 days.

G/A The affected lobe is firm and heavy. Cut surface is dry, granular and grey in appearance with liver-like consistency (Fig. 25.3).

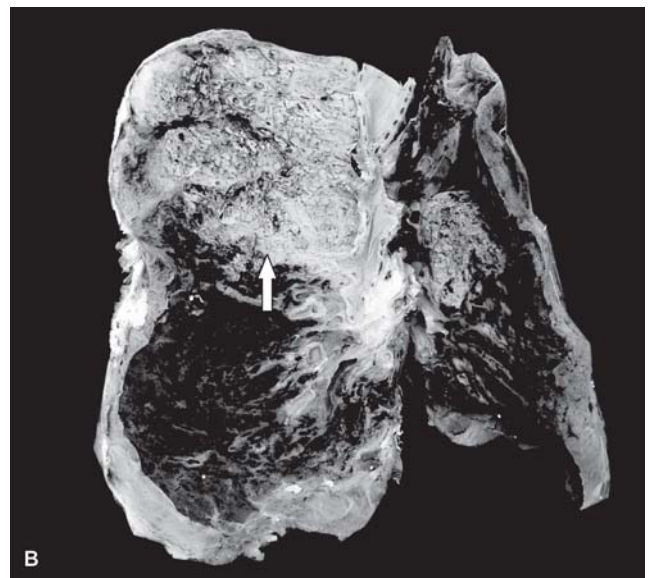
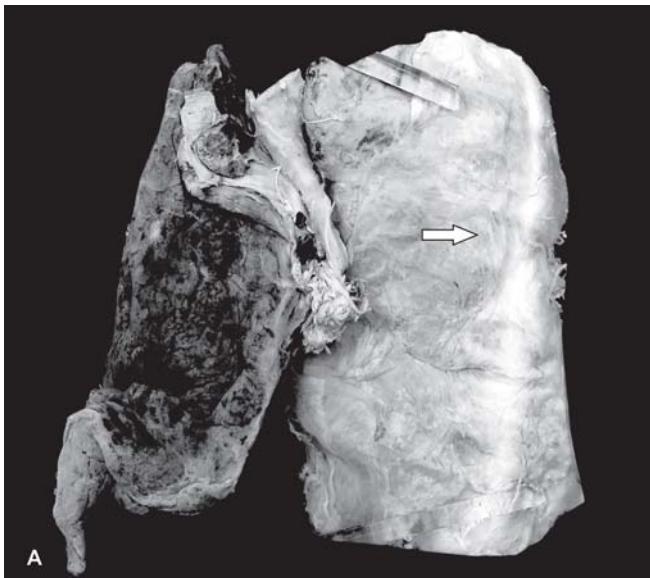


FIGURE 25.3: Grey hepatisation (late consolidation) (4-8 days). **A**, The pleural surface shows some serofibrinous deposits (arrow). **B**, Sectioned surface of the lung shows grey-brown, firm area of consolidation affecting a lobe (arrow) while the rest of the lung is spongy.

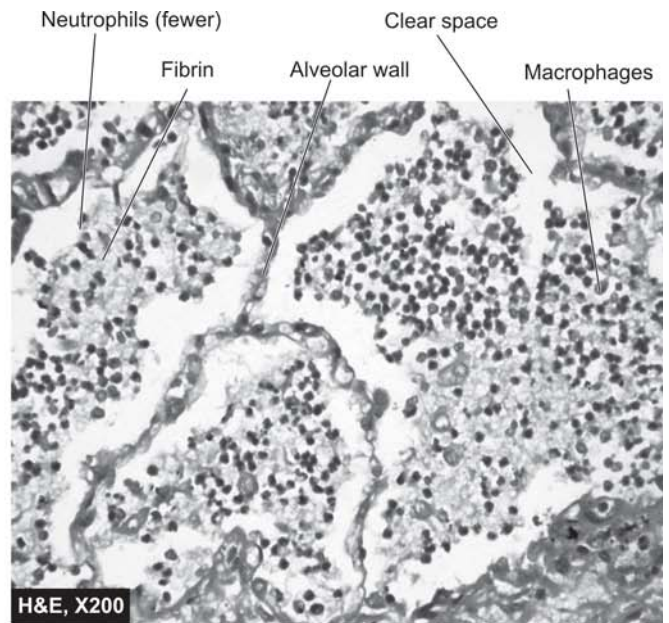
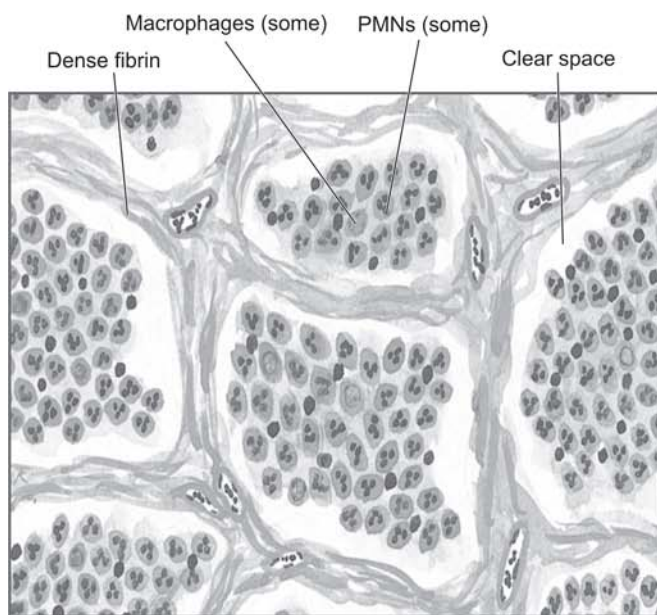


FIGURE 25.4: Lobar pneumonia, grey hepatisation. The cellular exudate is separated from septal wall by a clear space and consists of neutrophils as well as macrophages.

M/E

- i. The fibrin strands in the air spaces are dense.
- ii. The lumina of alveoli contain disintegrated neutrophils and many macrophages.
- iii. A clear space separates septal walls from the cellular exudate (Fig. 25.4).

BRONCHOPNEUMONIA

Bronchopneumonia or lobular pneumonia is infection of terminal bronchioles that extends into the surrounding alveoli resulting in patchy consolidation of the lung.

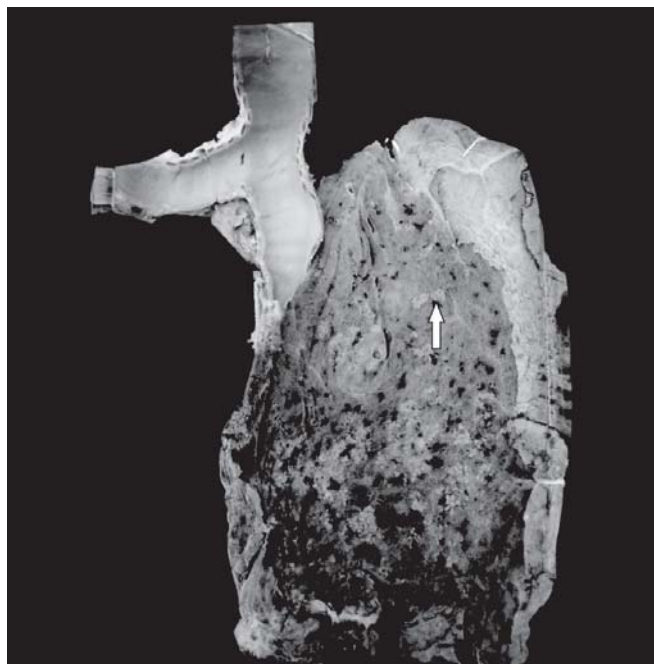


FIGURE 25.5: Bronchopneumonia. The pleural surface shows some serofibrinous deposits. Sectioned surface of the lung shows multiple, small, grey-brown, firm, patchy or granular areas of consolidation around bronchioles (arrow). These areas are seen affecting a lobe while the rest of the lung is spongy.

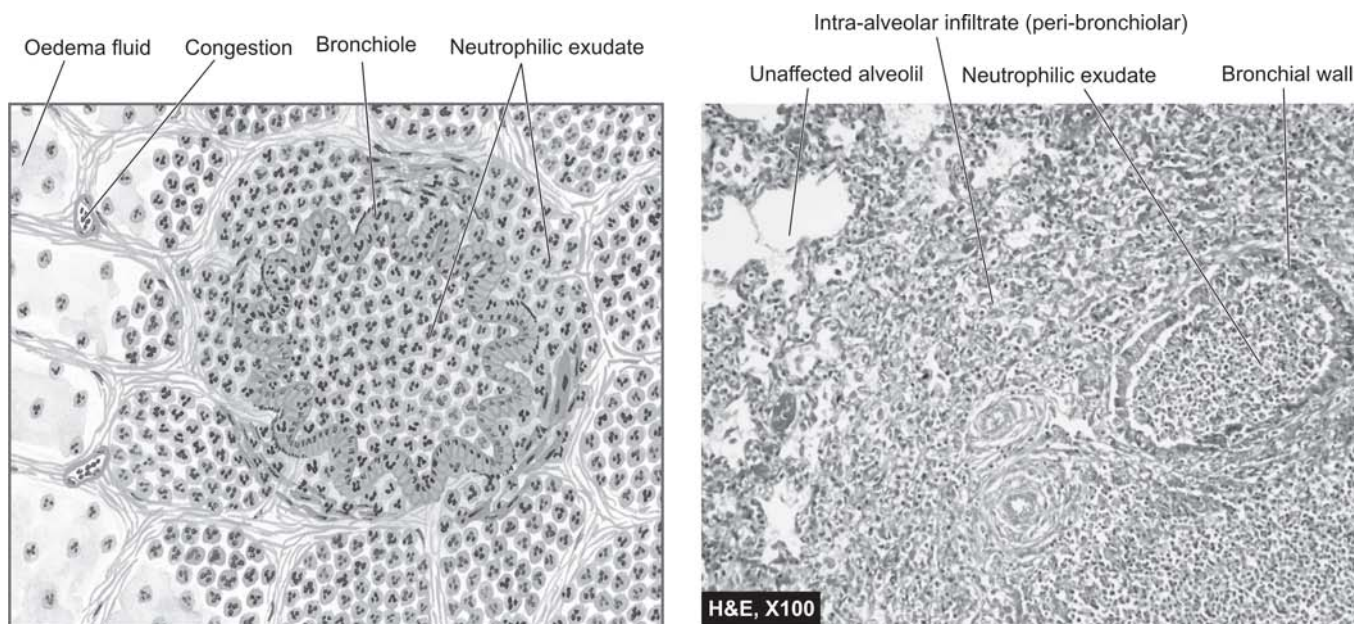


FIGURE 25.6: Microscopic appearance of bronchopneumonia. The bronchioles as well as the adjacent alveoli are filled with exudate consisting chiefly of neutrophils. The alveolar septa are thickened due to congested capillaries and neutrophilic infiltrate.

G/A Bronchopneumonia is identified by patchy areas of red or grey consolidation affecting one or more lobes, more often bilaterally and involving lower zones of lungs more frequently. On cut surface, patchy consolidated lesions appear dry, granular, firm, red or grey in colour, 3 to 4 cm in diameter. These lesions are slightly elevated over the surface centred around a bronchiole, best picked up by feeling with fingers on cut section (Fig. 25.5).

M/E

- Changes of acute bronchiolitis characterised by acute inflammatory cells in the bronchiolar walls.
- Suppurative exudate of neutrophils in the peri-bronchiolar alveoli.
- Widening of alveolar septa by congested capillaries and leucocytic infiltration.
- Alveoli away from the involved area contain oedema fluid (Fig. 25.6).



Respiratory System II

- ◆ Emphysema
- ◆ Bronchiectasis
- ◆ Small Cell Carcinoma Lung
- ◆ Squamous Cell Carcinoma Lung

Exercise

26

EMPHYSEMA

Emphysema is permanent dilatation of air spaces distal to the terminal bronchiole resulting in destruction of the walls of dilated air spaces.

G/A The lungs show varying-sized subpleural bullae and blebs. These spaces are air-filled cyst-like or bubble-like structures, 1 cm or larger in diameter (Fig. 26.1).

M/E

- Dilatation of air spaces and destruction of septal walls of part of acinus involved.
- Ruptured alveolar walls with spurs of broken septa between adjacent alveoli.
- Capillaries in the septal walls are thinned and stretched.
- Changes of bronchitis are often present (Fig. 26.2).

BRONCHIECTASIS

Bronchiectasis is abnormal and irreversible dilatation of the bronchi and larger bronchioles.

G/A Bilateral involvement of lower lobes of lungs is seen more frequently. The pleura is usually fibrotic and thickened. Cut surface of affected lower lobes shows characteristic honey-combed appearance due to dilated airways containing muco-pus and thickening of their walls (Fig. 26.3).

M/E

- Infiltration of the bronchial walls by acute and chronic inflammatory cells with destruction of normal muscle and elastic tissue with replacement fibrosis.
- Fibrosis of the intervening lung parenchyma and interstitial pneumonia.
- Normal, ulcerated or squamous metaplastic, bronchial epithelium (Fig. 26.4).

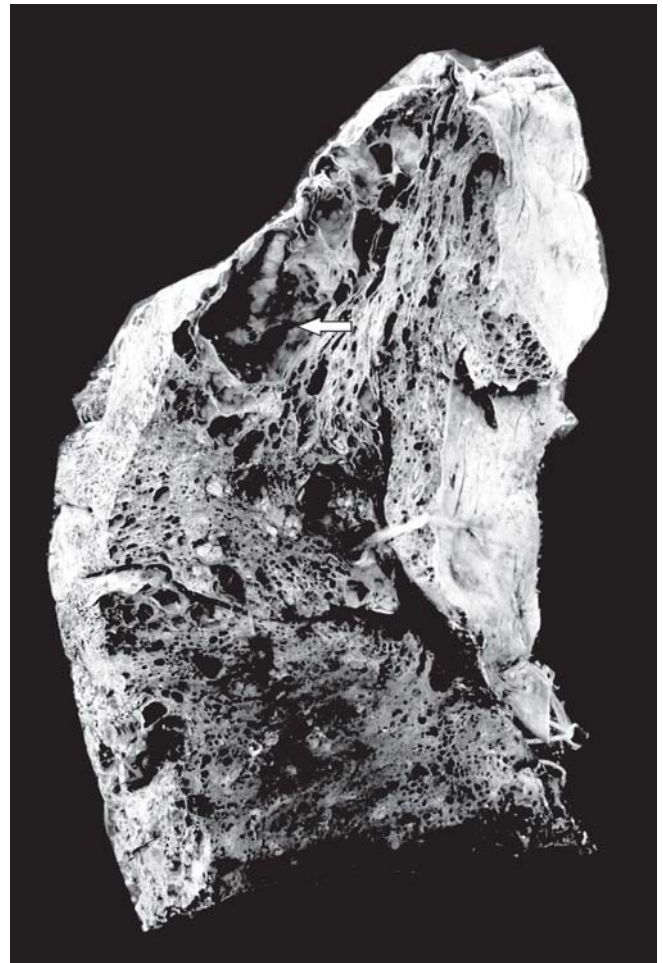


FIGURE 26.1: Bullous emphysema of the lung. The lung is expanded and has thin-walled cysts or bullae visible on the pleural surface. Sectioned surface of the lung shows many large air-filled sacs, a few centimeters in diameter (arrow), located under the pleura.

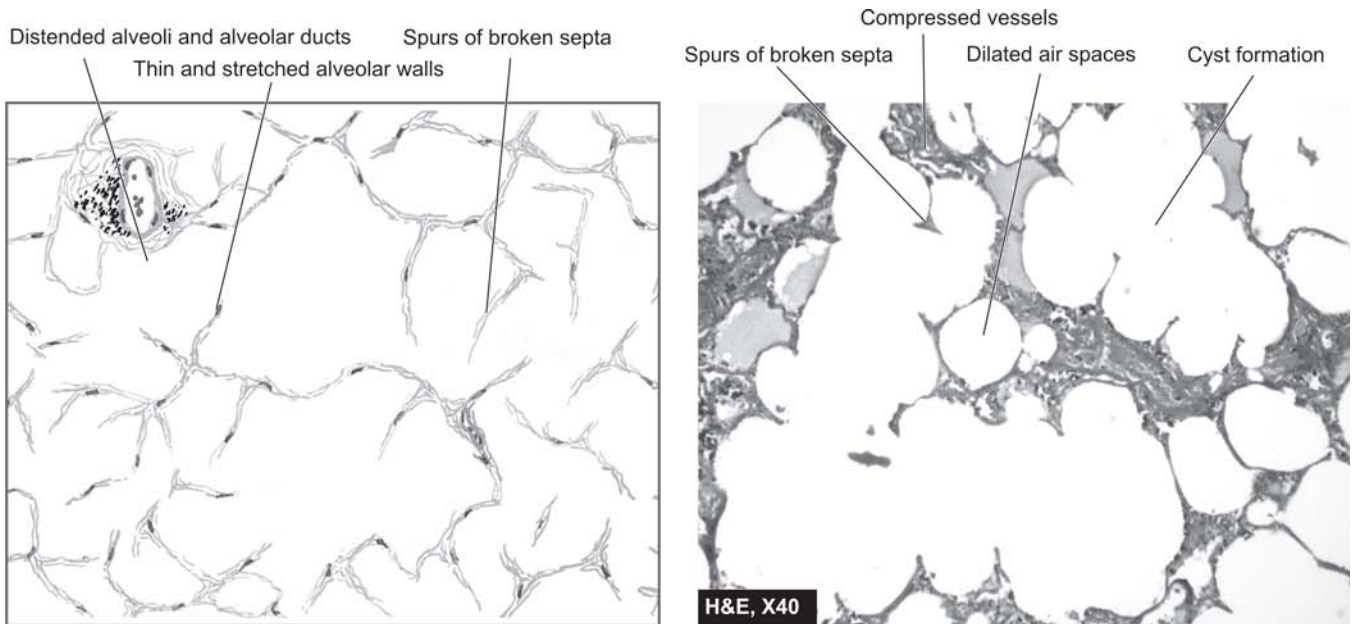


FIGURE 26.2: Emphysema. There is dilatation of air spaces and destruction of septal walls.

SMALL CELL CARCINOMA LUNG

Small cell carcinoma is a variant of bronchogenic carcinoma and is a highly malignant tumour.

G/A The tumour is frequently hilar or central in location. The tumour appears as a nodule 1-5 cm in diameter with ulcerated surface. Cut surface of the tumour is

yellowish-white with areas of necrosis and haemorrhages.

M/E

- i. Tumour cells are uniform, small, larger than lymphocytes with dense round or oval nuclei having diffuse chromatin, inconspicuous nucleoli and scanty cytoplasm.

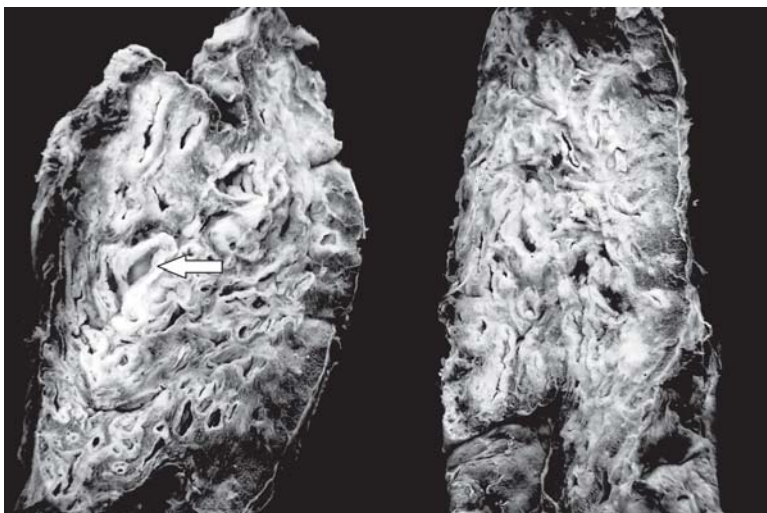


FIGURE 26.3: Bronchiectasis of the lung. Sectioned surface of the lung shows honey-combed appearance of the lung in its lower lobes where many thick-walled dilated cavities with cartilaginous wall are seen (arrow).

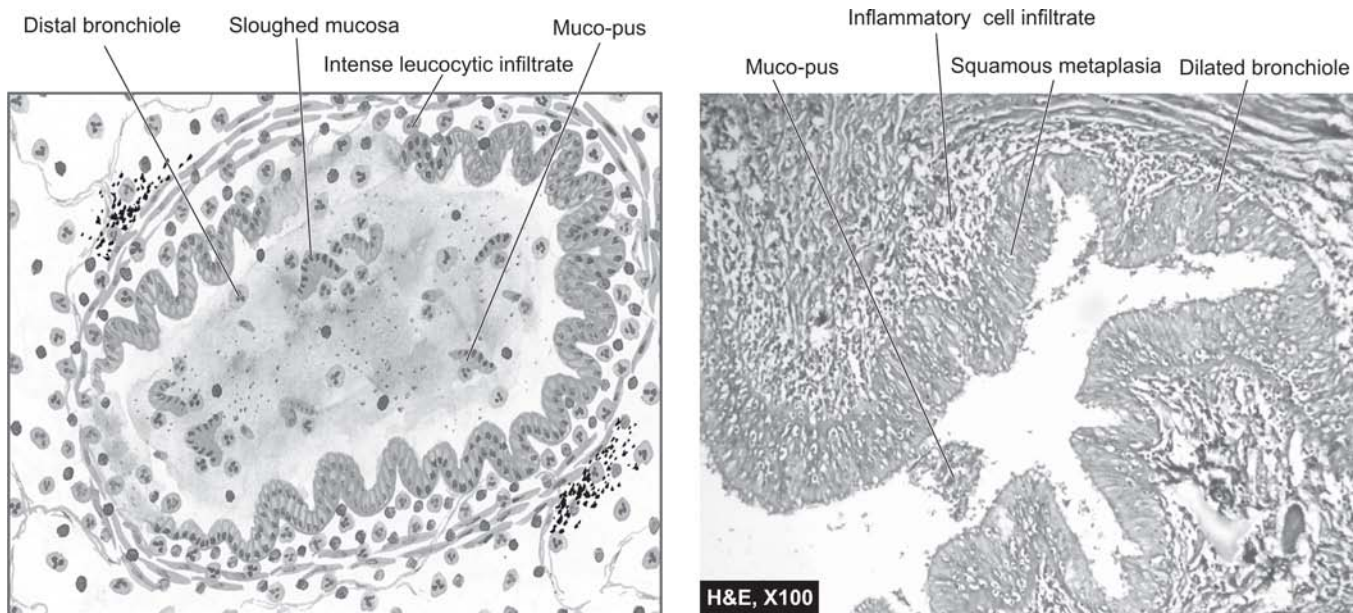


FIGURE 26.4: Microscopic appearance of a dilated distal bronchiole in bronchiectasis. The bronchial wall is thickened and infiltrated by acute and chronic inflammatory cells. The mucosa is sloughed off at places with exudate of muco-pus in the lumen.

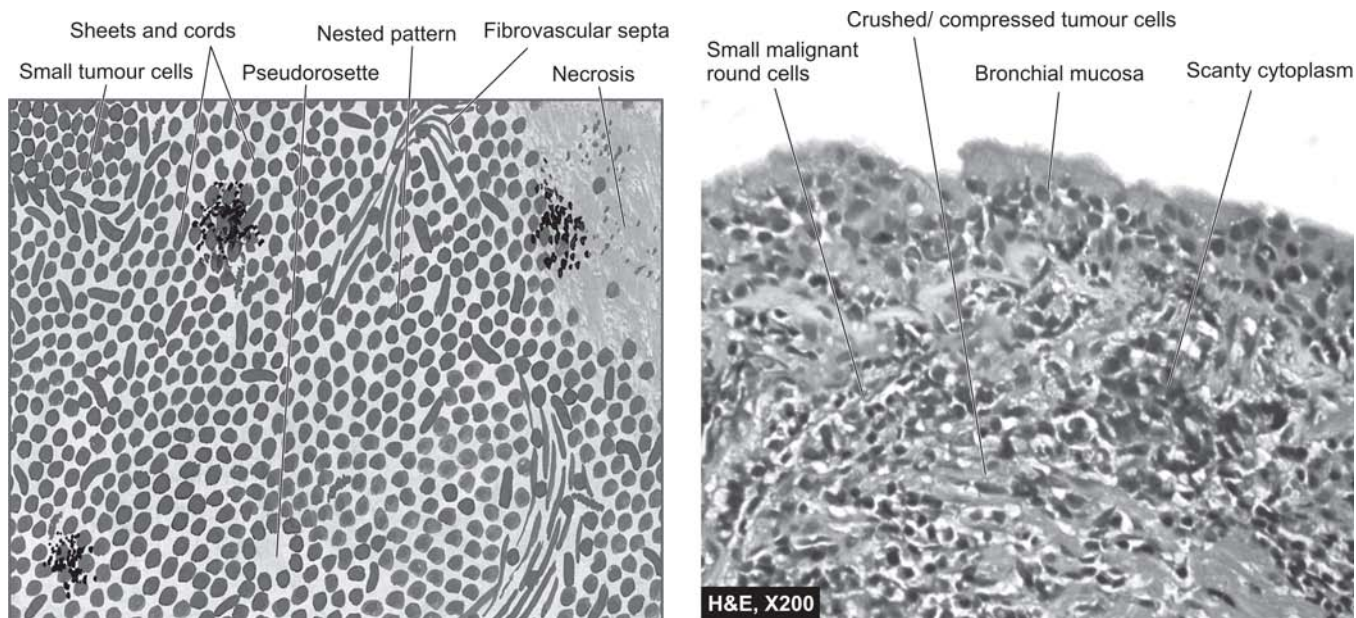


FIGURE 26.5: Small cell carcinoma of the lung. The tumour cells are arranged in sheets, cords, aggregates and at places form pseudorosettes. The individual tumour cells are small, uniform, lymphocyte-like with scanty cytoplasm.

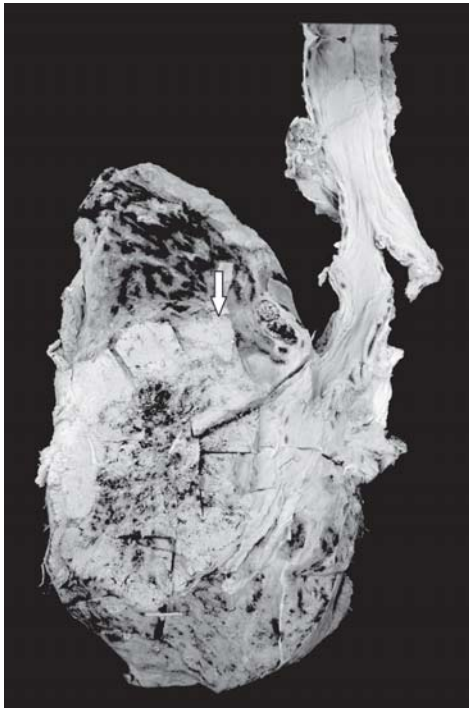


FIGURE 26.6: Squamous cell carcinoma of the lung, hilar type macroscopic pattern of bronchogenic carcinoma. Sectioned surface shows grey-white, fleshy, thickening of the bronchus at its bifurcation, partly occluding the lumen (arrow). The tumour is also seen extending irregularly into adjacent lung parenchyma and hilar lymph nodes.

- ii. Tumour cells are arranged in cords, aggregates and ribbons, or around small blood vessels forming pseudorosettes (Fig. 26.5).

SQUAMOUS CELL CARCINOMA LUNG

This is the most common type of bronchogenic carcinoma.

G/A The tumour is often hilar or central arising from a large bronchus, may be of variable size and invades the adjacent lung parenchyma. Cut surface of the tumour shows extensive necrosis and cavitation (Fig. 26.6).

M/E

- i. Varying grades of differentiation from well-differentiated with keratinisation (Fig. 26.7) to poorly-differentiated and sarcoma-like spindle cell carcinoma are seen.
- ii. Intercellular bridges or keratinisation are often seen in better differentiated tumour.
- iii. The edge of the tumour often shows squamous metaplasia, epithelial dysplasia and carcinoma in situ.

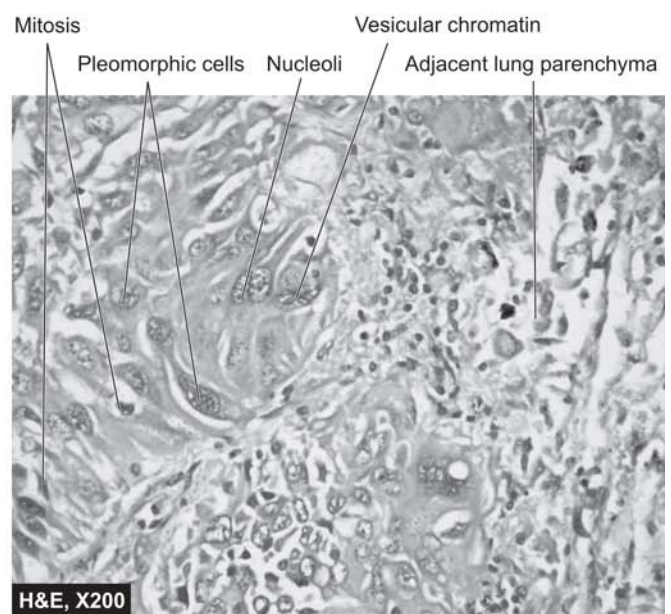
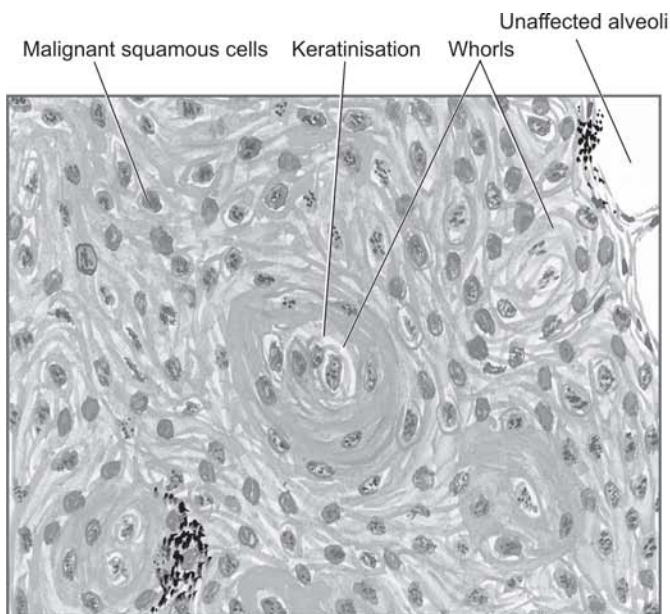


FIGURE 26.7: Squamous cell carcinoma of the lung. Islands of invading malignant squamous cells are seen. A few well-developed cell nests with keratinisation are evident.



GIT I

- ◆ Ameloblastoma
- ◆ Pleomorphic Adenoma
- ◆ Peptic Ulcer
- ◆ Ulcerative Colitis

Exercise

27

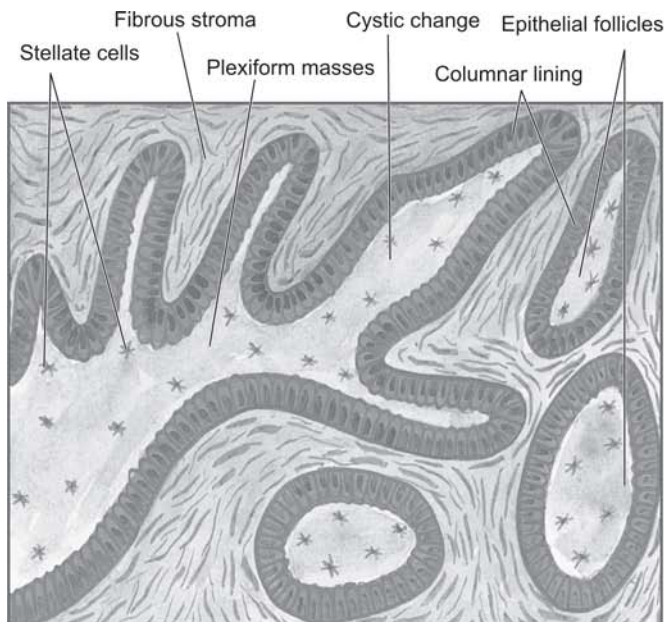
AMELOBLASTOMA

Ameloblastoma is the common benign but locally aggressive epithelial odontogenic tumour, commonly in the mandible and maxilla.

G/A The tumour is grey-white, usually solid, sometimes cystic, replacing the affected bone.

M/E

- i. Follicular pattern is the most common, characterised by follicles of varying size and shape which are separated by fibrous tissue.
- ii. The follicles consist of central area of stellate cells and peripheral layer of cuboidal or columnar cells (Fig. 27.1).



- iii. Other less common patterns include plexiform masses, acanthomatous pattern, basal cell pattern, and granular cell pattern.

PLEOMORPHIC ADENOMA

This is the commonest tumour in the parotid gland.

G/A The tumour is circumscribed, pseudoencapsulated, rounded and multilobulated, firm mass, 2-5 cm in diameter (Fig. 27.2). The cut surface is grey-white and bluish, variegated, with soft to mucoid consistency.

M/E The pleomorphic adenoma has two components: epithelial and mesenchymal (Fig. 27.3):

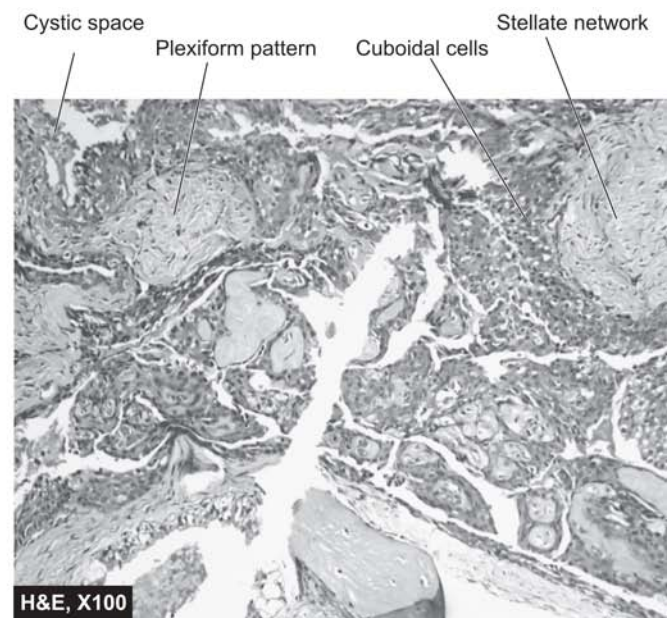


FIGURE 27.1: Ameloblastoma, follicular pattern. Epithelial follicles are seen in fibrous stroma. The follicles are composed of central area of stellate cells and peripheral layer of cuboidal or columnar cells. A few follicles show central cystic change .

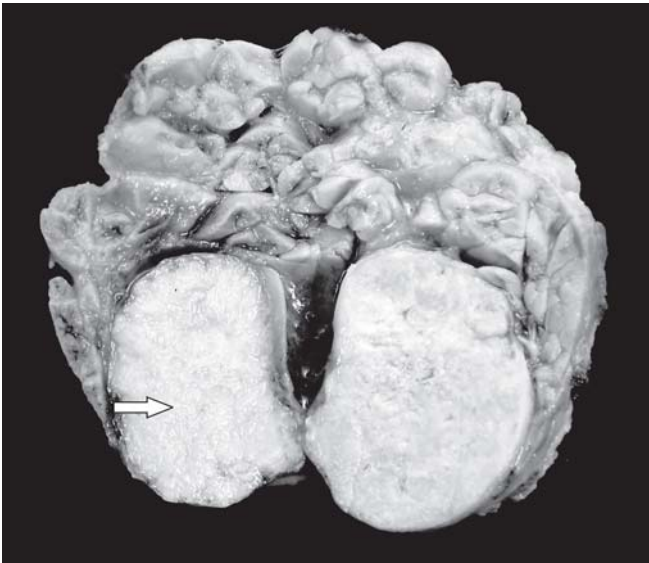


FIGURE 27.2: Pleomorphic adenoma (mixed salivary tumour) of the parotid gland. The salivary tissue is identified on section by lobules of soft tissue separated by thin septa. The cut surface of the tumour shows grey-white and light-bluish variegated semitranslucent parenchyma (arrow).

- i. *Epithelial component* consists of various patterns like ducts, acini, tubules, sheets and strands of monomorphic cells of ductal or myoepithelial origin. These ductal cells are cuboidal or columnar while myoepithelial cells are polygonal or spindle-shaped.

- ii. *Mesenchymal component* present in loose connective tissue includes myxoid, mucoid and chondroid matrix which simulates cartilage (pseudocartilage).

PEPTIC ULCER

Peptic ulcers are areas of degeneration and necrosis of mucosa of stomach and duodenum.

G/A Gastric ulcers are found predominantly along the lesser curvature in the region of pyloric antrum, more commonly on the posterior wall. Duodenal ulcers are commonly found in first part of the duodenum, more commonly on the anterior wall. Typically, peptic ulcers of either gastric or duodenal mucosa are small (1-2.5 cm in diameter), round to oval and characteristically punched out. The mucosal folds converge towards the ulcer (Fig. 27.4).

M/E Chronic peptic ulcers have 4 histologic zones (*from within outside*) (Fig. 27.5):

- i. *Necrotic zone* lies in the floor of the ulcer. The tissue elements show coagulative necrosis giving eosinophilic smudgy appearance with nuclear debris.
- ii. *Superficial exudative zone* lies underneath the necrotic zone and is composed of fibrinous exudate containing necrotic debris and a few leucocytes, predominantly neutrophils.

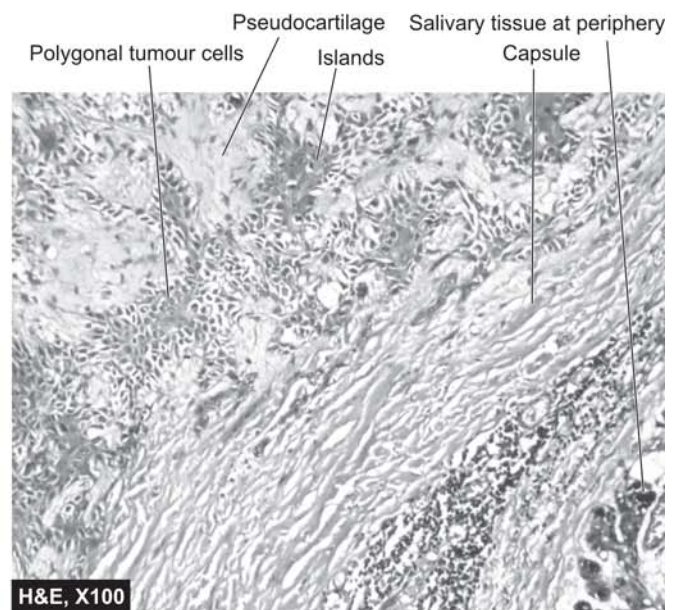
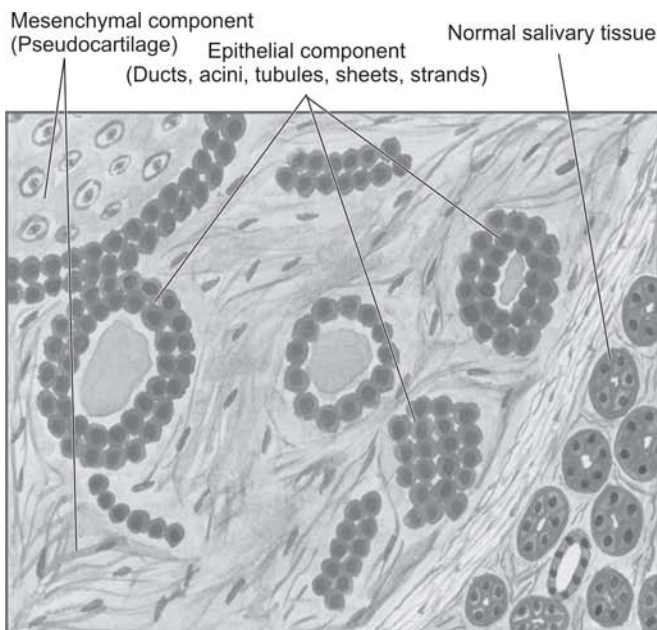


FIGURE 27.3: Pleomorphic adenoma, typical microscopic appearance. The epithelial element is composed of ducts, acini, tubules, sheets and strands of cuboidal and myoepithelial cells. These are seen randomly admixed with mesenchymal elements composed of pseudocartilage.

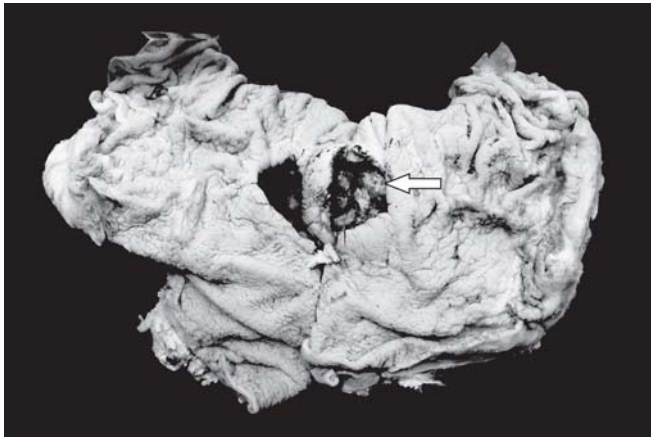
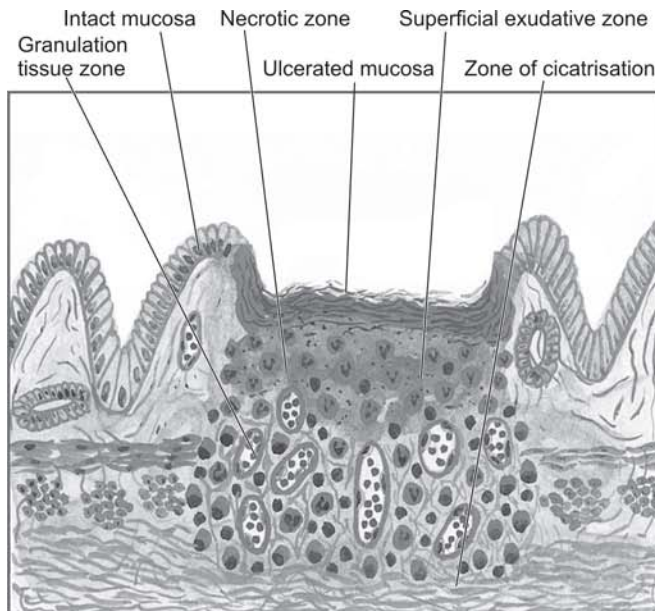


FIGURE 27.4: Benign chronic gastric ulcer. Partial gastrectomy specimen is identified by thick muscular wall and irregular mucosal folds. The luminal surface shows a punched out round to oval ulcer, about 1 cm in diameter (arrow) and penetrating in to muscularis layer.

- iii. *Granulation tissue zone* is seen merging into the necrotic zone. It is composed of nonspecific chronic inflammatory infiltrate and proliferating capillaries.



- iv. *Zone of cicatrisation* is seen outer to the layer of granulation tissue and is composed of dense fibrocollagenic scar tissue.

ULCERATIVE COLITIS

Ulcerative colitis is an inflammatory bowel disease affecting rectum and extending upwards into the sigmoid colon, descending colon, transverse colon and sometimes may involve the entire colon.

G/A The characteristic feature is the continuous involvement of rectum and colon without any skip areas. Mucosa shows linear and superficial ulcers while the intervening intact mucosa may form inflammatory pseudopolyps. The muscle layer is thickened due to contraction and produces loss of normal haustral folds giving 'garden-hose appearance' (Fig. 27.6).

M/E The *active disease process* shows the following changes (Fig. 27.7):

- Focal accumulation of neutrophils forming crypt abscesses.
- Marked congestion, dilatation and haemorrhages.
- Superficial mucosal ulcerations with crypt distortion.
- Diminution of goblet cells and mucodepletion.

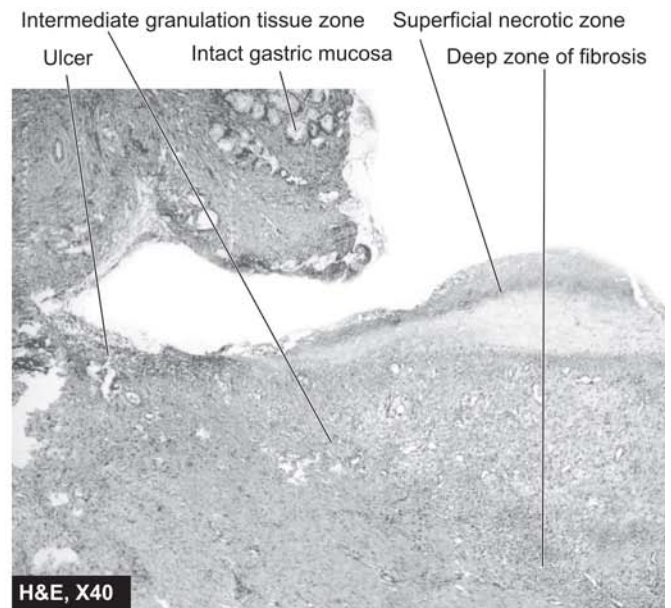


FIGURE 27.5: Chronic peptic ulcer. Histologic zones of the ulcer are illustrated. The mucosal surface shows necrosis, ulceration, and inflammation.



FIGURE 27.6: Ulcerative colitis. Continuous involvement of the rectum, sigmoid colon and descending colon are seen without any uninvolved skip areas **(A)**. The involved areas show ulcers and formation of mucosal polyps (arrows) with thickened wall and narrowed lumen which is better appreciated in close up **(B)**.

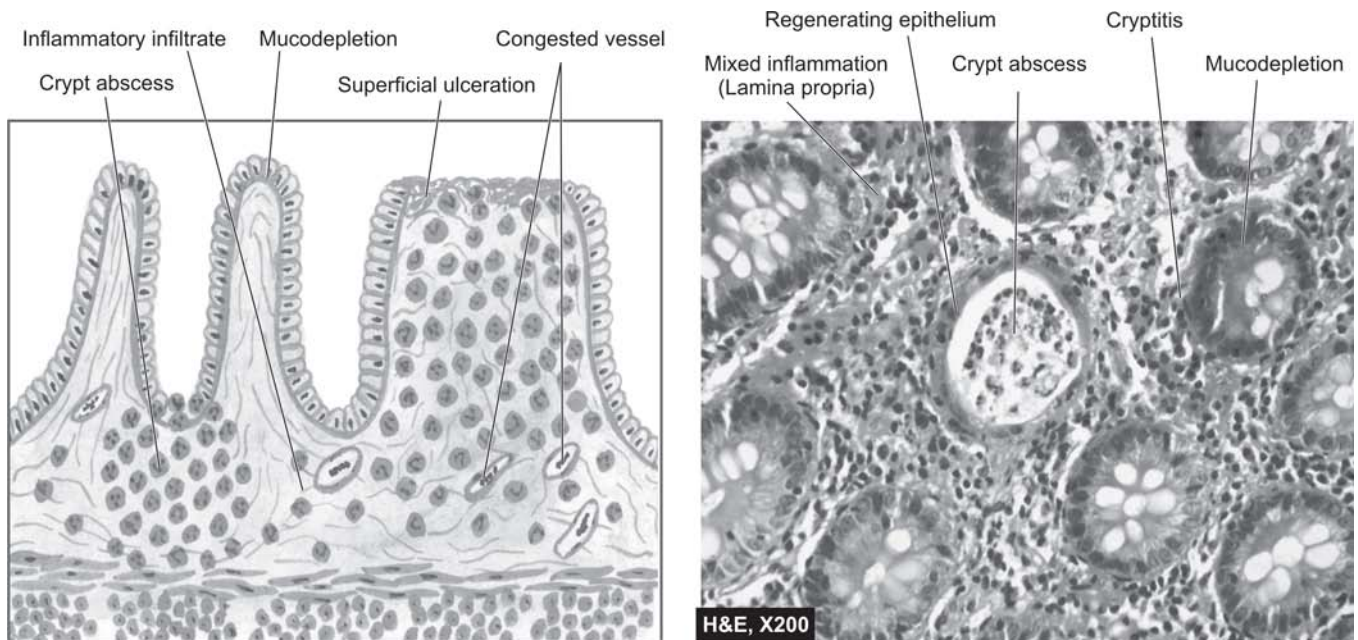


FIGURE 27.7: Ulcerative colitis in active phase. The microscopic features seen are superficial ulcerations, with mucosal infiltration by inflammatory cells and a 'crypt abscess.'

GIT II

- ◆ Acute Appendicitis
- ◆ Juvenile Polyp Rectum
- ◆ Adenocarcinoma Stomach
- ◆ Mucinous Adenocarcinoma Colon

Exercise

28

ACUTE APPENDICITIS

Acute appendicitis is the most common acute abdominal condition confronted by the surgeon.

G/A The appendix is swollen and serosa is hyperaemic and coated with fibrinopurulent exudate. The mucosa is ulcerated and sloughed.

M/E

- Most important diagnostic feature is neutrophilic infiltration of the muscularis.
- Mucosa is sloughed and blood vessels in the wall are thrombosed.

- Periappendiceal inflammation is seen in advanced cases (Fig. 28.1).

JUVENILE POLYP RECTUM

Juvenile or retention polyps are hamartomatous and occur more commonly in children under 5 years of age in the region of rectum.

G/A Juvenile polyp is often solitary, spherical, smooth-surfaced, about 2 cm in diameter, and pedunculated.

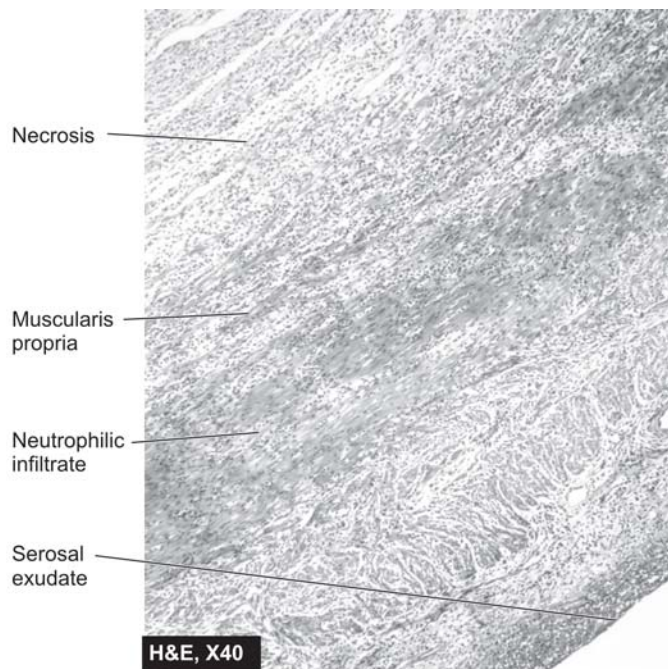
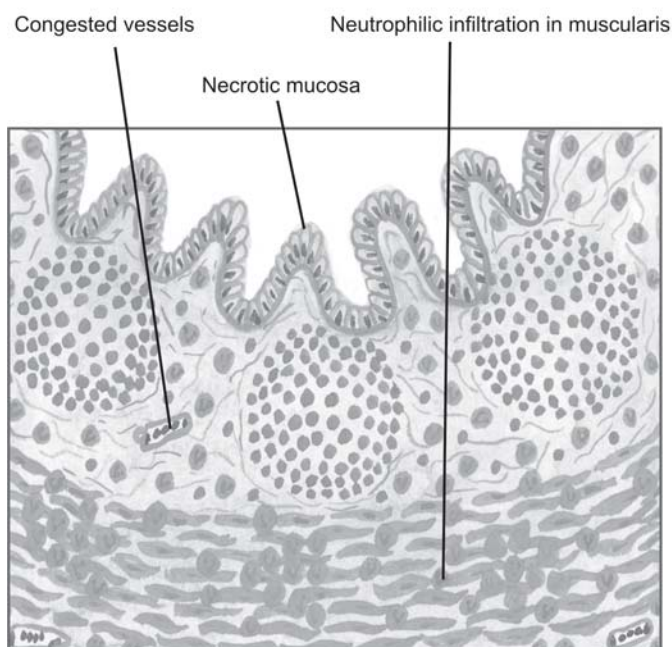


FIGURE 28.1: Acute appendicitis. Microscopic appearance showing diagnostic neutrophilic infiltration into the muscularis. The lumen of appendix shows exudate.

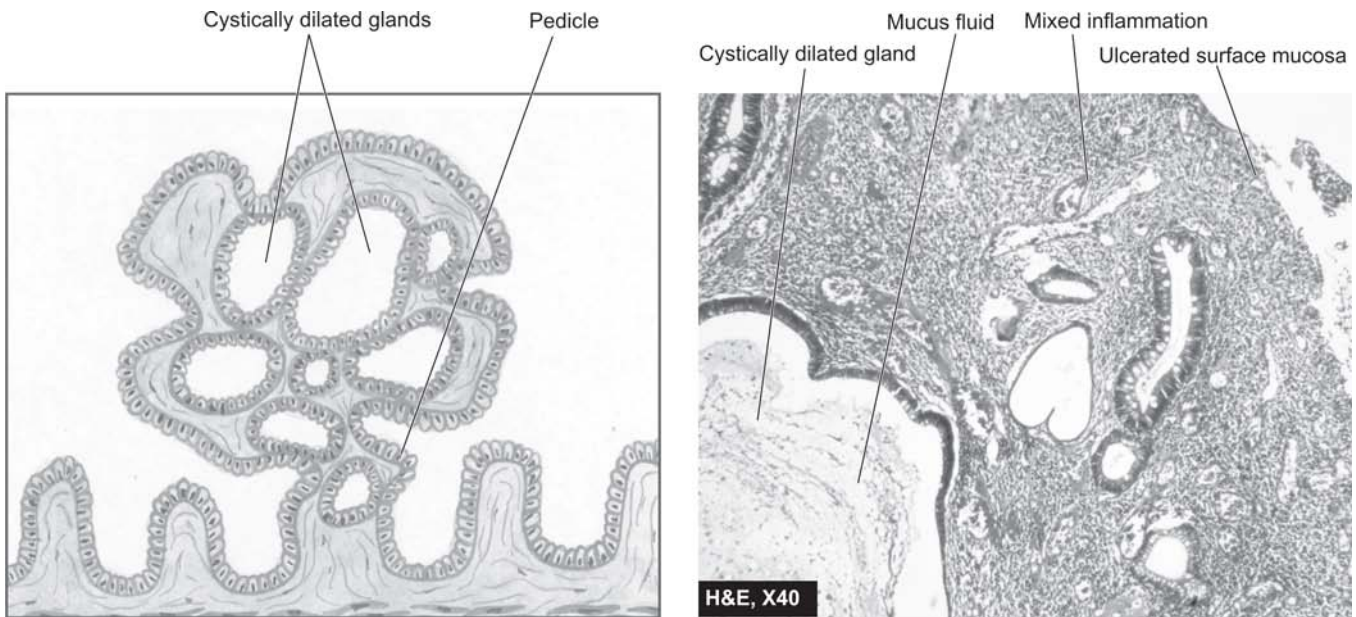


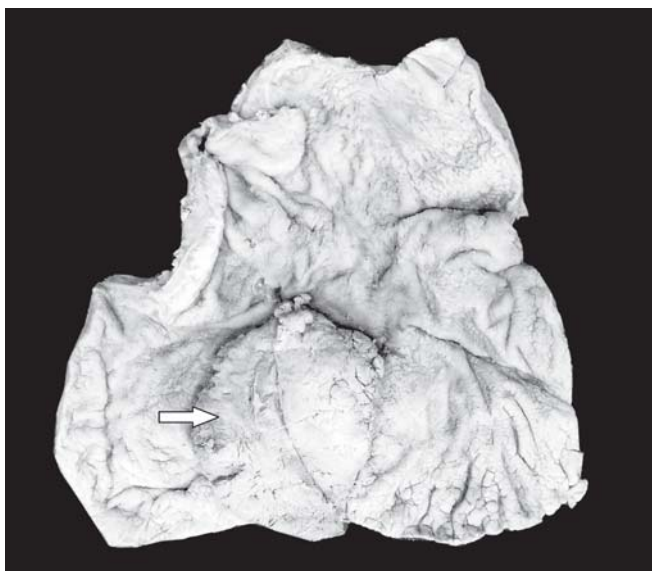
FIGURE 28.2: Juvenile polyp. There are cystically dilated glands while the stroma shows some inflammatory cells.

M/E

- i. Cystically dilated glands containing mucus and lined by normal mucin-secreting epithelium.
- ii. The stroma may show chronic inflammatory cell infiltrate (Fig. 28.2).

ADENOCARCINOMA STOMACH

Advanced gastric carcinoma extends beyond the basement membrane into the muscularis propria and is seen most often in the region of pyloric canal.



G/A Most common pattern is flat, infiltrating and ulcerative growth with irregular necrotic base and raised margin (Fig. 28.3). Other gross patterns include fungating (polypoid), scirrhous (linitis plastica), colloid (mucoid) and ulcer-cancer.

M/E

- i. Tubular and acinar pattern of growth infiltrating the stomach wall.
- ii. The tumour is more often poorly-differentiated and has high degree of anaplasia (Fig. 28.4).

FIGURE 28.3: Ulcerative carcinoma stomach. The luminal surface of the stomach in the region of the pyloric canal shows an elevated irregular growth with ulcerated surface and raised margins (arrow).

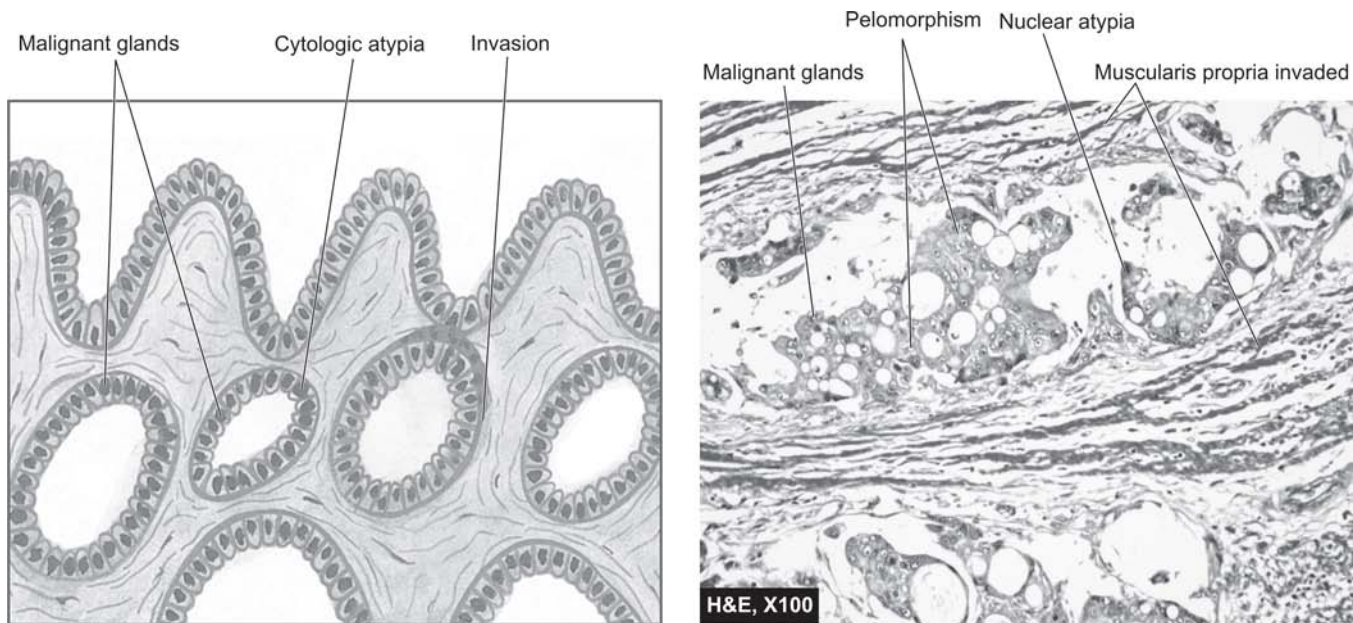


FIGURE 28.4: Adenocarcinoma stomach. Malignant glands invading the layers of wall of the stomach.

MUCINOUS ADENOCARCINOMA COLON

Colorectal carcinoma comprises the commonest form of visceral cancer. The most common location is rectum.

G/A The tumour has distinctive features in right and left-sided colonic cancer. *The right-sided growth*, tends

to be fungating, large, cauliflower-like, soft and friable mass projecting into the lumen (Fig. 28.5). The *left-sided growth*, on the other hand, has napkin-ring configuration i.e. it encircles the bowel wall circumferentially with increased fibrous tissue forming annular ring with central mucosal ulceration (Fig. 28.6).



FIGURE 28.5: Right-sided colonic carcinoma. The colonic wall shows thickening with presence of a luminal growth (arrow). The growth is cauliflower-like, soft and friable projecting into the lumen.

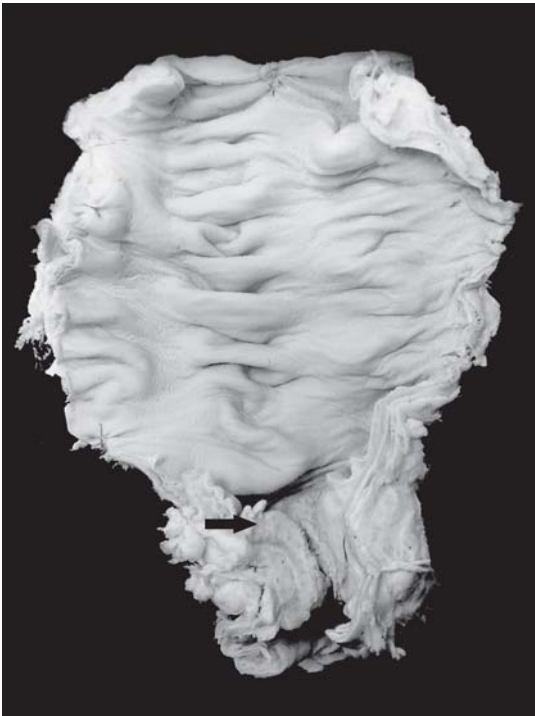


FIGURE 28.6: Left-sided colonic carcinoma. Sectioned surface shows napkin ring narrowing of the lumen while the colonic wall shows circumferential firm thickening (arrow).

M/E The microscopic appearance on right-sided and left-sided colonic cancer is similar:

- i. The tumour has infiltrating glandular pattern in the colonic wall with varying grades of differentiation of tumour cells.
- ii. About 10% cases show mucin-secreting colloid carcinoma with pools of mucin (Fig. 28.7).

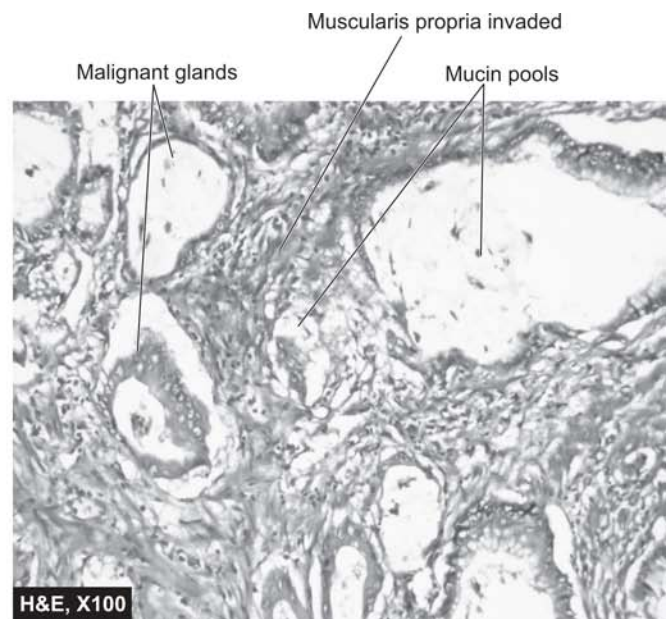
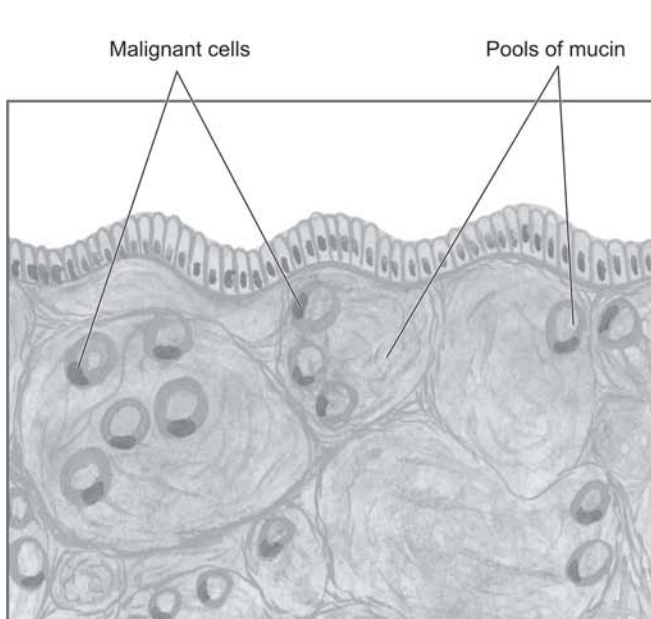


FIGURE 28.7: Mucinous adenocarcinoma colon. Pools of extracellular mucin as well as intracellular mucin in malignant glands.

Liver and Biliary System I

- ◆ Acute Viral Hepatitis
- ◆ Alcoholic Hepatitis
- ◆ Submassive Necrosis of Liver

Exercise

29

ACUTE VIRAL HEPATITIS

The most common consequence of all hepatotropic viruses is acute inflammatory involvement of the entire liver.

G/A The liver is slightly enlarged, soft and greenish.

M/E

- Earliest hepatocellular injury, most marked in centrilobular zone (zone 3), is ballooning degeneration in which the hepatocytes appear swollen and have granular cytoplasm.
- Presence of Councilman body or acidophil body identified by necrotic eosinophilic mass of cytoplasm.

- A few areas show dropout necrosis in which isolated or small clusters of hepatocytes undergo lysis.
- Mononuclear inflammatory cell infiltrate in the portal tracts (zone 1).
- Reactive hyperplasia of Kupffer cells (Fig. 29.1).

ALCOHOLIC HEPATITIS

Alcoholic hepatitis develops acutely, usually following a bout of heavy drinking.

G/A The liver is swollen, enlarged, soft and greenish. If repeated attacks of alcoholic hepatitis have superimposed on pre-existing fatty liver, changes of fatty liver in the form of yellow, greasy and smooth appearance may be present.

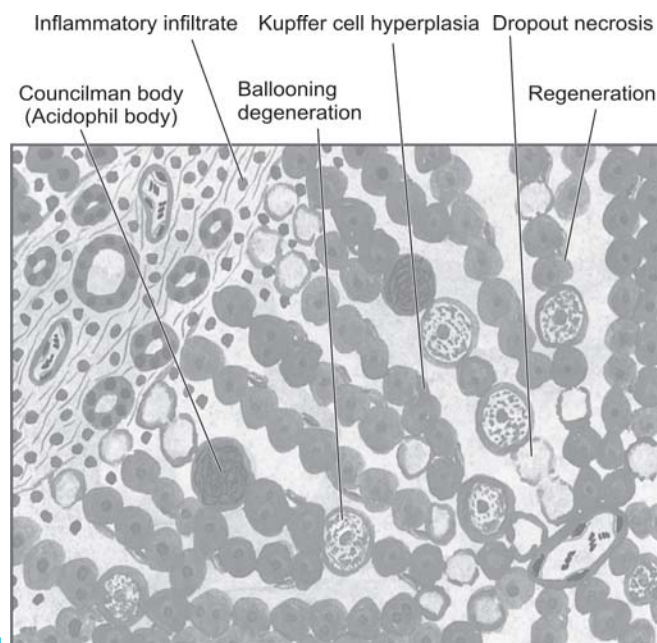


FIGURE 29.1: Acute viral hepatitis. There is lymphocytic infiltrate in the periportal area, zones of liver cell necrosis and shrunken hepatocytes called acidophil body.

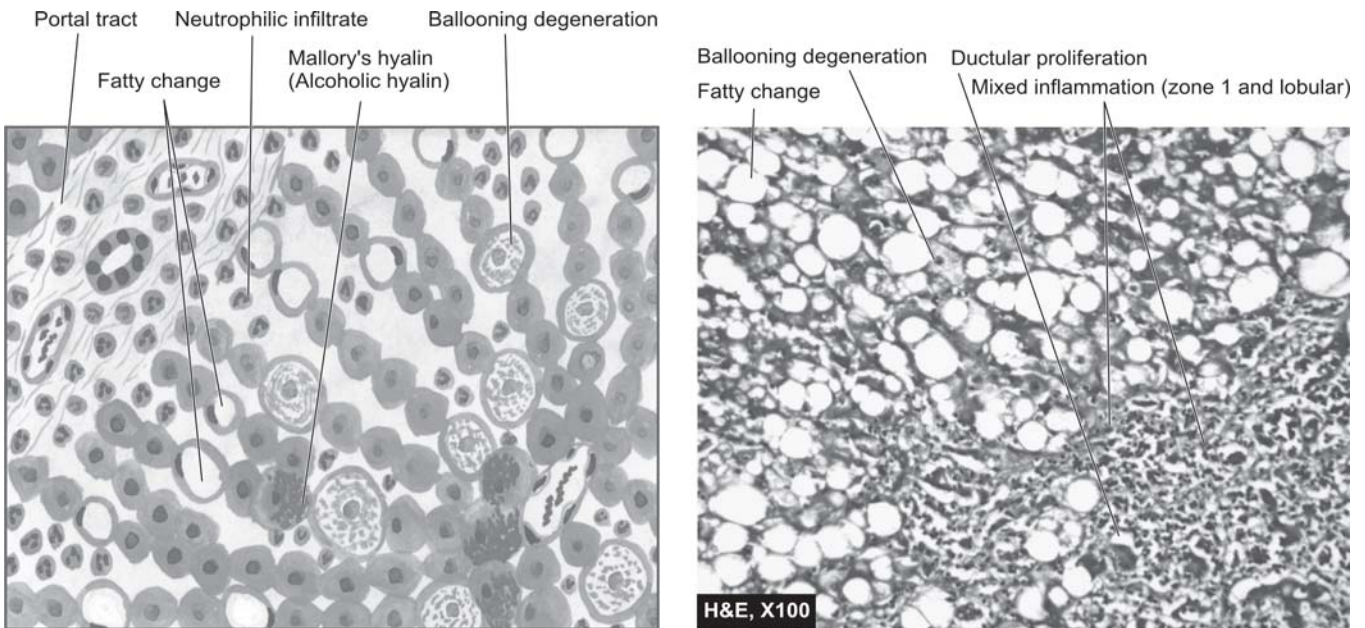


FIGURE 29.2: Alcoholic hepatitis. There is moderate fatty change, areas of liver cell necrosis and dense inflammatory cell infiltrate in the portal triad and periportal zone.

M/E

- i. Hepatocellular necrosis in the form of ballooned out hepatocytes, especially in the centrilobular zone.
- ii. Mallory body or alcoholic hyaline seen as eosinophilic intracytoplasmic inclusions in the perinuclear location in the swollen and ballooned hepatocytes.
- iii. Inflammatory cell infiltrate of polymorphs admixed with some mononuclear cells seen in the area of necrosis.

- iv. Web-like or chickenwire-like appearance of pericellular and perivenular fibrosis (Fig. 29.2).

SUBMASSIVE NECROSIS OF LIVER

Fulminant hepatitis is the most severe form of acute hepatitis of viral or non-viral etiologies and has two patterns—submassive and massive necrosis.

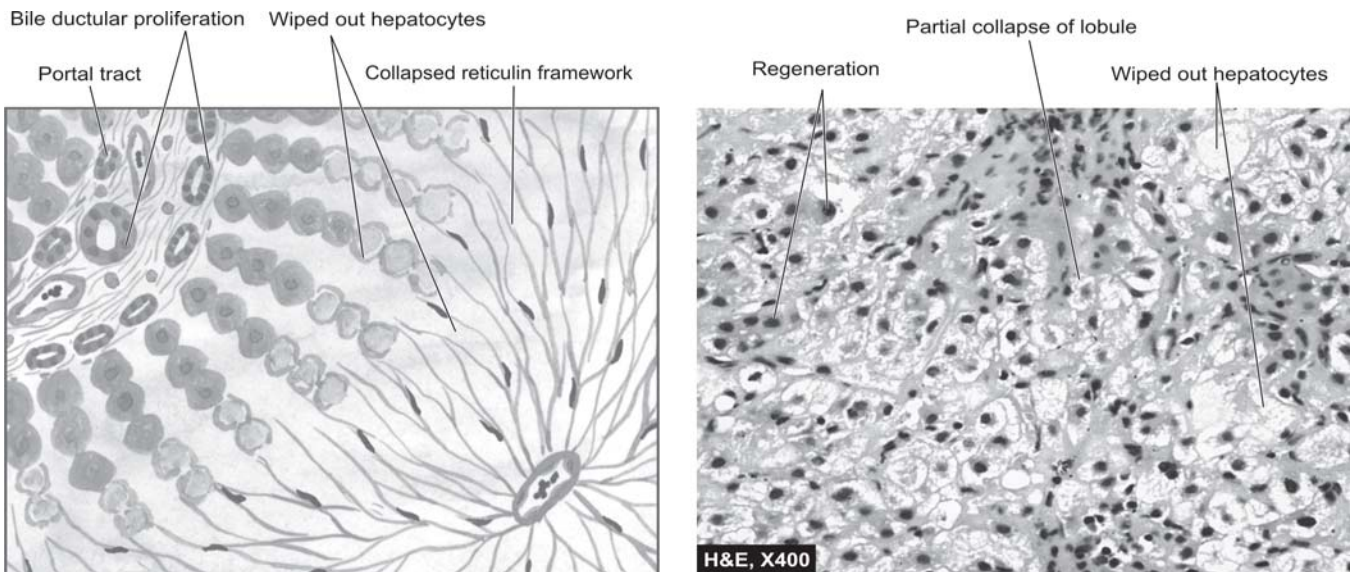


FIGURE 29.3: Fulminant hepatitis. There is complete wiping out of liver lobules with only collapsed reticulin framework left out in their place. There is no significant inflammation or fibrosis.

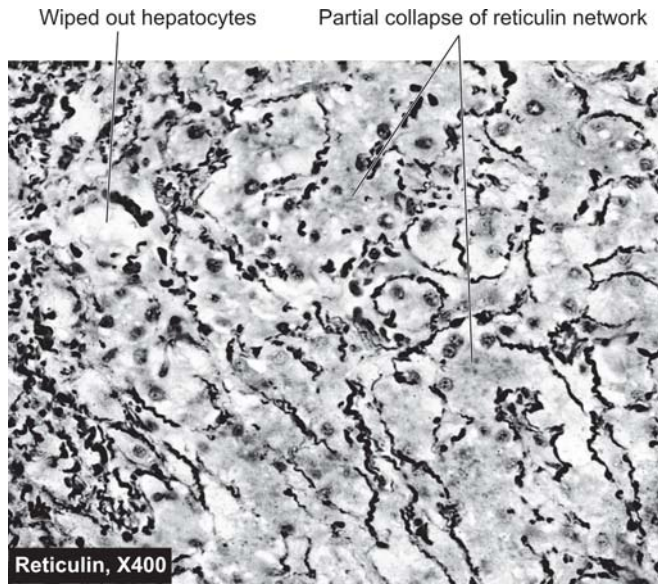


FIGURE 29.4: Fulminant hepatitis showing regeneration in the lobule while reticulin network is partly collapsed.

G/A The liver is small and shrunken and weighs 500-700 gm. The capsule is loose and wrinkled. The sectioned surface shows areas of muddy-red and yellow necrosis with patches of green bile staining.

M/E

- i. Wiping out of large groups of hepatocytes in centrilobular and mid-zone leading to collapsed reticulin framework (Fig. 29.3).
- ii. Areas of attempted regeneration are more orderly compared to massive necrosis (Fig. 29.4).



Liver and Biliary System II

- ◆ Cirrhosis Liver
- ◆ Hepatocellular Carcinoma
- ◆ Chronic Cholecystitis with Cholelithiasis
- ◆ Carcinoma Gallbladder

Exercise

30

CIRRHOSIS LIVER

Cirrhosis of the liver is a diffuse disease having disorganised lobular architecture and formation of nodules separated by irregular bands of fibrosis from one another.

G/A Cirrhosis is categorised by the size of nodules—*micronodular* if the nodules are less than 3 mm (Fig. 30.1), *macronodular* if the nodules are bigger than 3 mm (Fig. 30.2), and *mixed* if both small and large nodules are seen. On sectioned surface, the grey-brown nodules

are separated from one another by grey-white fibrous septa.

M/E The etiologic diagnosis in routine microscopy is generally not possible. The salient features of cirrhosis are as under:

- Lobular architecture of hepatic parenchyma is lost and central veins are hard to find.
- Fibrous septa divide the hepatic parenchyma into nodules.

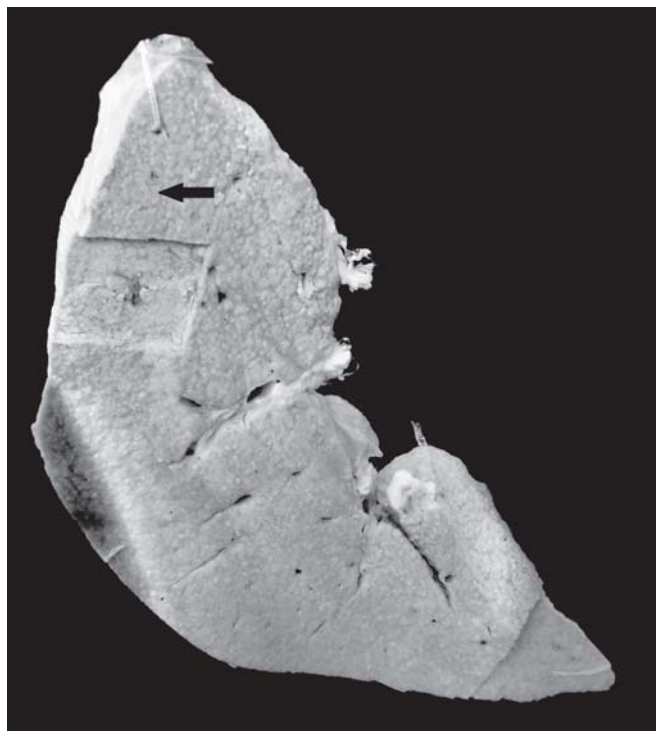


FIGURE 30.1: Alcoholic cirrhosis, showing the typical micronodular pattern in gross specimen.

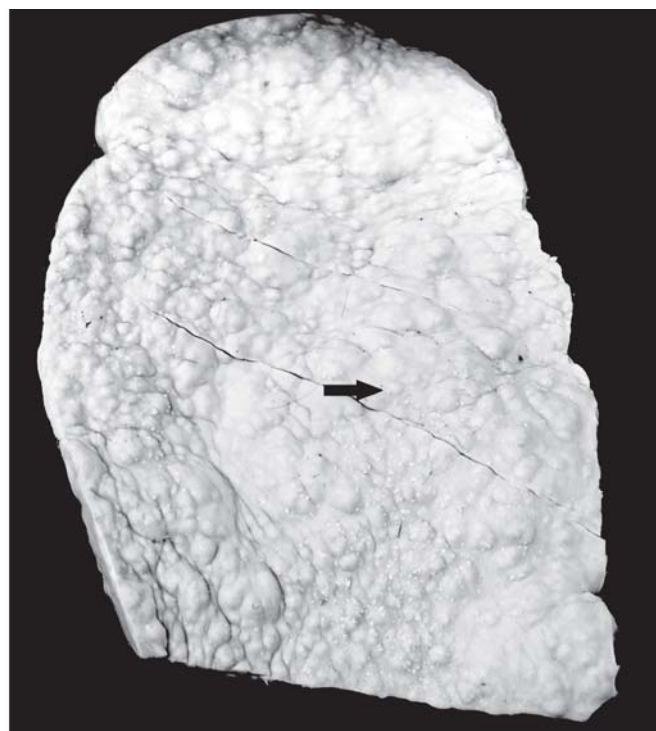


FIGURE 30.2: Post-necrotic cirrhosis, showing the typical irregular macronodular pattern in a small, distorted and irregularly scarred liver.

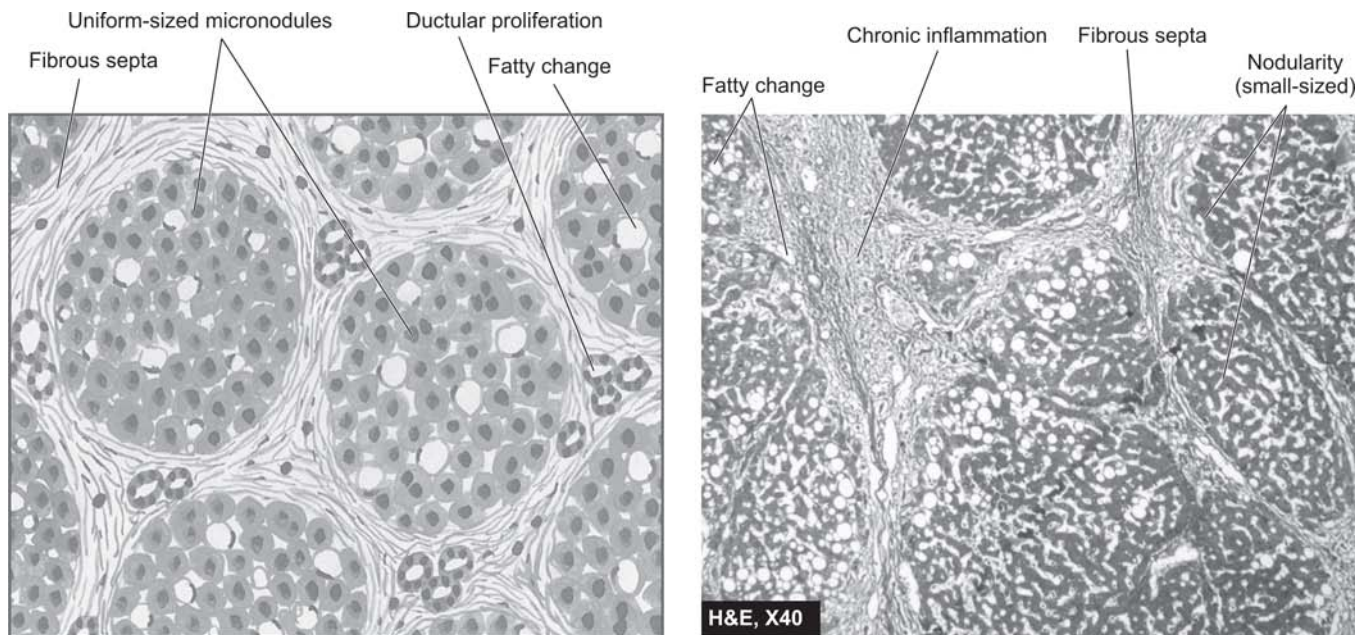


FIGURE 30.3: Alcoholic (micronodular) cirrhosis. The field shows dense fibrous septa forming nodules which have fatty change in many hepatocytes. There is minimal inflammation and some reactive bile duct proliferation in the septa.

- iii. The hepatocytes in the surviving parenchyma form regenerative nodules having disorganised masses of hepatocytes.
- iv. Fibrous septa contain some mononuclear inflammatory cell infiltrate and proliferated bile ductules (Fig. 30.3).

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) or hepatoma is the most common primary malignant tumour of the liver.

G/A The HCC may form one of the three patterns of growth (in decreasing order of frequency) (Fig. 30.4):

- i. *Expanding type* as a single large mass with central necrosis and haemorrhage.
- ii. *Multifocal type* as multiple masses scattered throughout the liver.
- iii. *Infiltrating type* is a diffusely spreading type and is less common.

M/E The features are as follows (Fig. 30.5):

- i. *Histologic patterns:* The tumour cells may be arranged in a variety of patterns. Most common is trabecular or sinusoidal pattern composed of 2-8 cell wide layers of tumour cells separated by endothelium-lined vascular spaces. Other patterns include pseudoglandular or acinar, compact and scirrhous.

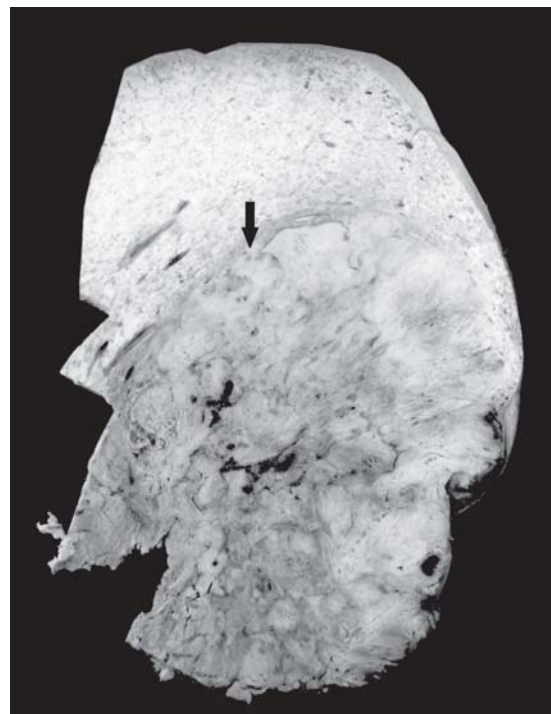


FIGURE 30.4: Hepatocellular carcinoma. Sectioned surface of the slice of liver shows a single, large mass (arrow) with irregular borders and having central areas of necrosis, while rest of the hepatic parenchyma shows many nodules of variable sizes owing to co-existent macronodular cirrhosis.

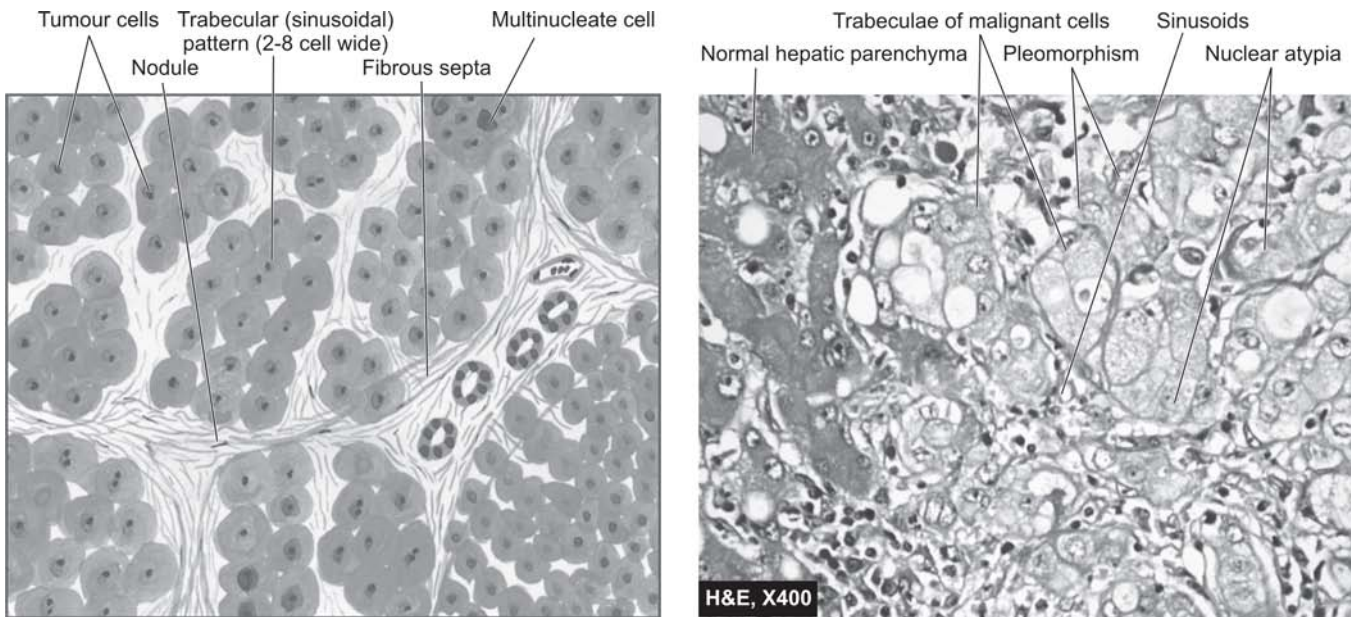


FIGURE 30.5: Hepatocellular carcinoma. It shows trabeculae of malignant hepatocytes separated by sinusoids.

- ii. *Cytologic features:* The tumour cells have features resembling hepatocytes having vesicular nuclei, prominent nucleoli, granular and eosinophilic cytoplasm. These tumour cells have pleomorphism, bizarre giant cell formation, and Mallory's hyaline.

CHRONIC CHOLECYSTITIS WITH CHOLELITHIASIS

Chronic cholecystitis is the commonest type of gall bladder disease.

G/A The gallbladder is generally contracted and the wall is thickened. Cut section of wall of gallbladder is grey-white due to dense fibrosis. The mucosal folds may be thickened, atrophied or flattened. The lumen commonly contains gallstones, most often multiple multifaceted mixed type, followed by pure cholesterol gallstones in descending order of frequency (Figs 30.6 and 30.7).

M/E

- i. Penetration of mucosa deep into the wall of the gall bladder upto the muscularis layer to form Rokitsky-Aschoff sinuses.
- ii. Variable degree of chronic inflammatory cells (lymphocytes, plasma cells and macrophages) in the lamina propria and subserosal layer.
- iii. Variable degree of fibrosis and thickening of perimuscular layer (Fig. 30.8).

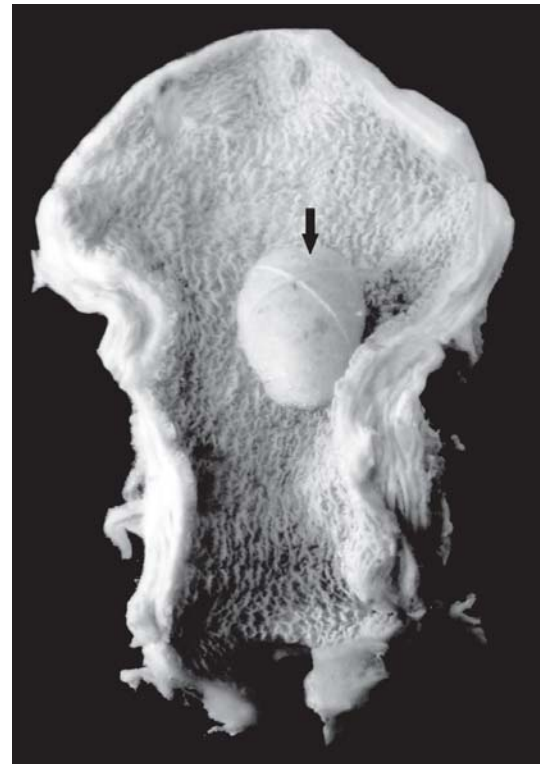


FIGURE 30.6: Chronic cholecystitis with cholesterol cholelithiasis: The wall of the gallbladder is thickened externally. Cut surface shows that gallbladder wall is thickened, fibrotic and grey-white. The mucosa is velvety. The lumen contains a single large, oval, hard, yellowish-white gallstone (arrow).

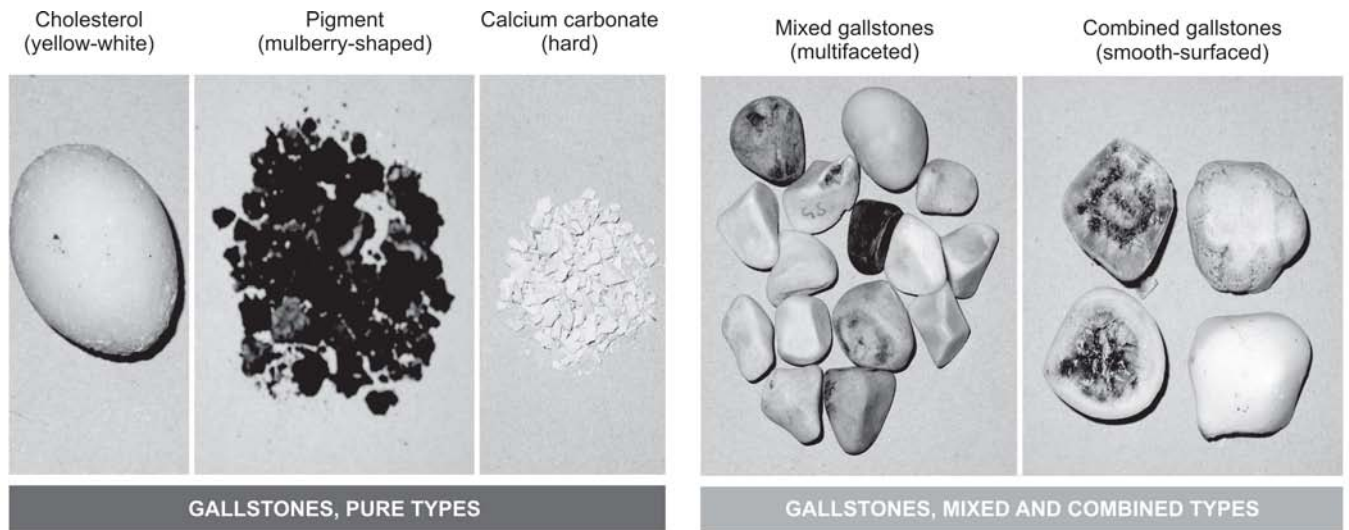


FIGURE 30.7: Gallstones of different types.

CARCINOMA GALLBLADDER

Primary carcinoma of the gallbladder is more common than cancer of the extra-hepatic biliary system.

G/A The commonest site for cancer of gallbladder is the fundus, followed next in frequency by the neck of the gallbladder. The tumour may be infiltrating type seen as

irregular area of diffuse thickening and induration in the gallbladder wall, or fungating type growing as irregular, friable, papillary or cauliflower-like growth into the lumen. Gallstones may coexist with carcinoma (Fig. 30.9).

M/E

- i. Most common is adenocarcinoma i.e. malignant glandular pattern.

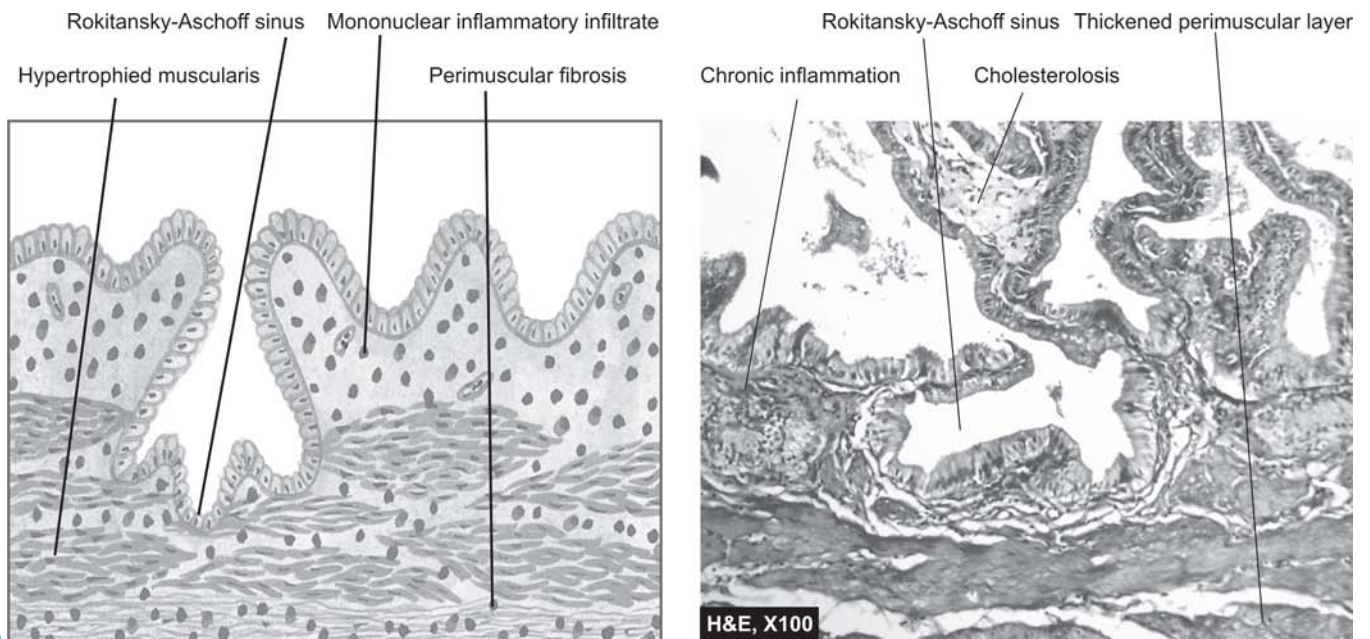


FIGURE 30.8: Chronic cholecystitis. There is perimuscular hyperplasia, chronic inflammatory cells in the wall and Rokitsky-Aschoff sinus in the mucosa.

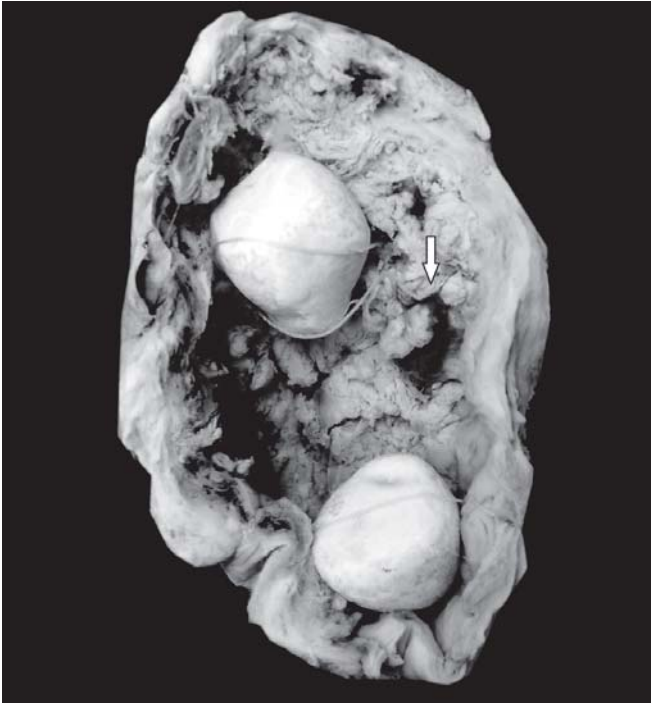


FIGURE 30.9: Carcinoma gallbladder with cholecystitis and mixed cholelithiasis. The wall of the gallbladder is thickened. Cut surface shows that gallbladder wall is thickened, fibrotic and grey-white. The lumen contains irregular, friable papillary growth arising from mucosa (arrow). The lumen also contains multiple, multi-faceted, gallstones.

ii. The tumour may have papillary or infiltrative growth pattern (Fig. 30.10).

iii. The tumour may be well-differentiated to poorly-differentiated, non-mucin secreting, or less commonly mucin-secreting type.

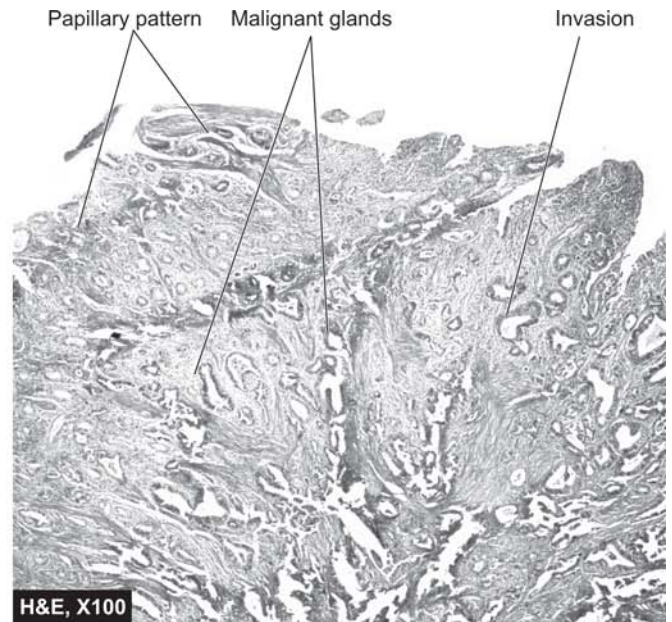
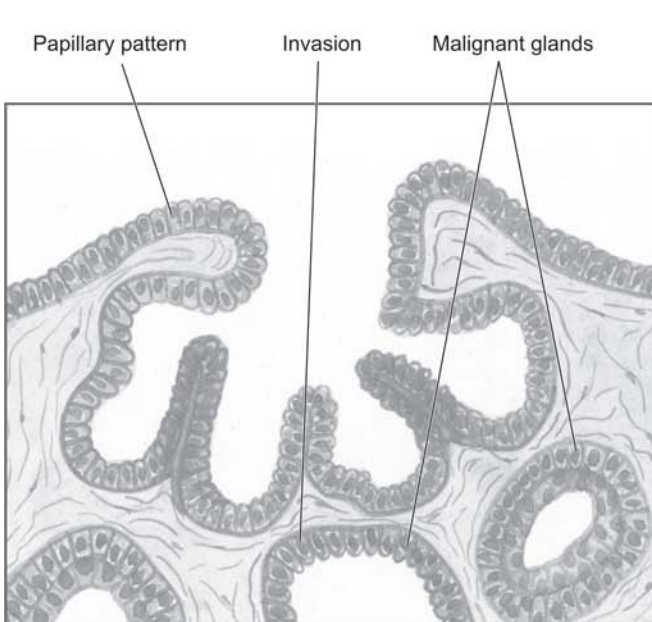


FIGURE 30.10: Adenocarcinoma gallbladder. Well-differentiated malignant glands are seen invading the wall of the gallbladder.

Urinary System I

- ◆ Acute Glomerulonephritis (GN)
- ◆ Rapidly Progressive Glomerulonephritis (RPGN)
- ◆ Chronic Glomerulonephritis
- ◆ Chronic Pyelonephritis

Exercise

31

ACUTE GLOMERULONEPHRITIS (GN)

Acute post-streptococcal GN is the most common form of GN in children 6 to 16 years of age.

G/A The kidneys are symmetrically enlarged, weighing one and a half to twice the normal weight. The cortical as well as sectioned surface show petechial haemorrhages giving the characteristic appearance of flea-bitten kidney (Fig. 31.1).

M/E

- Glomeruli are affected diffusely. They are enlarged and hypercellular.
- The diffuse hypercellularity of the tuft is due to proliferation of mesangial, endothelial and occasional epithelial cells (*acute proliferative lesions*) as well as polymorphs and monocytes (*acute exudative lesions*).
- Tubules may show swelling of tubular lining cells and their lumina may contain red cell casts.
- There may be some degree of interstitial oedema and leucocytic infiltration (Fig. 31.2).

RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (RPGN)

RPGN presents with acute renal failure in a few weeks and months and has a dismal prognosis.

G/A The kidneys are usually enlarged and pale with smooth outer surface (large white kidney). Cut surface shows pale cortex and congested medulla.

M/E

- Pathognomonic *crescents* are seen on the inside of Bowman's capsule. Crescents are collections of pale-staining polygonal cells formed from the proliferation of parietal epithelial cells.



FIGURE 31.1: Flea-bitten kidney. The kidney is enlarged in size and weight. The cortex shows tiny petechial haemorrhages visible through the capsule (arrow). Sectioned surface (not shown here) would show pale mottled appearance.

- Glomerular tufts frequently contain fibrin thrombi.
- Tubular epithelial cells may show hyaline droplets and tubular lumina may contain casts, red blood cells and fibrin.
- The interstitium is oedematous and may show early fibrosis.
- Arteries and arterioles may show associated changes of hypertension (Figs 31.3).

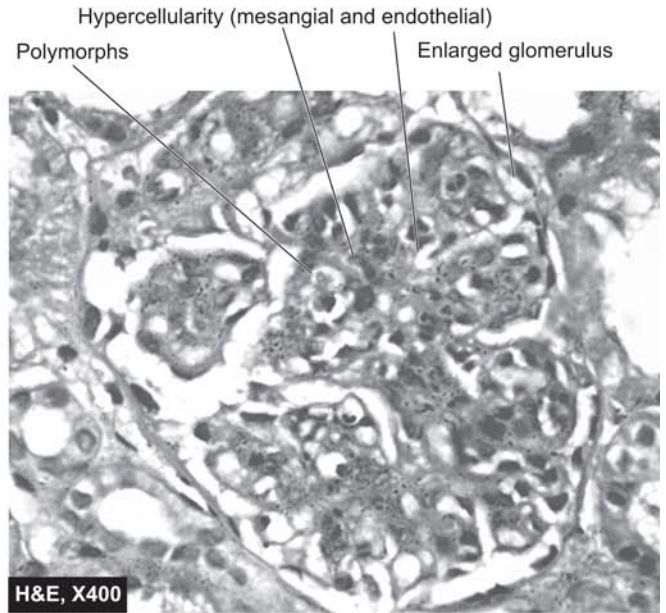
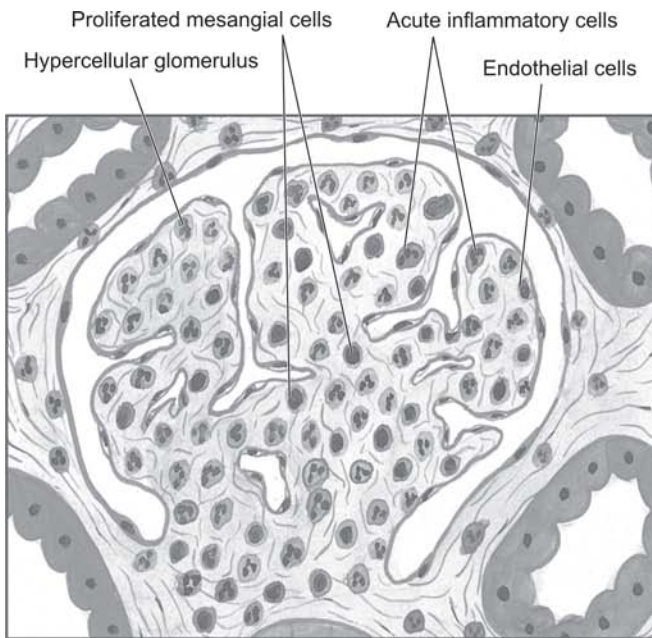


FIGURE 31.2: Acute glomerulonephritis. There is increased cellularity by proliferation of mesangial cells, endothelial cells and infiltration by polymorphs.

CHRONIC GLOMERULONEPHRITIS

Chronic GN or end-stage kidney is the final stage of a variety of glomerular diseases.

G/A The kidneys are usually small and contracted weighing as low as 50 gm each. The capsule is adherent to the cortex and the cortical surface is generally diffusely granular (Fig. 31.4). On cut section, the cortex is narrow and atrophic while the medulla is unremarkable.

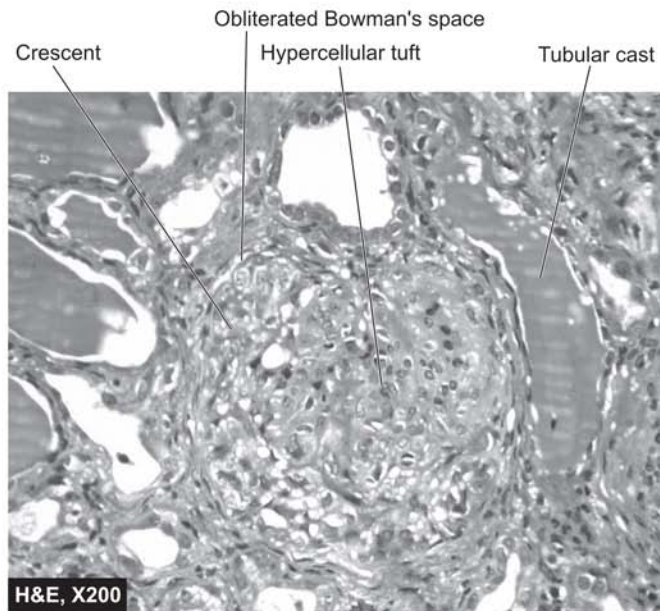
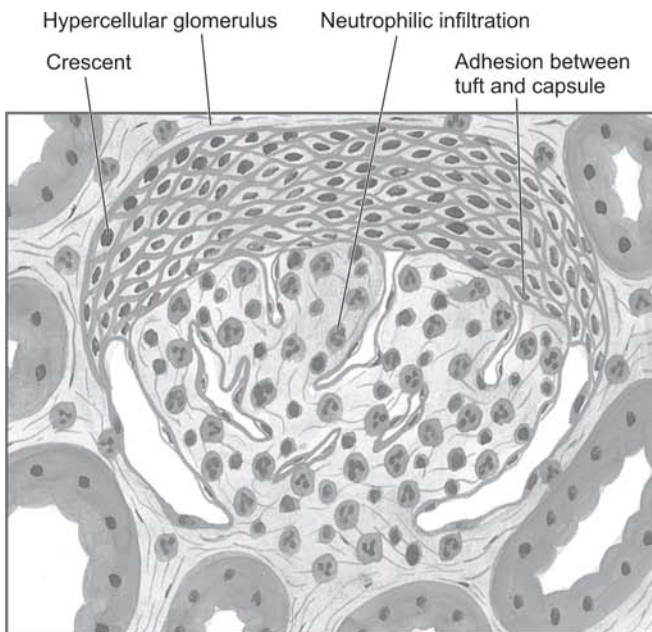


FIGURE 31.3: Post-infectious RPGN, light microscopic appearance. There are crescents in Bowman's space due to proliferation of visceral epithelial cells. These form adhesions between the glomerular tuft and Bowman's capsule.

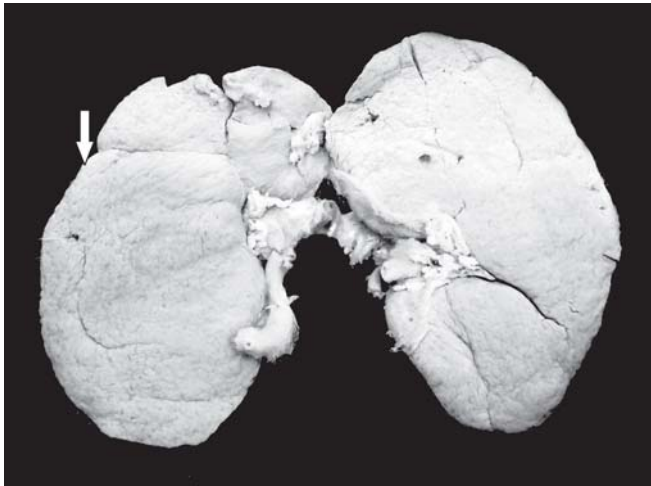
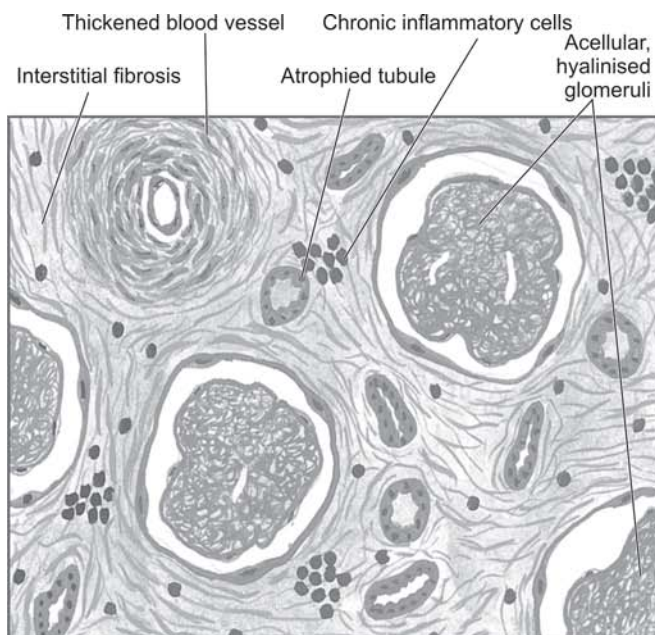


FIGURE 31.4: Short contracted kidney-end-stage kidney, chronic glomerulonephritis. The kidney is small and contracted, weighing less than normal. The capsule is adherent to the cortex and has granular surface (arrow).

M/E

- i. *Glomeruli* are reduced in number and most of them show completely hyalinised tufts appearing as acellular eosinophilic masses.
- ii. Many *tubules* completely disappear, there may be atrophy of tubules close to scarred glomeruli and tubular lumina contain eosinophilic homogeneous casts.



- iii. There is fine delicate fibrosis of the *interstitial tissue* and varying number of chronic inflammatory cells in the interstitium (Fig. 31.5).
- iv. Advanced cases associated with hypertension show conspicuous *arterial and arteriolar sclerosis*.

CHRONIC PYELONEPHRITIS

Chronic pyelonephritis is a chronic tubulointerstitial disease resulting from repeated attacks of inflammation and scarring.

G/A The kidneys are usually small and contracted, weighing less than 100 gm each, showing unequal reduction. The outer surface of the kidneys is irregularly scarred. These scars are of variable size and show irregular depressions on the cortical surface. The pelvis is dilated and calyces are blunted and may contain renal stone taking its shape called staghorn stone (Fig. 31.6).

M/E The predominant microscopic changes are seen in the interstitium and tubules:

- i. The *interstitium* shows chronic inflammatory infiltrate, chiefly composed of lymphocytes, plasma cells and macrophages with pronounced interstitial fibrosis.
- ii. The *tubules* show varying degree of atrophy and dilatation. Dilated tubules may contain colloid casts producing thyroidisation of tubules.

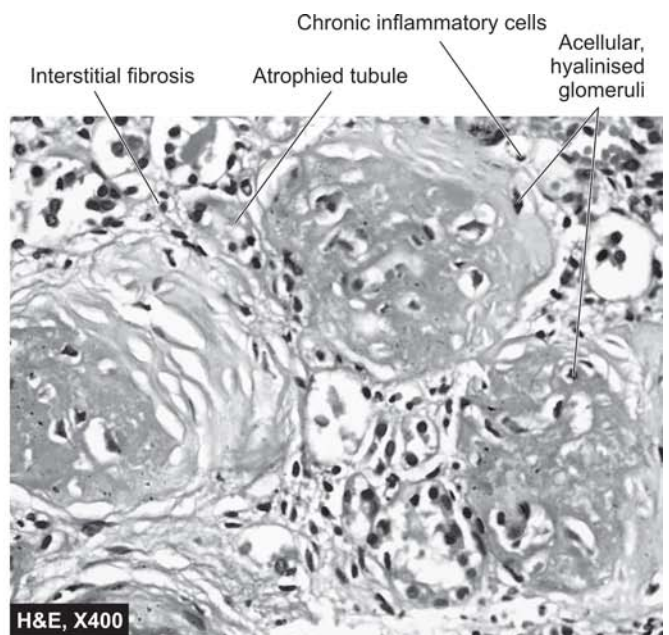


FIGURE 31.5: Chronic glomerulonephritis. Light microscopy shows acellular and completely hyalinised glomerular tufts, hyalinised and thickened blood vessels and fine interstitial fibrosis with a few chronic inflammatory cells and there is tubular atrophy.

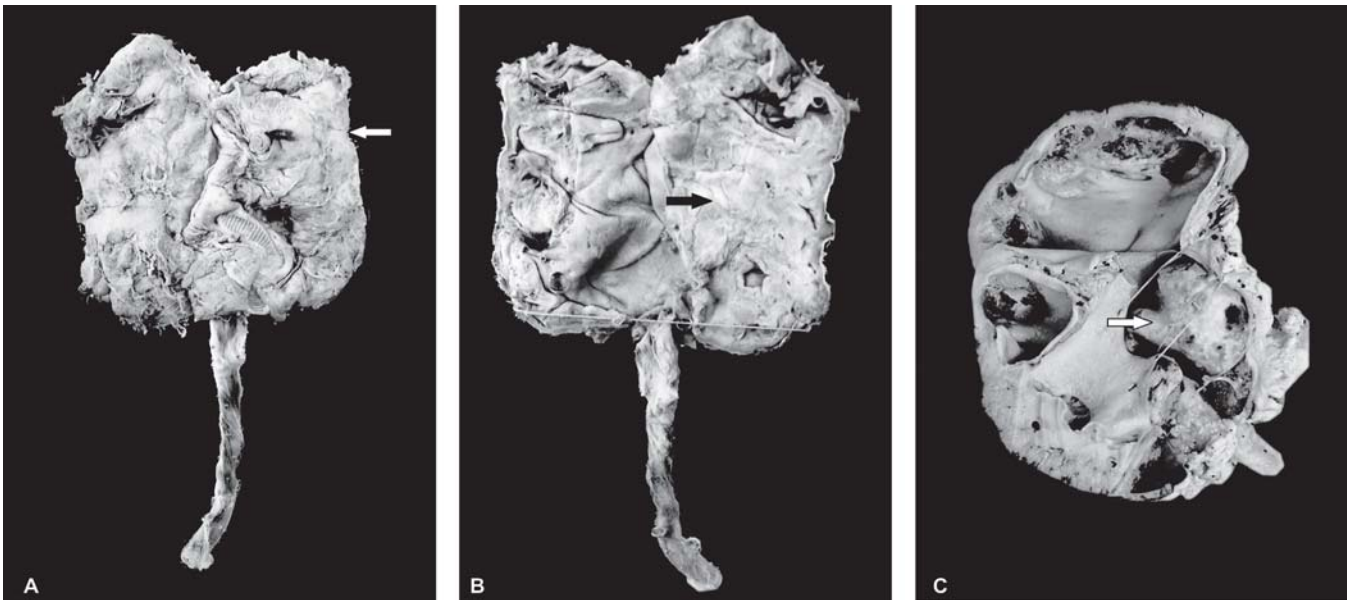


FIGURE 31.6: Chronic pyelonephritis—short contracted kidney. The kidney is small, contracted weighing less than normal. **A** (External surface): the capsule is adherent to the cortex and has irregular scars on the surface. **B** (Sectioned surface): shows dilated pelvi-calycal system with atrophied and thin peripheral cortex and increased hilar fat extending inside (arrow). **C**. Staghorn stone (arrow) lying in dilated pelvicalyceal system.

iii. The wall of *dilated pelvicalyceal system* shows marked chronic inflammation and scarring.

iv. There is often *periglomerular fibrosis* and hyalinisation of some glomeruli (Fig. 31.7).

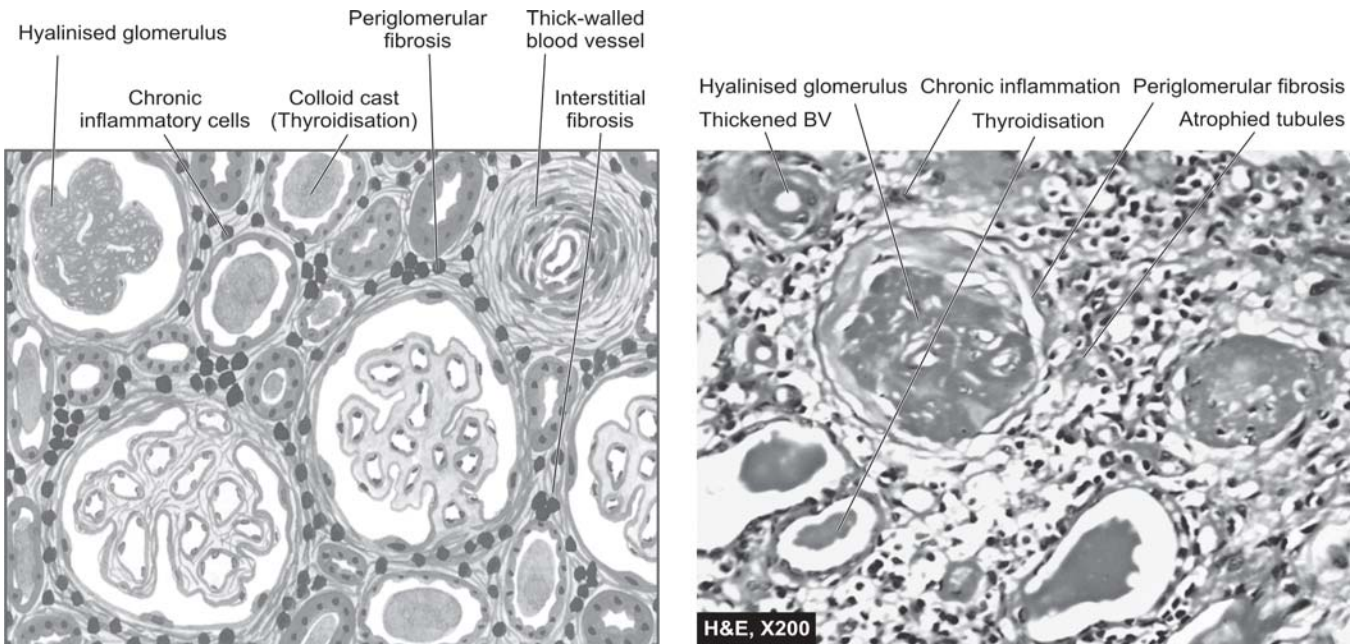


FIGURE 31.7: Chronic pyelonephritis. The tubules show atrophy of some tubules and dilatation of some others which contain colloid-like casts (thyroidisation). The interstitium shows chronic inflammatory cells and fibrosis. The blood vessels are thick-walled and the glomeruli show periglomerular fibrosis.

Urinary System II

- ◆ Diabetic Nephrosclerosis
- ◆ Renal Cell Carcinoma
- ◆ Wilms' Tumour
- ◆ Transitional Cell Carcinoma

Exercise

32

DIABETIC NEPHROSCLEROSIS

Renal involvement is an important complication of diabetes mellitus. Diabetic nephropathy encompasses 4 types of renal lesions—diabetic glomerulosclerosis, vascular lesions, diabetic pyelonephritis and tubular lesions.

G/A The kidneys are often small and contracted. Depending upon the nature of underlying renal lesions, the external surface shows irregular or granular appearance. The cut surface shows narrowed cortex.

M/E

- Diffuse glomerular lesions* are the most common. These include: diffuse involvement of all parts of

glomeruli, thickening of GBM, diffuse increase in mesangial matrix and exudative lesions.

- Exudative lesions* include capsular drops and fibrin caps. Capsular drop is an eosinophilic hyaline thickening of the parietal layer of Bowman's capsule and bulges into the glomerular space. Fibrin cap is homogeneous brightly eosinophilic material on the wall of a peripheral capillary of a lobule.
- Nodular lesions* of diabetic glomerulosclerosis (or Kimmelstiel-Wilson lesions) are seen in juvenile-onset diabetes and show one or more nodules in some glomeruli. Nodule is spherical, laminated, hyaline acellular mass within a lobule of the glomerulus. The nodule is surrounded peripherally by

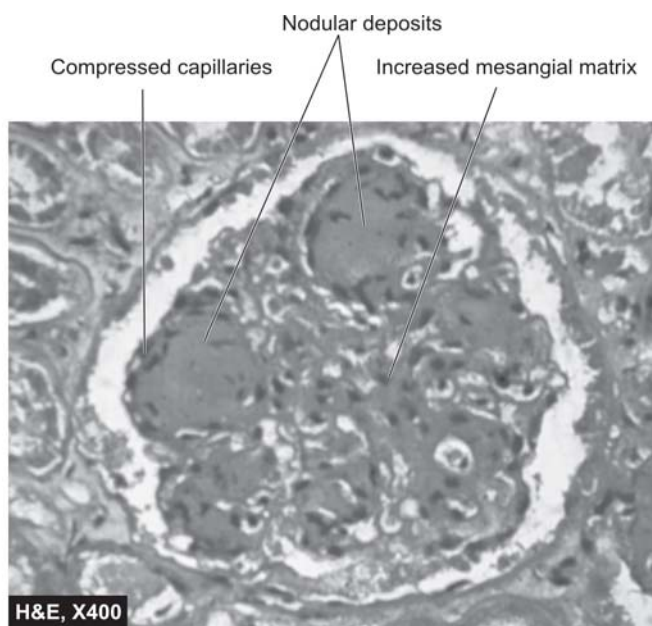
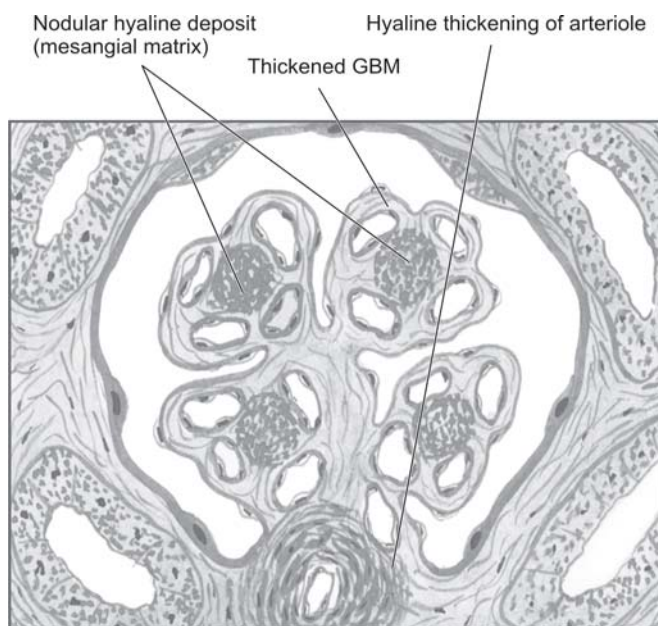


FIGURE 32.1: Diabetic glomerulosclerosis, microscopic appearance of nodular lesion (*Kimmelstiel-Wilson lesion*). There are hyaline nodules within the lobules of glomeruli, surrounded peripherally by glomerular capillaries with thickened walls.



FIGURE 32.2: Renal cell carcinoma. The upper pole of the kidney shows a large and tan mass while rest of the kidney has reniform contour. Sectioned surface shows irregular, circumscribed, yellowish mass with areas of haemorrhages and necrosis (arrow). The residual kidney is compressed on one side and shows obliterated calyces and renal pelvis.

glomerular capillary loops having thickened GBM (Fig. 32.1).

- iv. *Vascular lesions* consist of hyaline arteriosclerosis affecting afferent and efferent arterioles of the glomeruli.
- v. *Chronic pyelonephritis* is more common in diabetics than in others.
- vi. *Tubular lesions* (Armanni-Ebstein lesions) consist of glycogen deposits as cytoplasmic vacuoles.

RENAL CELL CARCINOMA

Renal cell carcinoma (RCC) or hypernephroma or adenocarcinoma comprises 70-80% of all renal cancers and occurs most commonly in 50 to 70 years of age.

G/A The tumour commonly arises from a pole, most often upper pole, of the kidney as a solitary and unilateral tumour. The tumour is generally large, golden yellow and circumscribed. Cut section of the tumour commonly shows large areas of ischaemic necrosis, cystic change and foci of haemorrhages. Another feature is the frequent presence of tumour thrombus in the renal vein (Fig. 32.2).

M/E

- i. A variety of patterns of tumour cells are seen such as solid, acinar, tubular, trabecular, cord and papillary arrangements in a delicate fibrous stroma.
- ii. Tumour cells are generally of 2 types—clear and granular. *Clear cells* comprise 70% of RCC and are

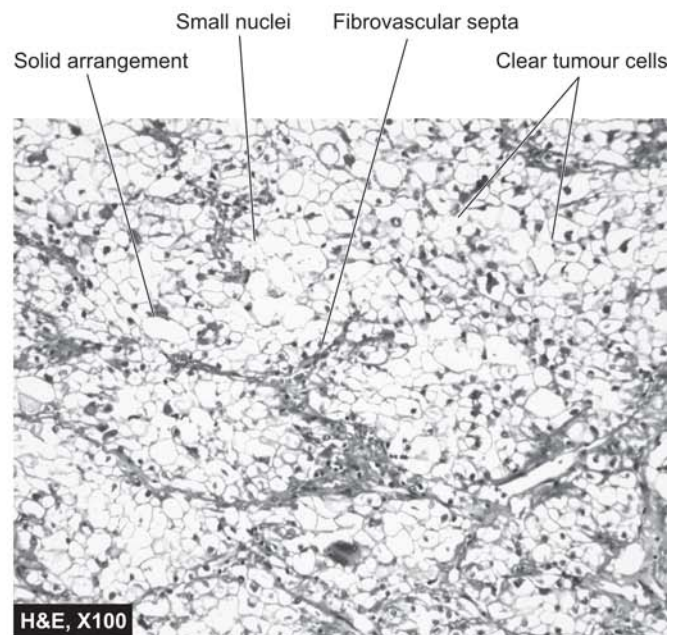
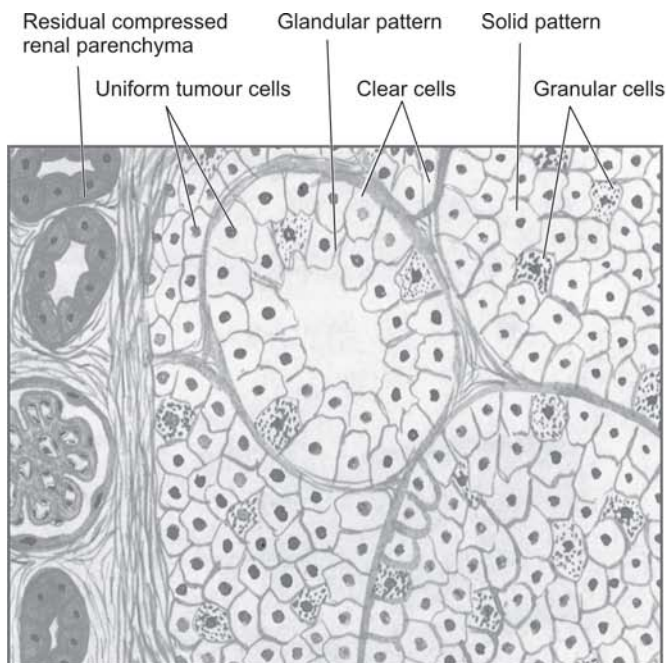


FIGURE 32.3: Adenocarcinoma kidney. Solid masses and glandular pattern of malignant cells having features of clear cells.

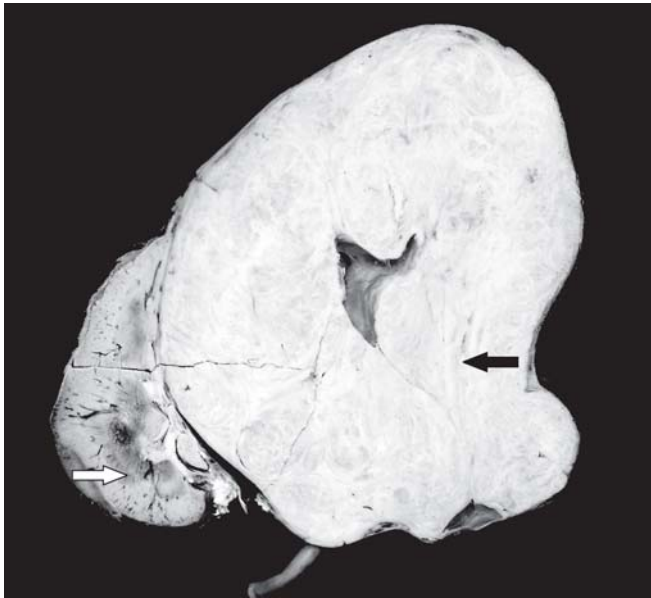


FIGURE 32.4: Nephroblastoma (Wilms' tumour). The kidney is enlarged and has ovoid and nodular appearance. The sectioned surface shows replacement of almost whole kidney by the tumour leaving a thin strip of compressed renal tissue at lower end (white arrow). Cut section of the tumour is grey-white, fleshy (black arrow) and has small areas of haemorrhages and necrosis.

large cells with well-defined borders, abundant clear cytoplasm and regular pyknotic nuclei. *Granular cells* have similar features but have moderate amount of pink granular cytoplasm (Fig. 32.3).

WILMS' TUMOUR

Wilms' tumour or nephroblastoma is an embryonic tumour commonly seen in children between 1 to 6 years of age.

G/A The tumour is generally quite large, spheroidal replacing most of the kidney. It is generally solitary and unilateral. On cut section, the tumour shows soft, fish-flesh like, grey-white appearance with foci of necrosis and haemorrhages. Sometimes, myxomatous and cartilaginous elements are identified (Fig. 32.4).

M/E

- The tumour shows mixture of primitive epithelial and mesenchymal elements.
- The tumour largely consists of small, round to spindled, anaplastic, sarcomatoid tumour cells.
- Abortive tubules and poorly formed glomerular structures are seen in these areas.
- Sometimes, mesenchymal elements such as smooth muscle, cartilage, bone and fat cells may be seen (Figs 32.5).

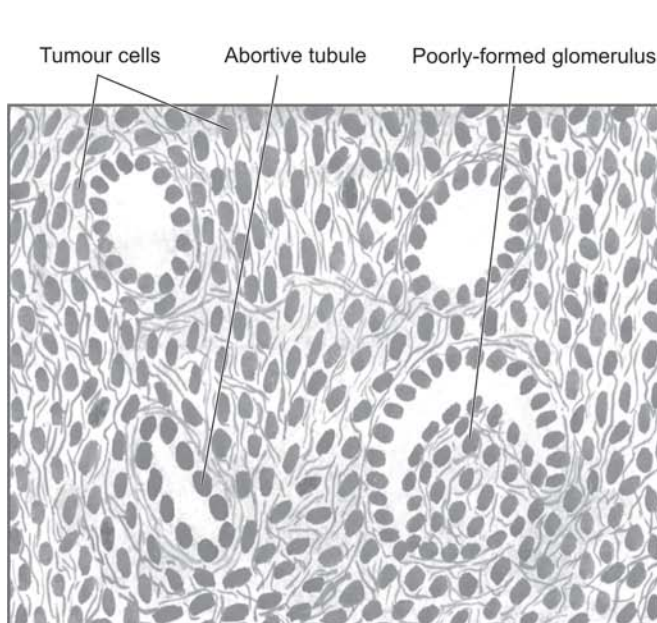


FIGURE 32.5: Nephroblastoma (Wilms' tumour), showing predominance of small round to spindled sarcomatoid tumour cells. A few abortive tubules and poorly-formed glomerular structures are present in it.

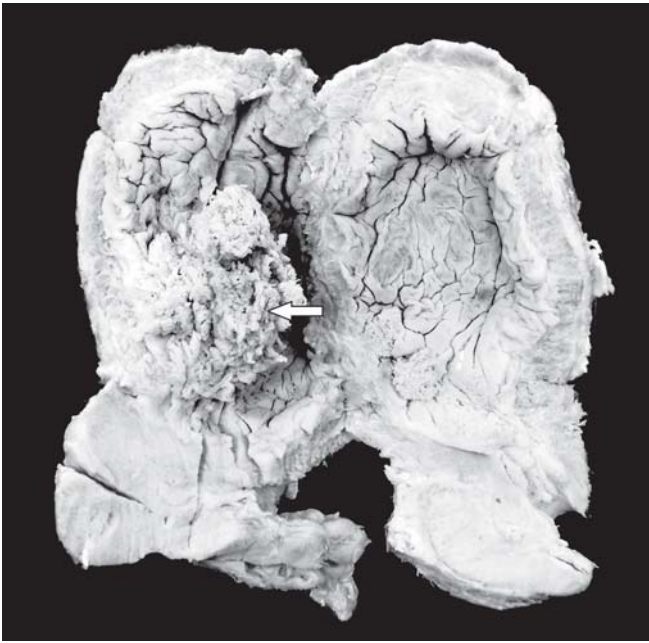


FIGURE 32.6: Transitional cell carcinoma urinary bladder. The urinary bladder wall is identified by thick muscular wall, narrow lumen and portions of ureters attached above. The mucosal surface shows papillary tumour floating in the lumen (arrow).

TRANSITIONAL CELL CARCINOMA

More than 90% of bladder tumours are transitional cell type.

G/A The tumour may be single or multiple. About 90% of the tumours are papillary (non-invasive or invasive) whereas remaining 10% are flat indurated (non-invasive or invasive). The papillary tumour has free floating fern-like arrangement with broad or narrow pedicle (Fig. 32.6). The non-papillary tumours are bulkier with ulcerated surface.

M/E The tumour is divided into 3 histologic grades according to the Mostofi classification (grades I, II and III). Type II transitional cell carcinoma presents classic features (Fig. 32.7):

- The tumour shows increased layers of cells.
- These tumour cells are still recognisable as transitional cells but they have features of anaplasia such as nuclear crowding, nuclear hyperchromatism, mitotic activity and loss of polarity.
- The tumour may or may not show invasion beyond basement membrane.

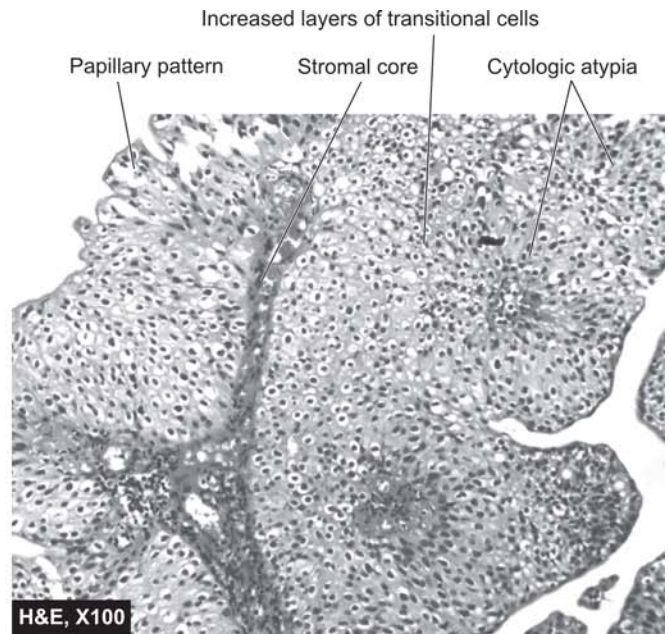
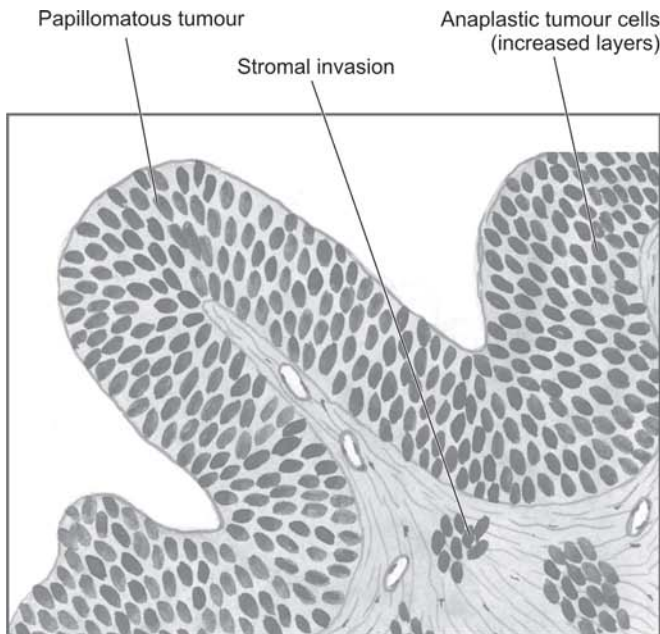


FIGURE 32.7: Microscopic features of grade II transitional cell carcinoma. The tumour has papillary pattern and marked increase in the layers of transitional cells depicting features of cytologic atypia.

Lymphoid System

- ◆ Non-Hodgkin's Lymphoma
- ◆ Hodgkin's Disease—Nodular Sclerosis and Mixed Cellularity

Exercise

33

NON-HODGKIN'S LYMPHOMA (NHL)

NHL is more common than Hodgkin's disease. Majority (65%) arise in lymph nodes while the remaining 35% take origin in extranodal lymphoid tissues. Out of various classifications of NHL, *Working Formulations for Clinical Usage* is widely used which divides all NHLs into 3 prognostic groups—low grade, intermediate grade and high grade.

G/A The affected groups of lymph nodes are enlarged and matted due to infiltration into the surrounding connective tissue. Sectioned surface appears grey-white and fleshy (Fig. 33.1).

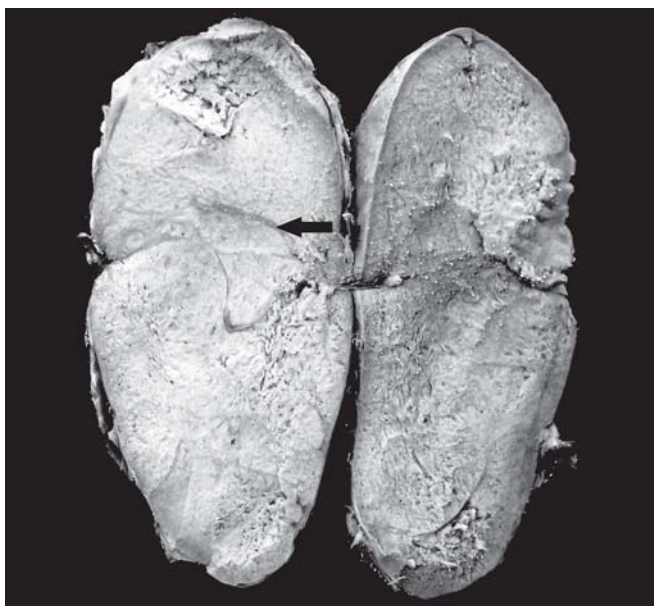


FIGURE 33.1: Non-Hodgkin's Lymphoma. Lymph nodes are identified by bean-shaped contour, peripheral capsule and fat. Sectioned surface shows matted lymph nodes which are homogeneous, grey-white and fleshy (arrow).

M/E The microscopic features of various prognostic groups are variable. The features of diffuse large cell lymphoma (intermediate grade) are outlined below (Fig. 33.2):

- There is diffuse effacement of nodal architecture.
- There is diffuse replacement of the node by mature, small and well differentiated lymphocytes.
- Infiltration by the tumour cells into perinodal soft tissue is common.

HODGKIN'S DISEASE (HD)

HD arises primarily within the lymph nodes. Unlike NHL, there is only one universally accepted classification of HD (*Rye classification*) which divided it into 4 subtypes: lymphocyte-predominance, nodular sclerosis, mixed cellularity and lymphocyte-depletion type. Central to diagnosis of HD is the essential identification of Reed-Sternberg (RS) cell.

G/A The affected lymph nodes are matted together. The sectioned surface is homogeneous and fleshy. *Nodular sclerosis* type shows nodular scarring while there is abundance of necrosis in *mixed cellularity* type.

M/E The characteristic microscopic feature of all types of HD is RS cell which has different morphologic variants. *Classic RS cell* is a large cell having bilobed nucleus appearing as mirror image of each other. Each lobe of the nucleus contains a prominent, eosinophilic, inclusion-like nucleolus with a clear halo around it giving it an owl-eye appearance. The cytoplasm of the cell is abundant and amphophilic (Fig. 32.3,A). *Lacunar type RS cell* is characterised by pericellular halo due to shrinkage of cell (Fig. 32.3,B).

The microscopic features of the two common types of HD are outlined below:

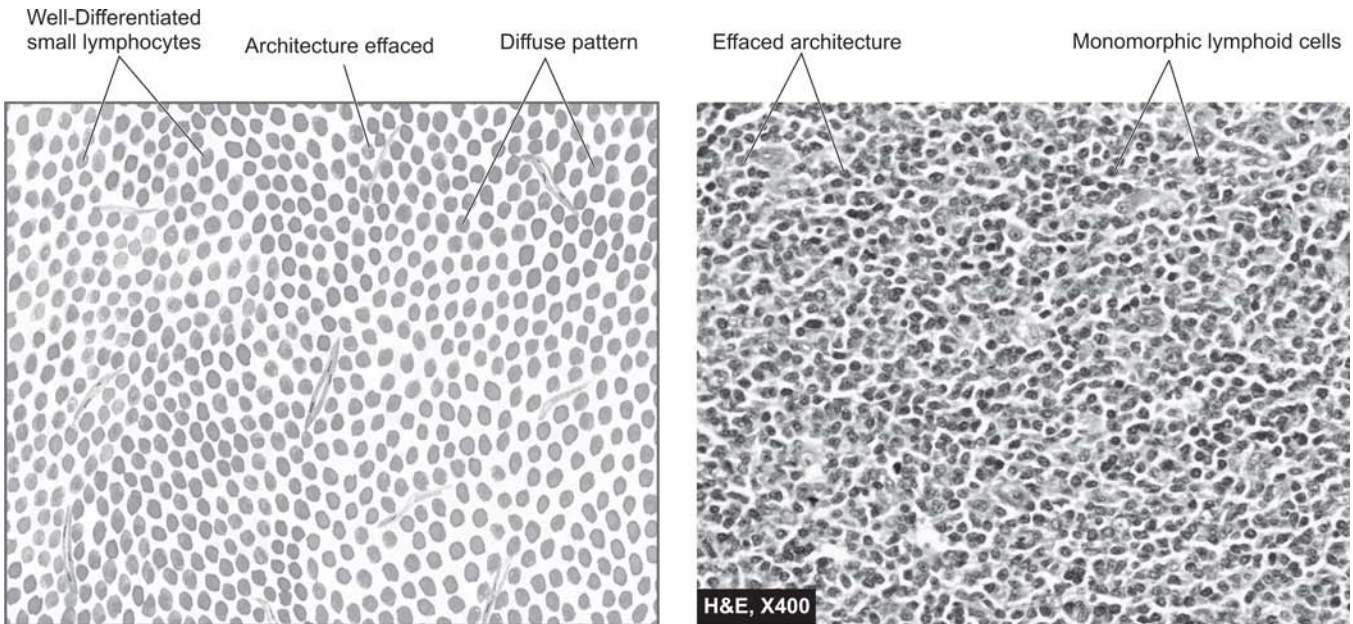
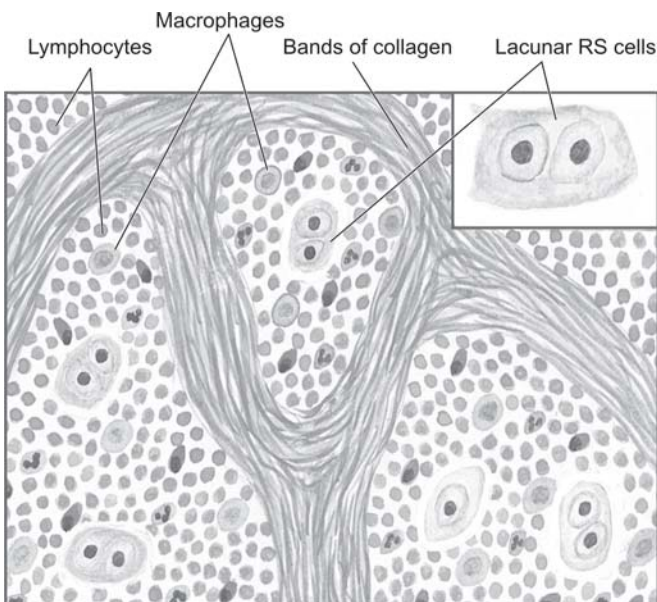


FIGURE 33.2: Non-Hodgkin's lymphoma, small lymphocytic type (SLL). There are sheets of uniform small lymphoid cells with little mitotic activity.

HD-Nodular Sclerosis Type

It is characterised by two essential features:

- Bands of collagen:* Variable amount of fibrous tissue is present in the involved lymph nodes.
- Lacunar type of RS cells:* Characteristic lacunar type of RS cells with distinctive pericellular halo are present (Figs 33.3).



HD-Mixed Cellularity Type

The features are as under:

- Replacement of the entire affected lymph nodes by heterogeneous mixture of various types of apparently normal cells that include proliferating lymphocytes, histiocytes, eosinophils, neutrophils and plasma cells.

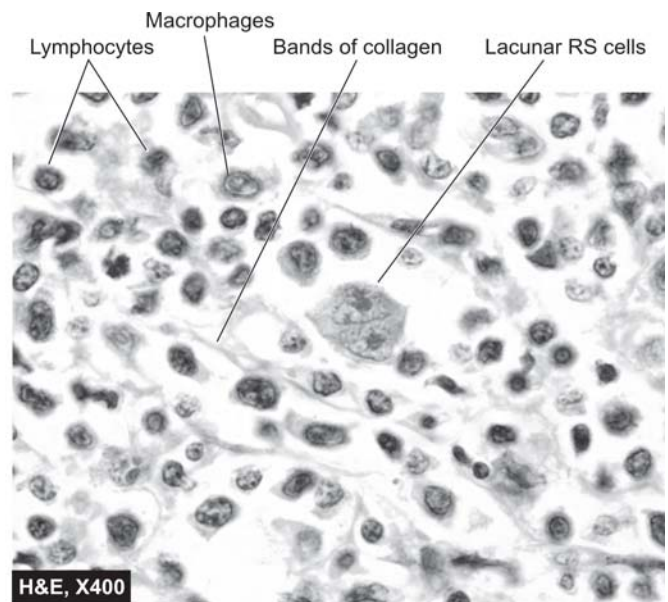


FIGURE 33.3: Hodgkin's disease, nodular sclerosis type. There are bands of collagen forming nodules and characteristic lacunar type RS cells.

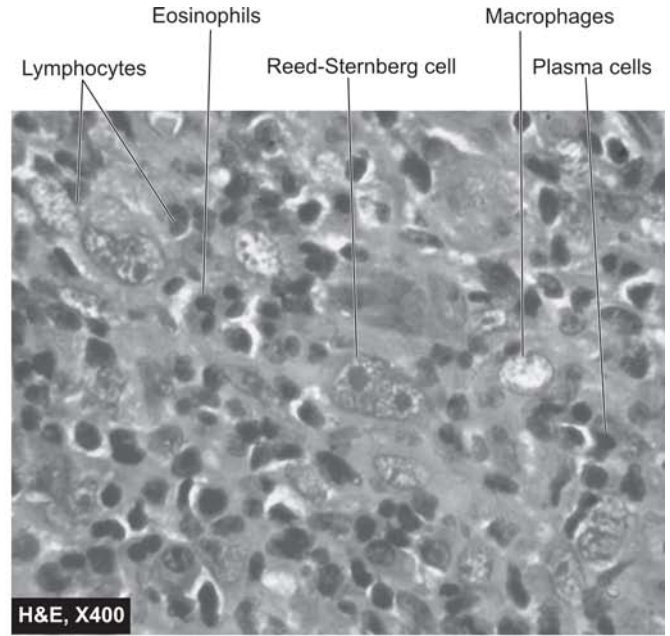
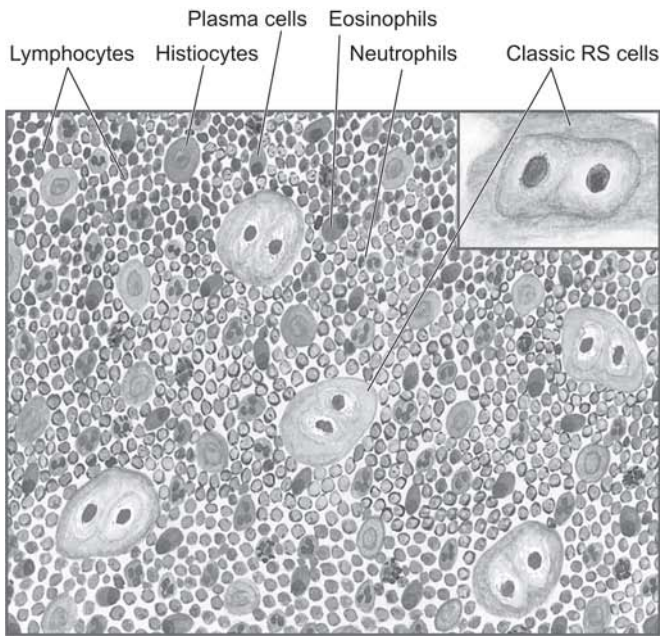


FIGURE 33.4: Hodgkin's disease, mixed cellularity. There is mixed infiltrate of lymphocytes, plasma cells, neutrophils and eosinophils and an RS cell in the middle.

- ii. Some amount of fibrosis and focal areas of necrosis are generally present.
- iii. Typical RS cells are frequent (Figs 33.4).



Male Reproductive System and Prostate

- ◆ Seminoma Testis
- ◆ Nodular Hyperplasia Prostate
- ◆ Adenocarcinoma Prostate

Exercise

34

SEMINOMA TESTIS

Seminoma is the commonest malignant tumour of the testis, constituting 45% of all testicular germ cell tumours and corresponds to dysgerminoma in the female gonad.

G/A The involved testis is enlarged (upto 10 times) but tends to maintain its normal contour since the tumour rarely invades the tunica. Cut section of the affected testis shows homogeneous, grey-white lobulated appearance (Fig. 34.1).

M/E

- Tumour cells:** The seminoma cells generally lie in cords, sheets or columns forming lobules. The tumor cells are typically uniform in size with clear cytoplasm and well-defined cell borders. The nuclei are central,

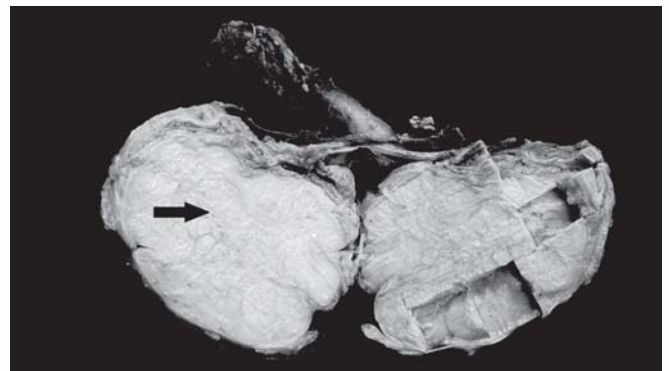


FIGURE 34.1: Seminoma testis. The testis is enlarged (normal weight 20-27 gm) but the testicular contour is maintained. Sectioned surface shows replacement of the entire testis by lobulated, homogeneous, grey-white mass (arrow).

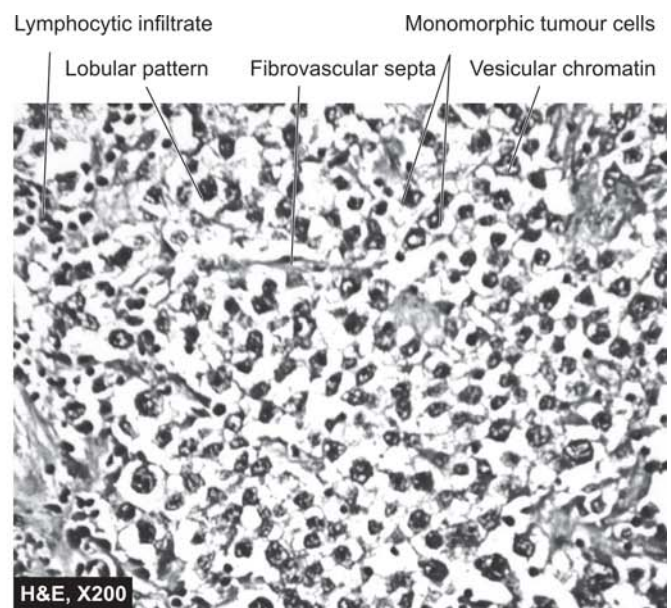
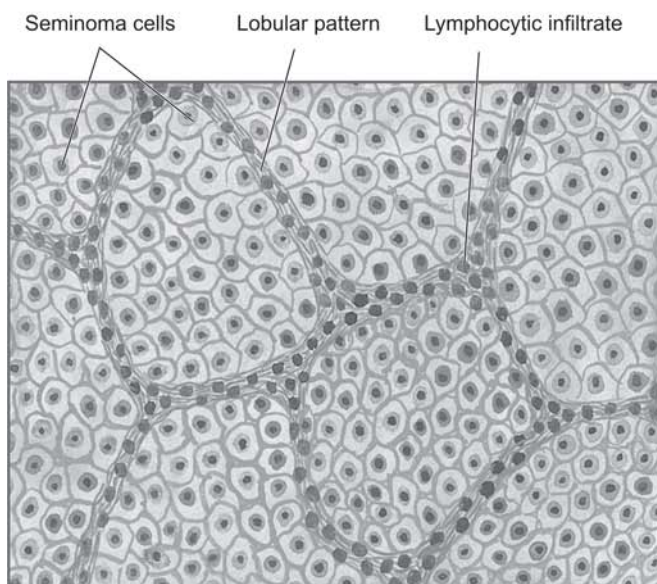


FIGURE 34.2: Seminoma testis. The tumour cells are monomorphic and uniform and forming lobular pattern with lymphocytic infiltrate.

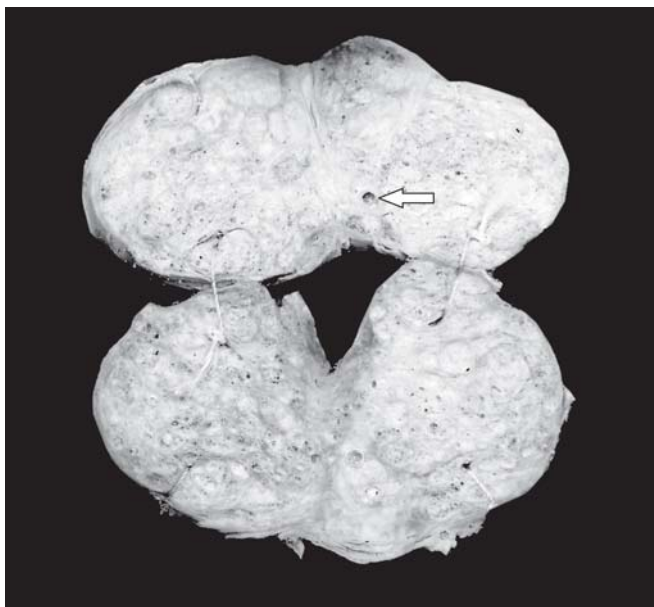
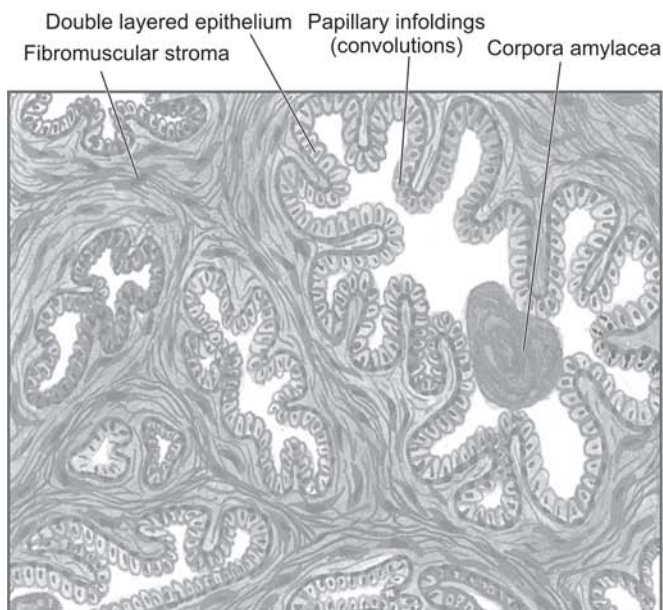


FIGURE 34.3: Nodular hyperplasia of the prostate. The prostate is enlarged (normal weight 20 gm). Sectioned surface shows soft to firm, grey-white, nodularity with microcystic areas (arrow).

large, hyperchromatic and usually contain 1-2 prominent nucleoli.



- ii. *Stroma*: The stroma is delicate fibrous tissue which divides the tumour into lobules. The stroma shows characteristic lymphocytic infiltration (Fig. 34.2).

NODULAR HYPERPLASIA PROSTATE

Non-neoplastic tumour-like enlargement of the prostate is a very common condition in men, frequently above the age of 50 years.

G/A The enlarged prostate is nodular, smooth and firm and weighs 2-4 times its normal weight (normal average weight 20 gm). The appearance on cut section shows nodularity having varying admixture of yellowish pink, soft, honey-combed appearance (glandular hyperplasia) and firm homogeneous appearance (fibromuscular hyperplasia) (Fig. 34.3).

M/E There is hyperplasia of all three tissue elements in varying proportions—glandular, fibrous and muscular (Fig. 34.4):

- i. *Glandular hyperplasia* predominates in most cases and is characterised by exaggerated intra-acinar papillary infoldings with delicate fibrovascular cores. Glands are lined by two layers of epithelium.
- ii. *Fibromuscular hyperplasia* appears as aggregates of spindle cells forming an appearance similar to fibromyoma of the uterus.

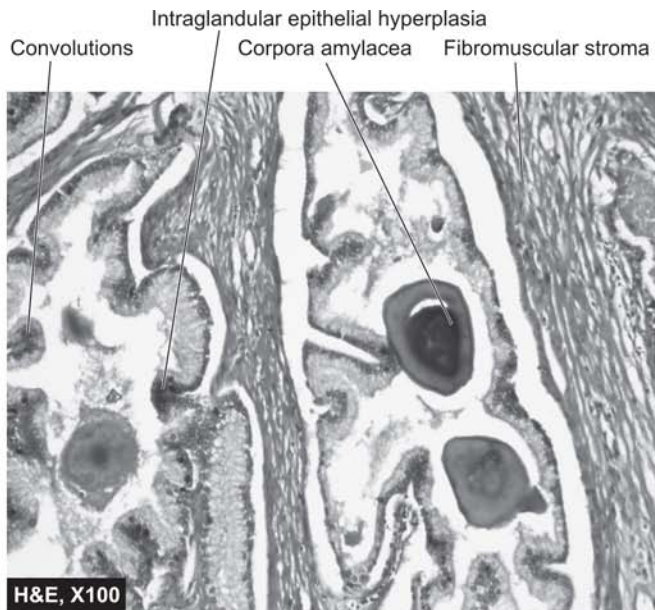


FIGURE 34.4: Nodular hyperplasia prostate. There are intra-acinar papillary infoldings (convolutions) lined by two layers of epithelium with basal polarity of nuclei and fibromuscular stromal hyperplasia.

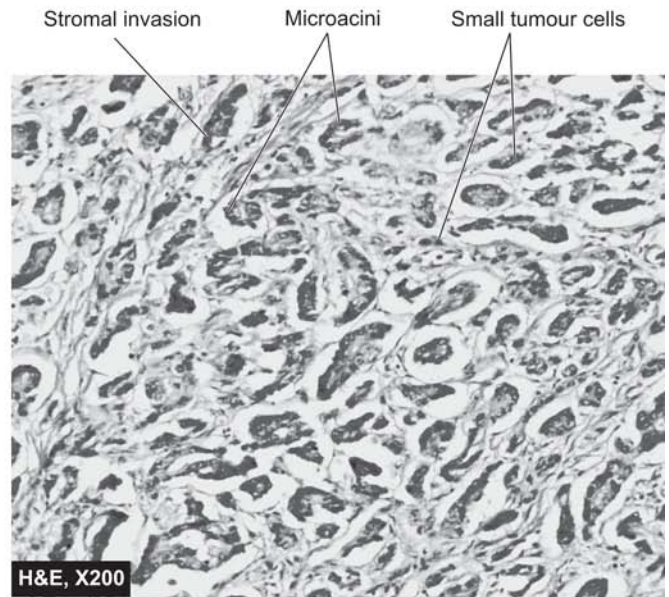
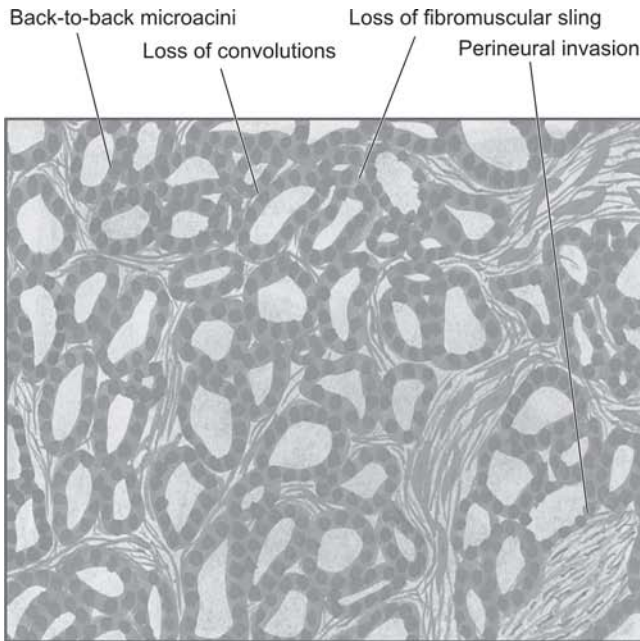


FIGURE 34.5: Adenocarcinoma prostate. The field shows micro-acini of small malignant cells infiltrating the prostatic stroma.

ADENOCARCINOMA PROSTATE

Cancer of the prostate is second most common form of cancer in males, followed in frequency by lung cancer.

G/A The prostate is often enlarged, firm and fibrous. Cut section is homogeneous and contains irregular yellowish areas.

M/E

- i. **Architectural disturbance:** There is loss of intra-acinar papillary infoldings. Instead, the groups of acini are either closely packed in back-to-back arrangement, or are haphazardly distributed.
- ii. **Stroma:** Malignant acini have little or no stroma between them.
- iii. **Gland pattern:** Often the glands are small or medium-sized, lined by a single layer of cuboidal or low columnar cells.
- iv. **Tumour cells:** The individual tumour cells may be clear, dark and eosinophilic type and do not show usual morphologic features of malignancy (Fig. 34.5).
- v. **Invasion:** There is often early and frequent invasion of intraprostatic perineural spaces (Fig. 34.6).

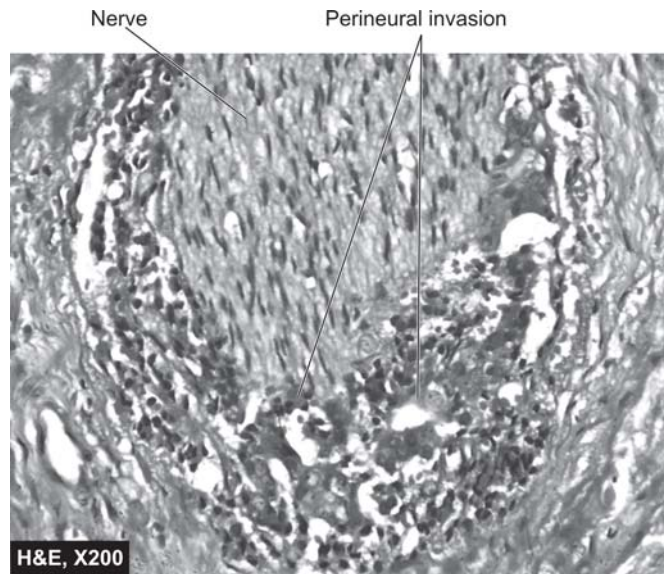


FIGURE 34.6: Adenocarcinoma prostate showing perineural invasion.



Female Reproductive System I

- ◆ Simple (Cystoglandular) Hyperplasia
- ◆ Hydatidiform Mole
- ◆ Invasive Cervical Cancer

Exercise

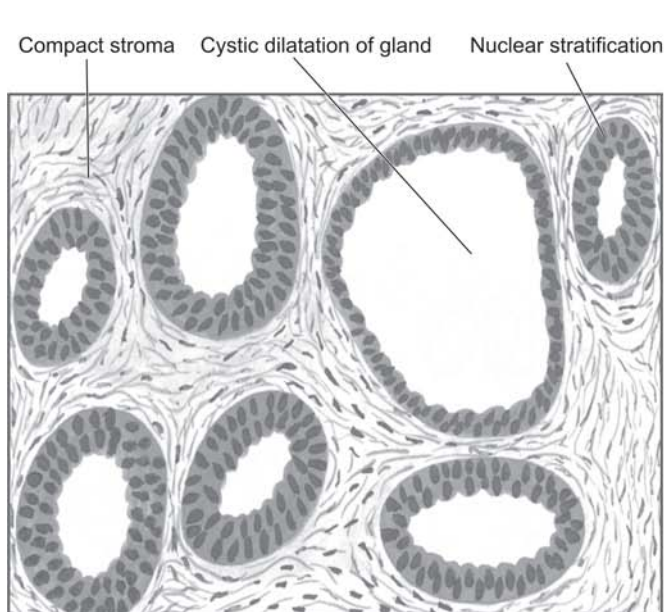
35

SIMPLE (CYSTOGLANDULAR) HYPERPLASIA

Endometrial hyperplasia is a condition characterised by proliferative patterns of glandular and stromal tissues. Currently employed classification of endometrial hyperplasia divides it into 3 types—simple (cystic) hyperplasia, complex hyperplasia without atypia (adenomatous), and complex hyperplasia with atypia (atypical).

M/E The features of *simple hyperplasia* are as under:

- Presence of varying-sized glands, many of which are large and cystically dilated and are lined by atrophic epithelium.
- The stroma is sparsely cellular and oedematous (Fig. 35.1).



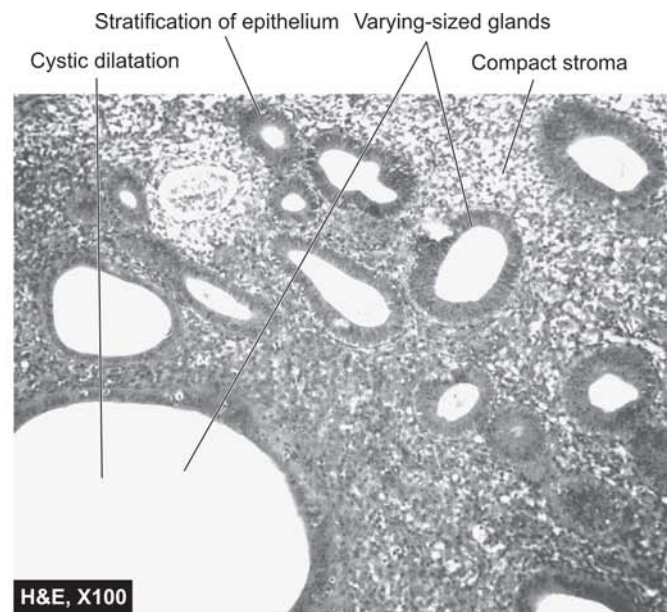
HYDATIDIFORM MOLE

Hydatidiform mole is a benign condition of placental tissue but has potential for developing into choriocarcinoma.

G/A Molar tissue consists of grape-like vesicles upto 3 cm in diameter. The vesicles contain clear watery fluid (Fig. 35.2).

M/E

- Large, round oedematous chorionic villi.
- Hydropic degeneration and decreased vascularity of villous stroma.
- Trophoblastic proliferation seen as masses and sheets of both cytotrophoblast and syncytiotrophoblast (Fig. 35.3).



140 **FIGURE 35.1:** Simple hyperplasia endometrium. Varying-sized glands, some cystically dilated, are lined by atrophied epithelium.



FIGURE 35.2: Hydatidiform mole. The specimen shows numerous, variable-sized, grape-like translucent vesicles containing clear fluid (black arrow). Tan areas of haemorrhage are also seen (white arrow).

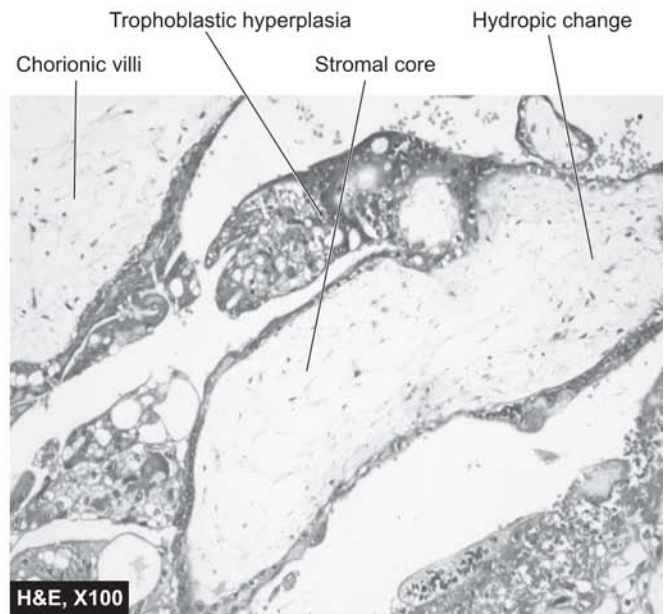
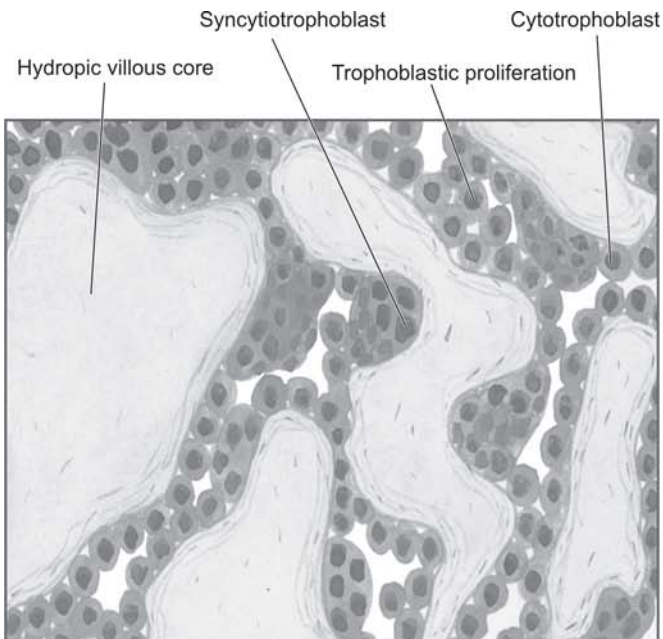


FIGURE 35.3: Hydatidiform mole characterised by hydropic and avascular enlarged villi with trophoblastic proliferation in the form of masses and sheets.

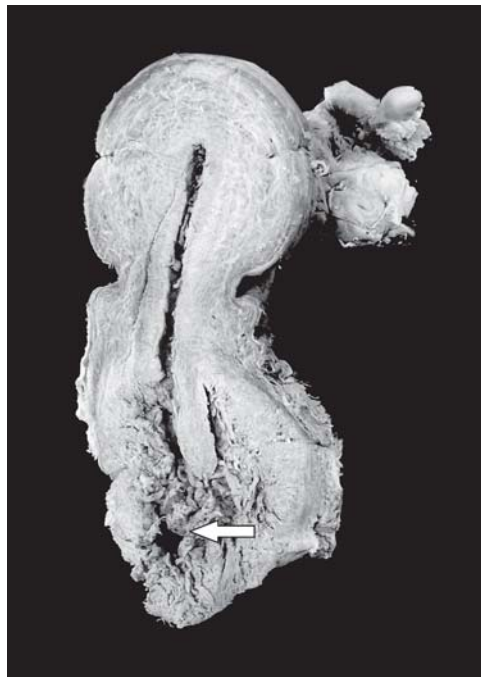


FIGURE 35.4: Invasive carcinoma of the cervix. Sectioned surface shows normal uterine cavity while the cervix is replaced by irregular grey-white friable growth (arrow) extending into cervical canal as well as distally into attached vaginal cuff.

INVASIVE CERVICAL CANCER

Invasive cervical cancer in about 80% of cases is epidermoid (squamous cell) carcinoma.

G/A Invasive cervical carcinoma may present 3 types of patterns: fungating, ulcerating and infiltrating. The fungating or exophytic pattern appears as cauliflower-like growth infiltrating the adjacent vaginal wall (Fig. 35.4).

M/E

- Most commonly, the tumour is moderately-differentiated, non-keratinising, large cell type.
- The tumour cells are seen as masses of anaplastic cells of varying size and have abundant cytoplasm.
- Intervening stroma shows prominent inflammatory cell infiltrate (Fig. 35.5).

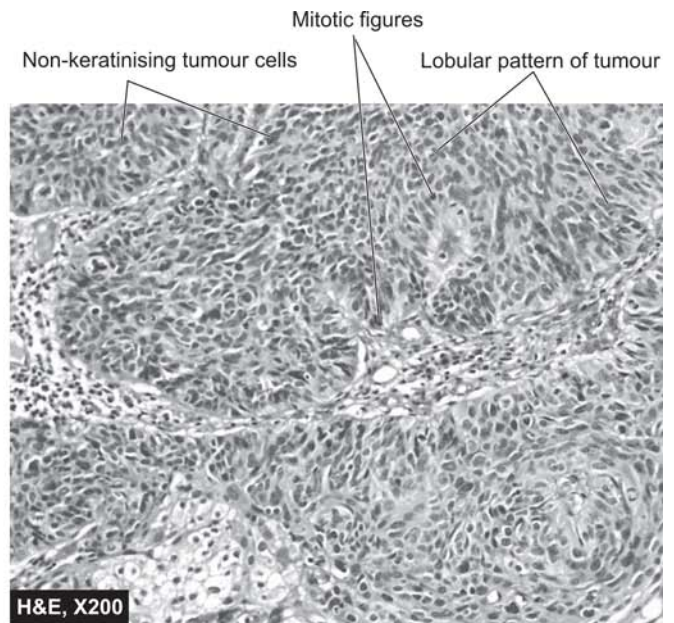
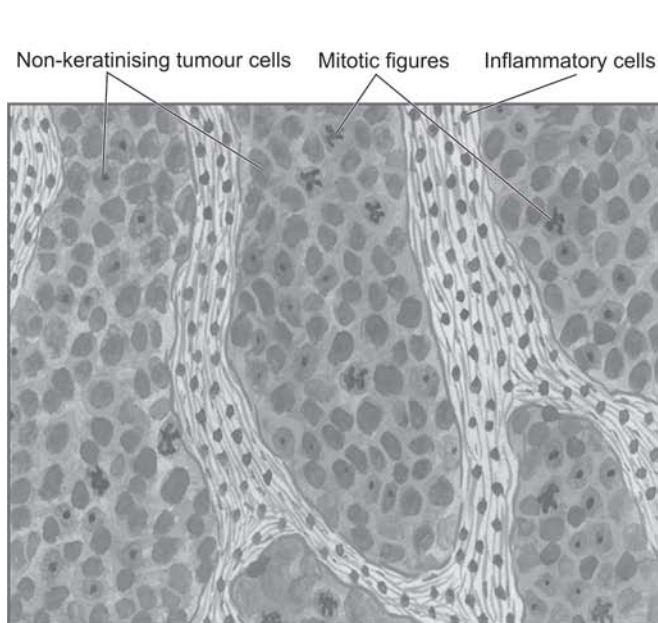


FIGURE 35.5: Squamous cell carcinoma cervix, moderately differentiated large cell type, non-keratinising.

Female Reproductive System II

- ◆ Serous Ovarian Tumours—Cystadenoma and Papillary Serous Cystadenocarcinoma
- ◆ Mucinous Ovarian Tumours—Cystadenoma
- ◆ Benign Cystic Teratoma Ovary

Exercise

36

SEROUS OVARIAN TUMOURS

Serous tumours comprise the largest group constituting about 20% of all ovarian tumours. These tumours arise from the ovarian surface (coelomic) epithelium which differentiates along tubal-type of epithelium.

G/A Serous tumours of benign, borderline and malignant type are large and spherical masses. Cut section of benign tumours is unilocular while larger cysts are multilocular and contain clear watery fluid. Malignant serous tumours have solid areas in the cystic mass and may contain exophytic as well as intracystic papillary projections (Fig. 36.1).

M/E Features of benign and malignant serous tumours are as follows:

Serous Cystadenoma Ovary

- The cyst is lined by properly-oriented low columnar epithelium.



FIGURE 36.1: Papillary serous cystadenoma of the ovary. Cut surface shows a large unilocular cyst containing numerous papillary structures projecting into it (arrow).

- The lining cells may be ciliated and resemble tubal epithelium (Fig. 36.2).

Papillary Serous Cystadenocarcinoma Ovary

- Lining of the cyst is by multilayered malignant cells having features such as loss of polarity, presence of solid sheets of anaplastic epithelial cells.
- There is definite evidence of stromal invasion by malignant cells.
- Papillae formations are more frequent in malignant variety and may be associated with psammoma bodies (Fig. 36.3).

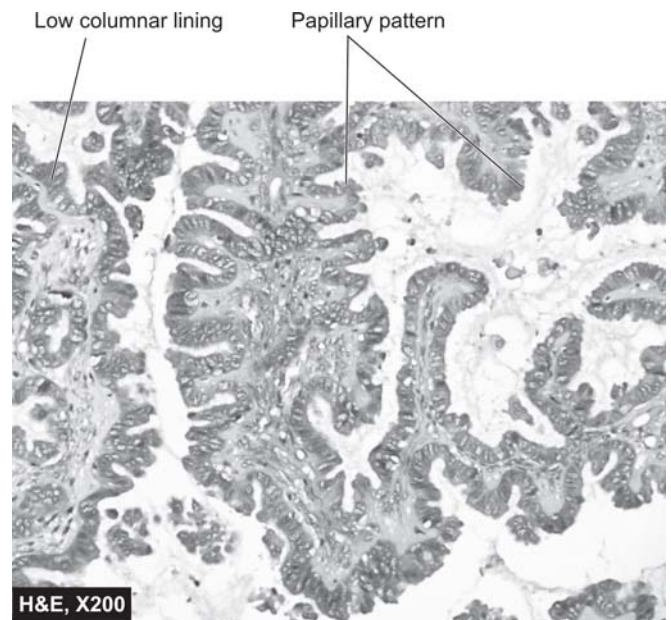


FIGURE 36.2: Papillary serous cystadenoma of the ovary. Microscopic features include single layer of low columnar, at places ciliated, epithelium lining with pronounced papillary pattern.

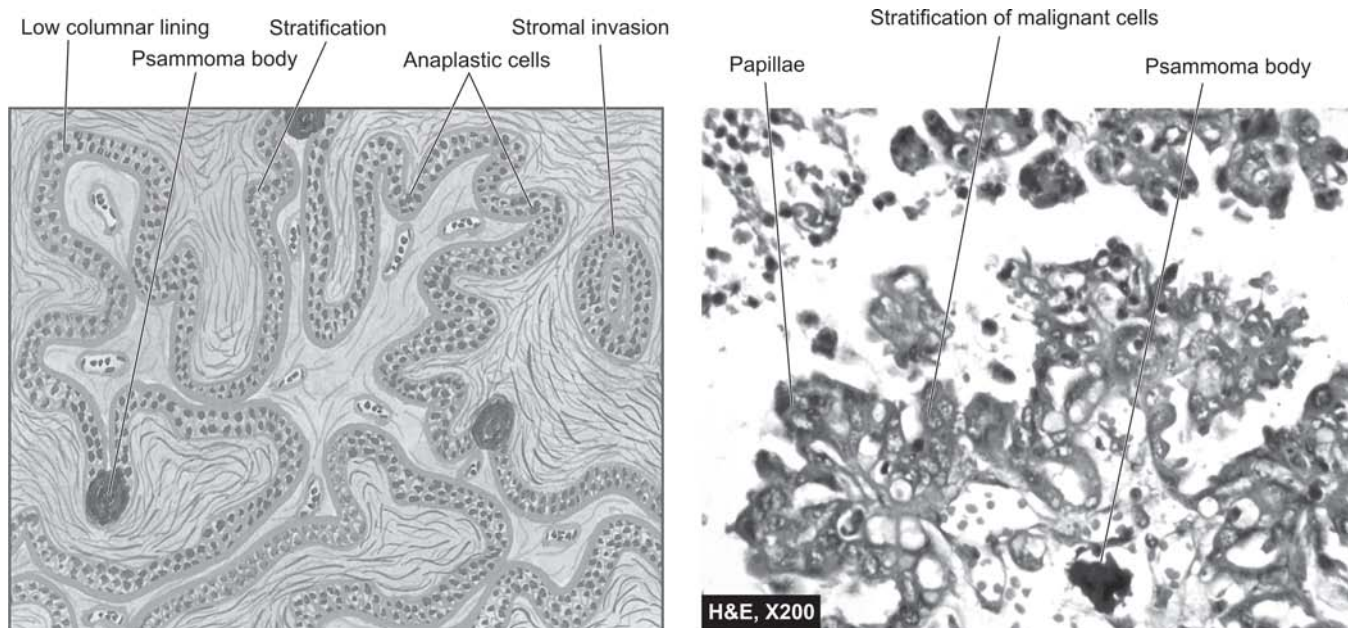


FIGURE 36.3: Papillary serous cystadenocarcinoma of the ovary.

MUCINOUS OVARIAN TUMOURS

Mucinous tumours are more commonly unilateral than serous tumours. These tumours arise from coelomic epithelium that differentiates along endocervical type or intestinal type of mucosa. Like serous ovarian tumours, mucinous tumours too may be benign, borderline and malignant. However, bilateral mucinous cystadeno-

carcinoma of the ovary is rare and is frequently due to metastasis.

Mucinous Cystadenoma Ovary

G/A Mucinous tumours are larger than serous type. They are smooth-surfaced cysts with characteristic multiloculations containing thick and viscid gelatinous

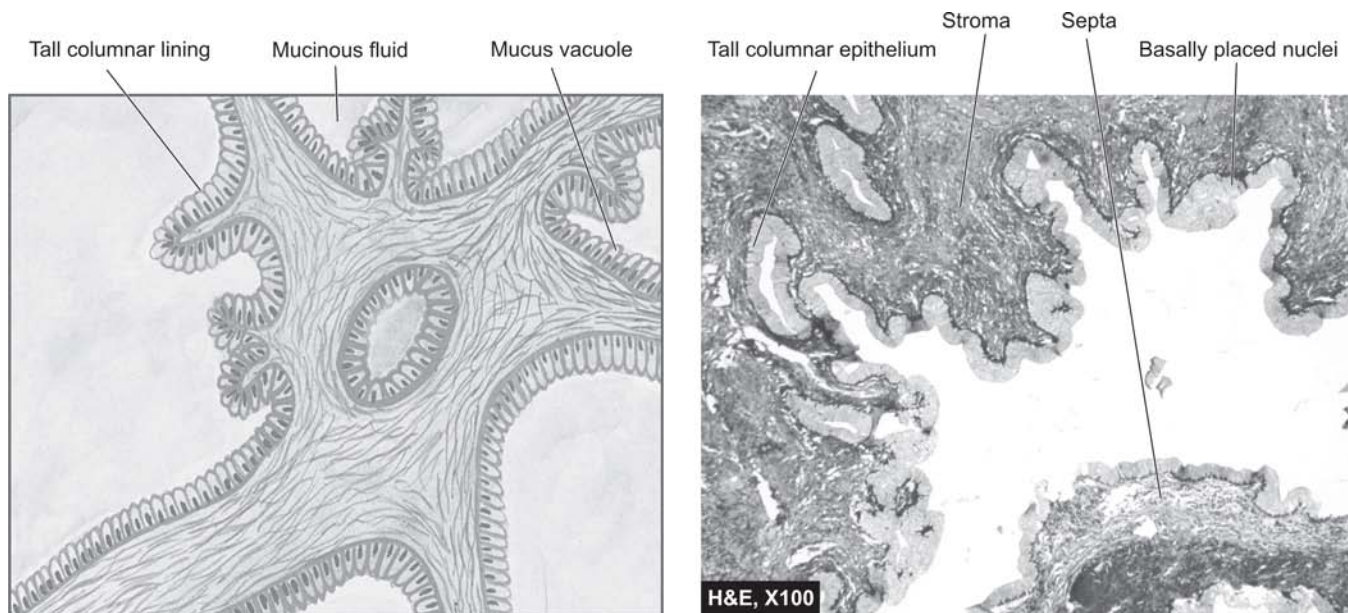


FIGURE 36.4: Mucinous cystadenoma of the ovary. The cyst wall and the septa are lined by a single layer of tall columnar mucin-secreting epithelium with basally-placed nuclei and large apical mucinous vacuoles.

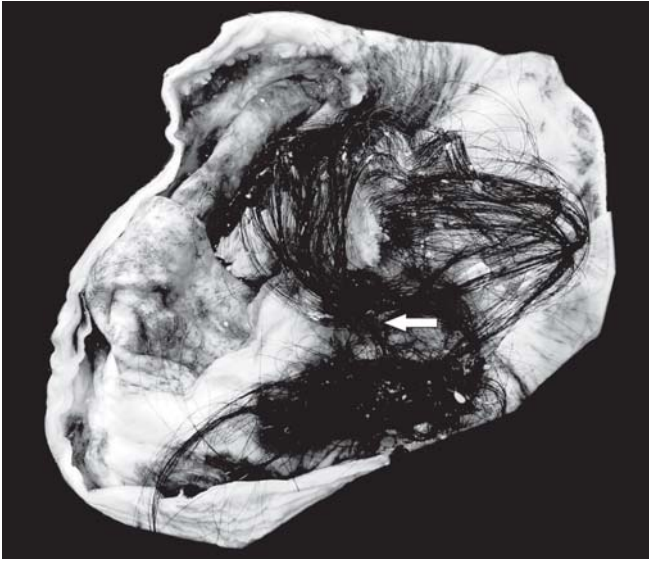
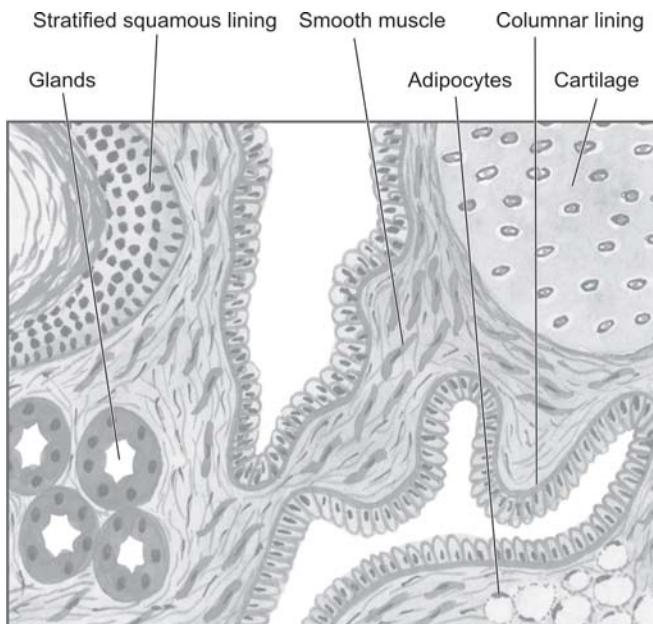


FIGURE 36.5: Benign cystic teratoma (dermoid cyst) of the ovary. Cut surface shows a large unilocular cyst containing hair, pultaceous material and bony tissue.

fluid. Benign tumours have thin wall and septa which are translucent while malignant variety has thickened areas.

M/E The features of *mucinous cystadenoma* are as follows (Fig. 36.4):

- The cyst is lined by a single layer of cells having basal nuclei and apical mucinous vacuoles.
- There is no invasion or papillae formation.



BENIGN CYSTIC TERATOMA OVARY

Benign cystic teratoma or dermoid cyst of the ovary is more frequent in young women in their active reproductive life. Teratoma is a tumour composed of tissue derived from three germ cell layers—ectoderm, mesoderm and endoderm.

G/A Benign cystic teratoma is characteristically a unilocular cyst, 10-15 cm in diameter. On sectioning, the cyst is filled with paste-like sebaceous secretions and desquamated keratin admixed with masses of hair. The cyst wall is thin and opaque grey-white. Quite often, the cyst wall shows a solid prominence where tissue elements such as tooth, bone, cartilage and other odd tissues are present (Fig. 36.5).

M/E

- Viewing a benign cystic teratoma in different microscopic fields reveals a variety of mature differentiated tissues, producing kaleidoscopic appearance.
- Ectodermal derivatives are most prominent. The lining of the cyst wall is by stratified squamous epithelium and its adnexal structures such as sebaceous glands, sweat glands and hair follicles.
- Tissues of mesodermal and endodermal origin are commonly present and include bronchus, intestinal epithelium, cartilage, bone, smooth muscle, neural tissue, salivary gland, retina, pancreas and thyroid tissue (Fig. 36.6).

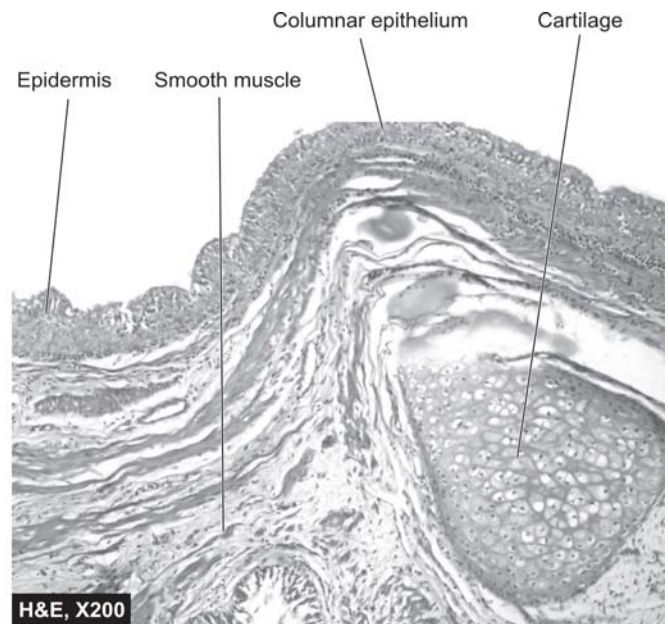


FIGURE 36.6: Benign cystic teratoma ovary. The field shows epidermis-lined cyst, islands of cartilage and some adipose tissue.



Breast

- ◆ Fibroadenoma
- ◆ Simple Fibrocystic Change
- ◆ Infiltrating Duct Carcinoma-NOS

Exercise

37

FIBROADENOMA

Fibroadenoma is a benign tumour of fibrous and epithelial elements of the breast. It is the most common benign tumour of the breast in reproductive life.

G/A Typically fibroadenoma is a small (2-4 cm diameter), solitary, well-encapsulated, spherical or discoid mass. The cut surface is firm, grey-white, slightly myxoid and may show slit-like spaces.

M/E The arrangement between fibrous overgrowth and ducts may produce 2 types of patterns: intracanalicular and pericanalicular (Fig. 37.1):

- Intracanalicular pattern* is one in which the stroma compresses the ducts so that they are reduced to

slit-like clefts lined by ductal epithelium and may appear as cords of epithelial elements surrounding masses of fibrous tissue.

- Pericanalicular pattern* is characterised by encircling masses of fibrous tissue around the patent or dilated ducts.

SIMPLE FIBROCYSTIC CHANGE

Fibrocystic change is characterised by formation of cysts of varying size and increase in fibrous stroma.

G/A The cysts are usually multifocal and bilateral, varying in size from microcysts to 5-6 cm in diameter. On sectioning, the cysts contain thin serous to haemorrhagic fluid.

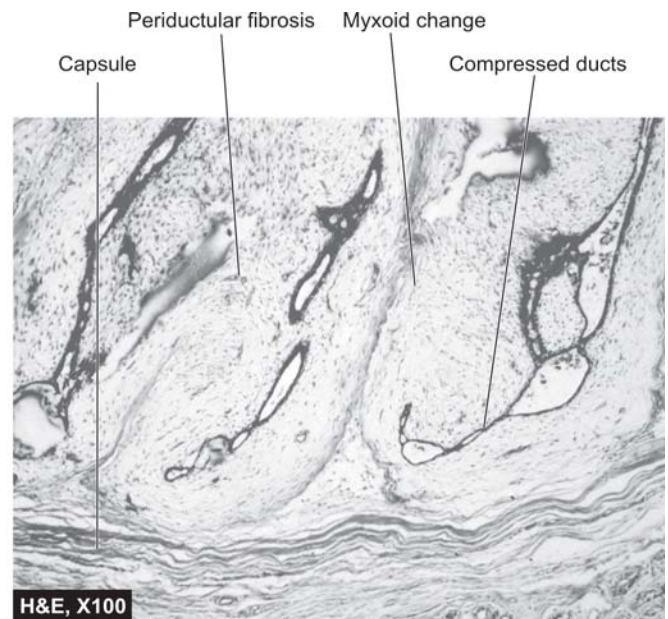
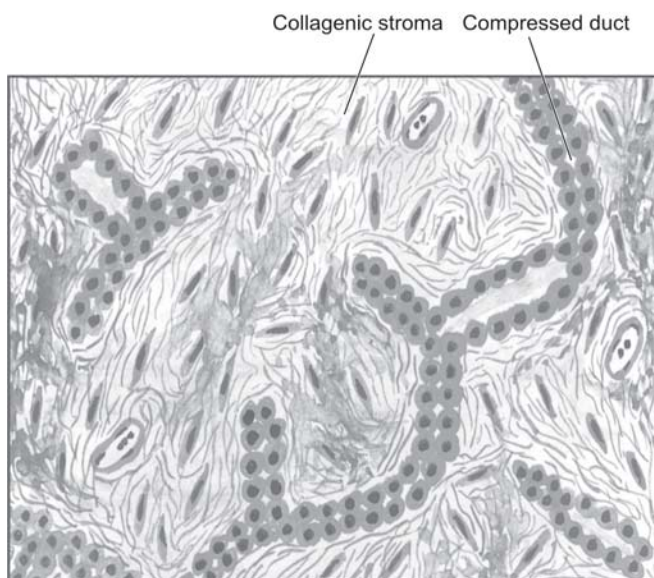


FIGURE 37.1: Fibroadenoma breast. The tumour is encapsulated and composed of intracanalicular growth pattern of stroma and compressed ducts.

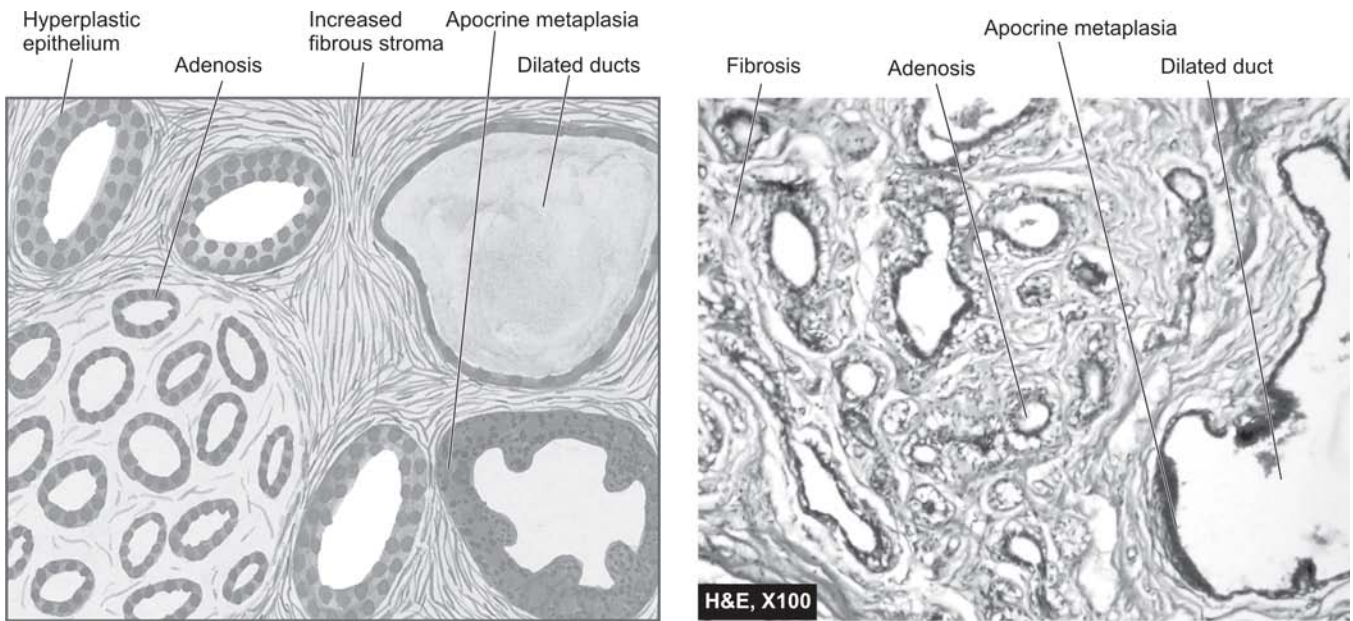


FIGURE 37.2: Fibrocystic change breast. Simple fibrocystic change showing cystic dilatation of ducts and increase in fibrous stroma.

M/E

- The cyst lining is often by flattened or atrophic epithelium.
- Apocrine change in the lining of the cyst is frequently present.
- There may be epithelial hyperplasia forming tiny intracystic papillary projections.
- There is increased fibrous stroma surrounding the cyst and varying degree of lymphocytic infiltrate (Fig. 37.2).

INFILTRATING DUCT CARCINOMA-NOS

Infiltrating duct carcinoma–NOS (not otherwise specified) is the classic breast cancer and is the most common histologic pattern accounting for 70% cases of breast cancer.

G/A The tumour is irregular, 1-5 cm in diameter, hard, cartilage-like mass that cuts with a grating sound. Sectioned surface of the tumour is grey-white to yellowish with chalky streaks and often extends irregularly into the surrounding fat (Fig. 37.3).

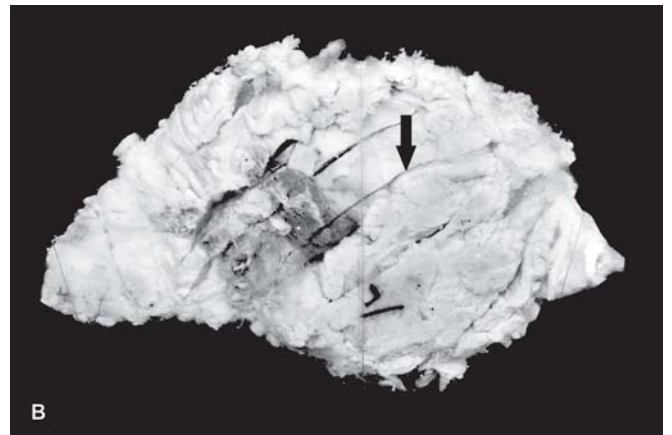
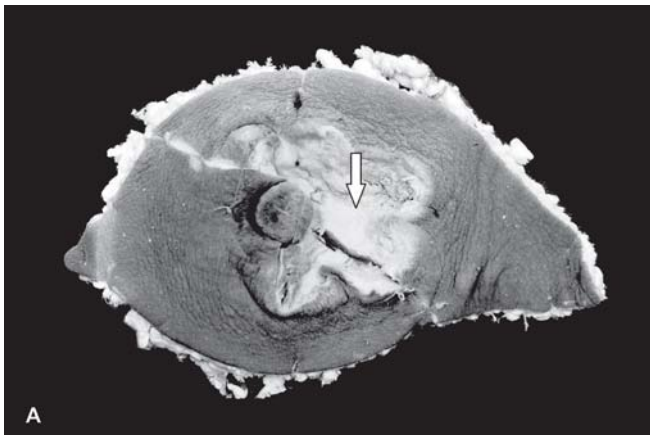


FIGURE 37.3: Infiltrating duct carcinoma-NOS. **A**, Specimen of the breast shows a tumour extending upto nipple and areola (white arrow). **B**, Cut surface shows a grey-white firm tumour (black arrow) extending irregularly into adjacent breast parenchyma.

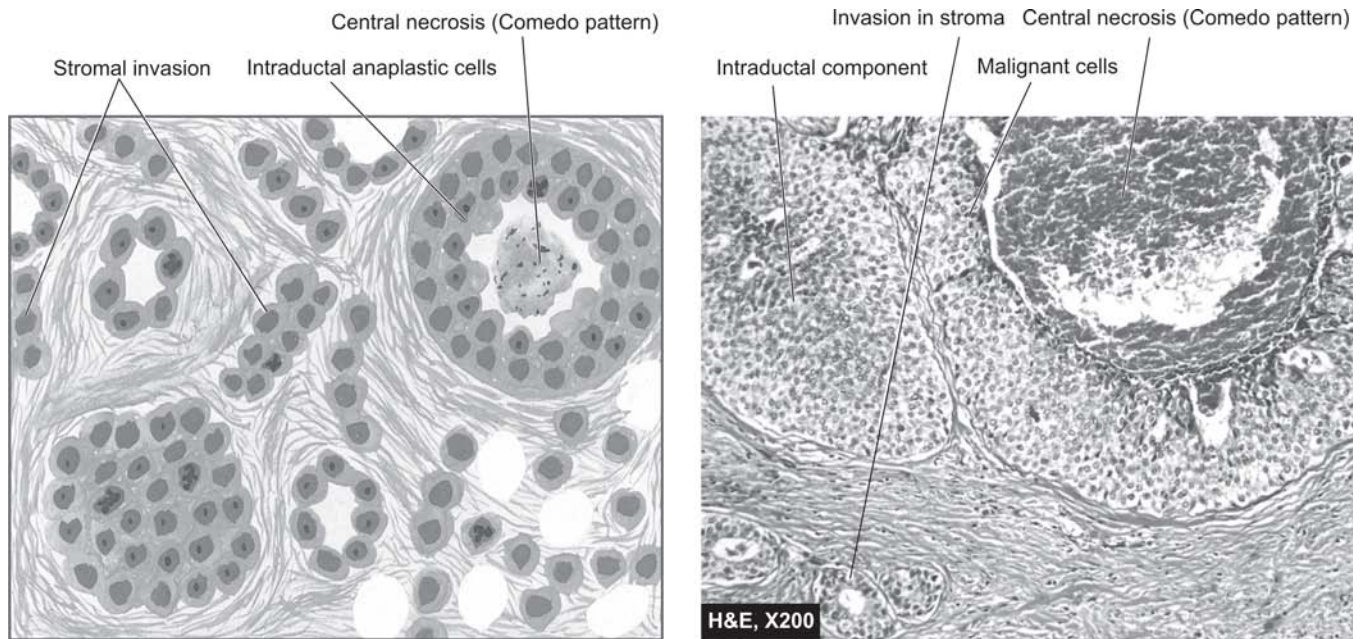


FIGURE 37.4: Infiltrating duct carcinoma breast, NOS. Anaplastic tumour cells proliferating in the ducts with central areas of necrosis termed comedo pattern.

M/E

- i. Anaplastic tumour cells forming solid nests, cords, poorly-formed glandular structures and some intraductal foci.
- ii. Infiltration by these patterns of tumour cells into diffuse fibrous stroma and fat.
- iii. Invasion by the tumour cells into perivascular and perineural space, besides lymphatic and vascular emboli (Fig. 37.4).



Thyroid

- ◆ Follicular Adenoma
- ◆ Nodular Goitre
- ◆ Hashimoto's Thyroiditis
- ◆ Papillary Carcinoma

Exercise

38

FOLLICULAR ADENOMA

Follicular adenoma is the most common benign thyroid tumour seen more frequently in adult women.

G/A Follicular adenoma is characterised by *4 cardinal features*: solitary nodule, complete encapsulation, clearly distinct architecture inside and outside the capsule, and compressed thyroid parenchyma outside the capsule. Usually, the adenoma is small (upto 3 cm in diameter) and spherical. On cut section the tumour is grey-white to red-brown, and less colloidal than the surrounding thyroid parenchyma (Fig. 38.1). Less commonly, secondary changes such as fibrous scarring, focal calcification, haemorrhages and cyst formation are seen.

M/E

- The tumour shows complete fibrous encapsulation.
- The tumour cells are benign follicular epithelial cells forming follicles of varying size.
- Variety of patterns of growth may be seen, most commonly being microfollicular (foetal) pattern characterised by small follicles containing little or no colloid (Fig. 38.2).

NODULAR GOITRE

Nodular goitre is regarded as end-stage of long-standing simple goitre. It is characterised by tumour-like enlargement of the thyroid gland and characteristic nodularity.

G/A The thyroid shows asymmetric and extreme enlargement weighing 100-500 gm (normal weight 15-40 gm). The *5 cardinal gross features* are: nodularity with poor encapsulation, fibrous scarring, haemorrhages,

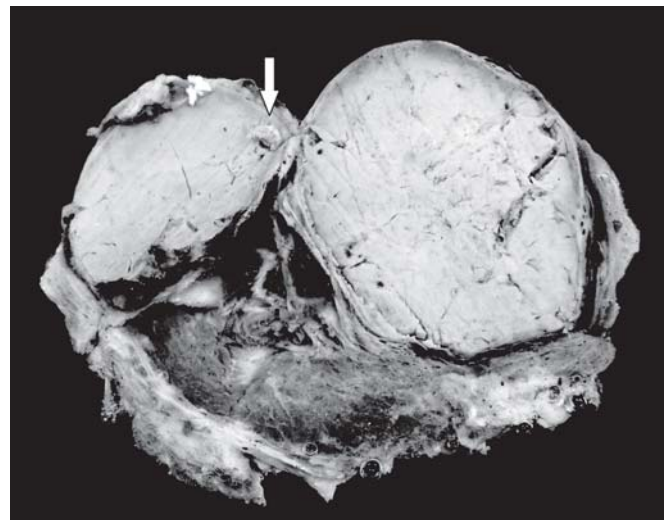


FIGURE 38.1: Follicular adenoma. The thyroid gland is identified by light brown colloid. Sectioned surface shows a solitary nodule having complete encapsulation (arrow). The nodule is grey-white and is distinct from the adjoining thyroid parenchyma.

focal calcification, and cystic degeneration. Cut surface of the gland shows multinodularity (Fig. 38.3)

M/E

- Partial or incomplete encapsulation.
- The follicles of varying size from small to large and lined by flat to high epithelium.
- Areas of haemorrhages, haemosiderin-laden macrophages and cholesterol crystals.
- Fibrous scarring and calcification in the nodules.
- Cystic degeneration (Fig. 38.4).

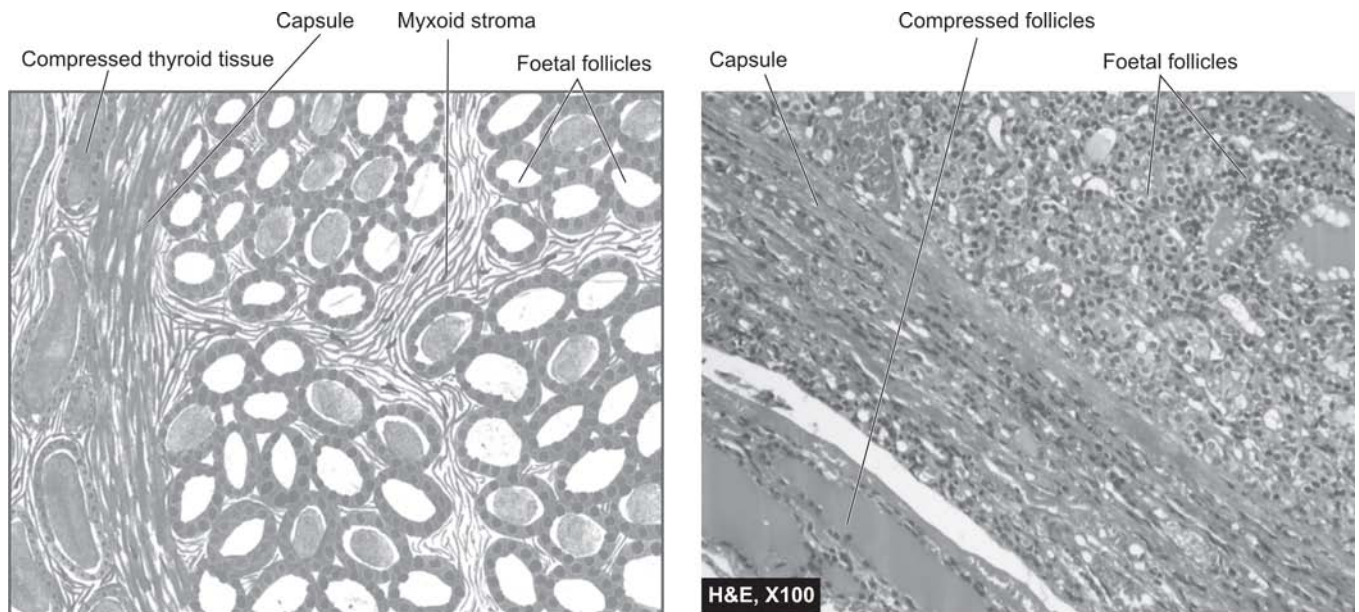


FIGURE 38.2: Follicular adenoma thyroid, foetal (microfollicular) type. The tumour is well-encapsulated with compression of surrounding thyroid parenchyma. The tumour consists of small follicles lined by cuboidal epithelium and contain little or no colloid and separated by abundant loose stroma.

HASHIMOTO'S THYROIDITIS

Hashimoto's thyroiditis occurs more frequently between the age of 30 and 50 years and shows about 10 times



FIGURE 38.3: Nodular goitre. The thyroid gland is enlarged and nodular (normal weight 15-40 gm). Cut surface shows multiple nodules separated from each other by incomplete fibrous septa. Areas of haemorrhage (arrow) and cystic change are also seen.

higher preponderance in females. Pathologically, two types of Hashimoto's thyroiditis are seen: classic and fibrosing.

G/A The classic form is characterised by diffuse, symmetric, firm and rubbery enlargement of the thyroid. Sectioned surface of the gland is fleshy with accentuation of normal lobulations. The fibrosing variant has a firm enlarged gland with compression of the surrounding tissues.

M/E

- Extensive infiltration of the gland by lymphocytes, plasma cells, immunoblasts and macrophages with formation of lymphoid follicles having germinal centres.
- Decreased number of thyroid follicles, atrophic follicles and often devoid of colloid.
- Follicular epithelial cells are transformed into their degenerated state termed Hurthle's cells (oxyphil cells or oncocytes). These cells have abundant eosinophilic and granular cytoplasm due to numerous mitochondria.
- Variable amount of fibrous replacement of thyroid parenchyma (Fig. 38.5).

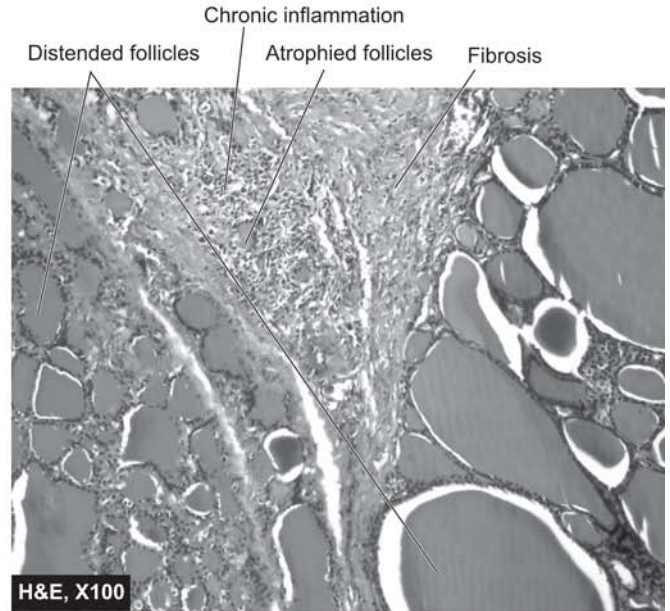
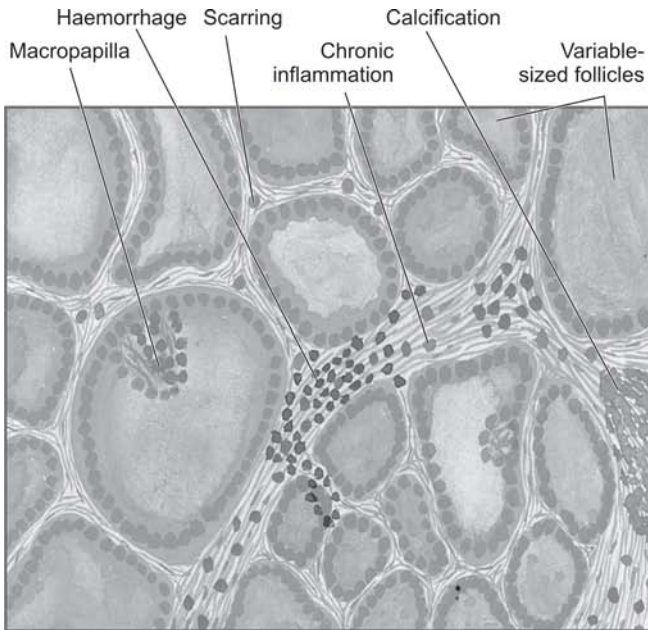


FIGURE 38.4: Nodular goitre. The predominant histologic features are: nodularity, extensive scarring with foci of calcification, areas of haemorrhages and variable-sized follicles lined by flat to high epithelium and containing abundant colloid.

PAPILLARY CARCINOMA

Papillary carcinoma is the most common type of thyroid cancer comprising about 60% of cases, seen more frequently in females.

G/A Papillary carcinoma may range from microscopic foci to nodules up to 10 cm in diameter and is generally poorly delineated. Cut surface of the tumour is greyish-white, hard to scar-like (Fig. 38.6).

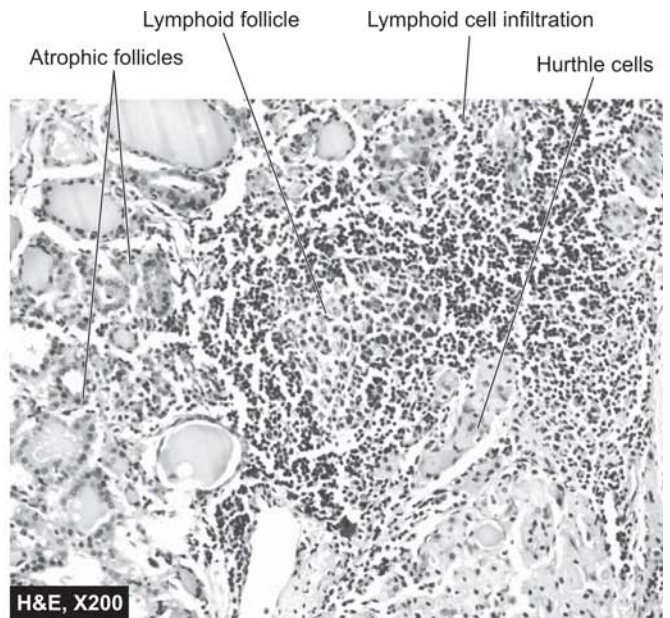
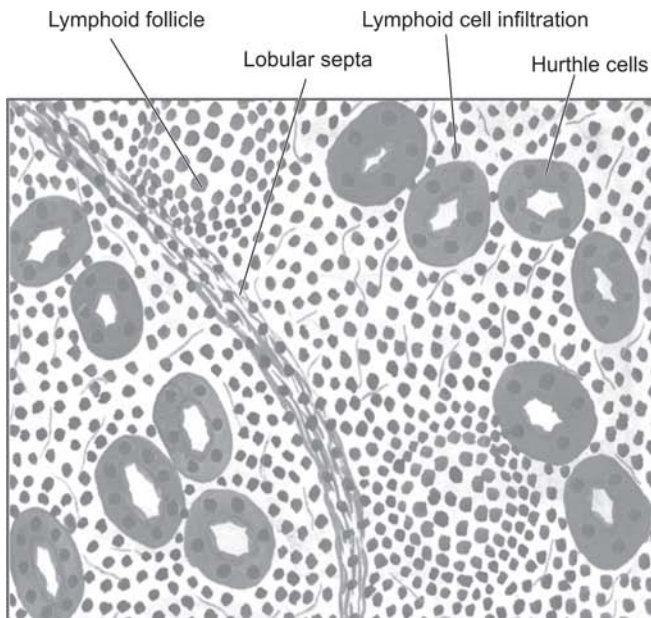


FIGURE 38.5: Hashimoto's thyroiditis. There are lymphoid follicles with germinal centres, atrophic thyroid follicles and a few Hurthle's cells.

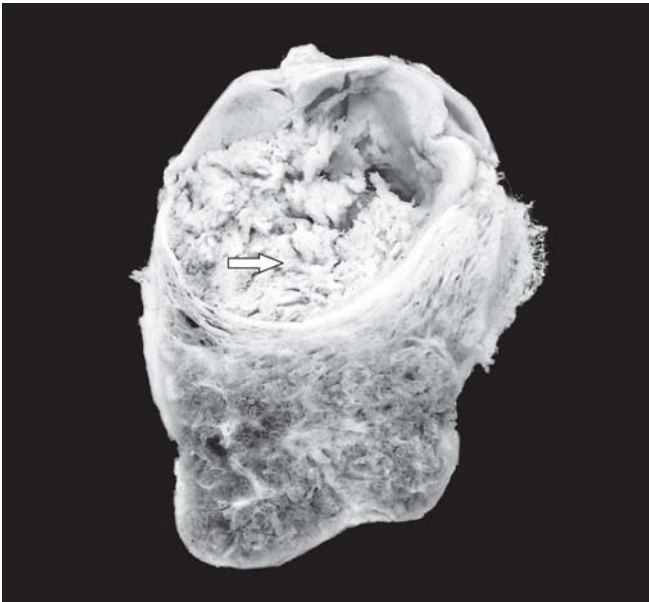


FIGURE 38.6: Papillary carcinoma of the thyroid. The thyroid gland is enlarged and nodular (normal weight 15-40 gm). Cut surface shows a single nodule separated from the rest of thyroid parenchyma by incomplete fibrous septa. The nodule on cut section shows grey-white soft papillary tumour (arrow).

M/E

- i. **Papillary pattern:** Papillae composed of fibrovascular stalk and covered by single layer of tumour cells.
- ii. **Tumour cells:** The tumour cells have overlapping pale nuclei (ground glass appearance) and clear or oxyphil cytoplasm.
- iii. **Invasion:** The tumour cells invade the capsule, and intrathyroid lymphatics.
- iv. **Psammoma bodies:** Almost half of papillary carcinomas show typical, small concentric calcified spherules in the stroma (Fig. 38.7).

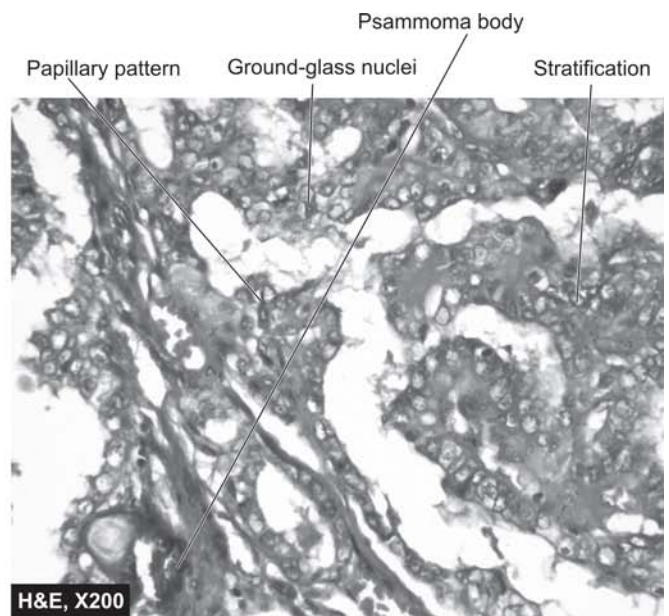
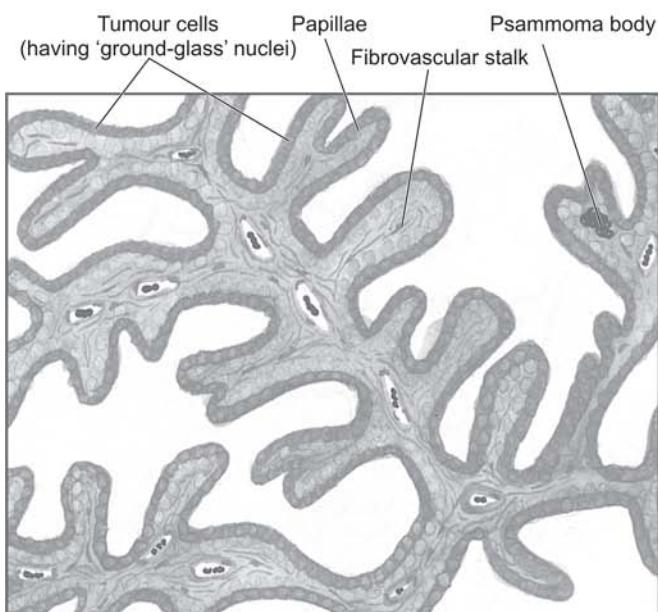


FIGURE 38.7: Papillary carcinoma of the thyroid. Microscopy shows branching papillae having fibrovascular stalk covered by a single layer of cuboidal cells having ground glass nuclei. Colloid-filled follicles and solid sheets of tumour cells are also present.



Bones and Joints I

- ◆ Chronic Osteomyelitis
- ◆ Tuberculous Osteomyelitis
- ◆ Osteochondroma
- ◆ Osteoclastoma

Exercise

39

CHRONIC OSTEOMYELITIS

Chronic osteomyelitis is pyogenic or suppurative infection of the bone. Infection may occur by haematogenous route or by direct penetration or extension of bacteria.

G/A Residual and fragmented necrotic bone is seen as sequestrum while the surrounding reactive new bone is involucrum.

M/E

- Marked infiltrate of polymorphs and chronic inflammatory cells (lymphocytes, plasma cells and macrophages).
- Chips of necrotic bone are seen in the pus.

- Repair reaction consisting of osteoclasts, fibroblastic proliferation and new bone formation (Fig. 39.1).

TUBERCULOUS OSTEOMYELITIS

Infection of the bone marrow by tubercle bacilli is a common condition in the underdeveloped and developing countries of the world. It occurs secondary to pulmonary tuberculosis by haematogenous spread. Tuberculosis of the vertebral bodies or Pott's disease is one of the important forms of tuberculous osteomyelitis.

G/A The appearance of necrotic bone (sequestrum) and surrounding new bone (involucrum) is similar to

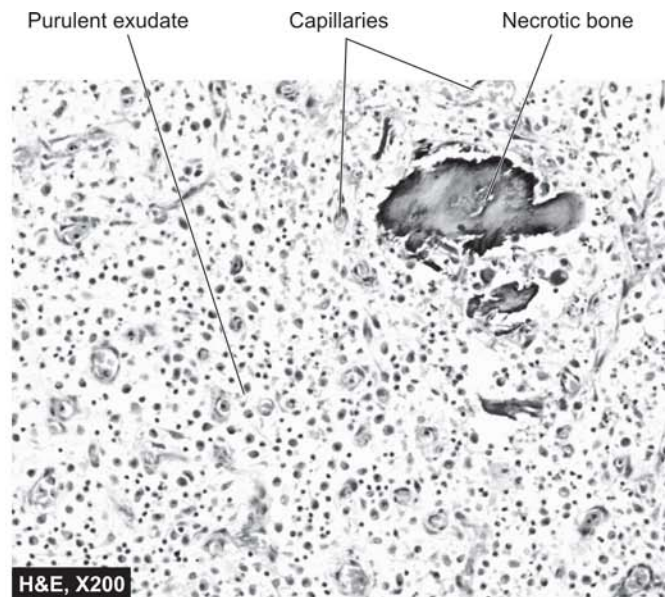
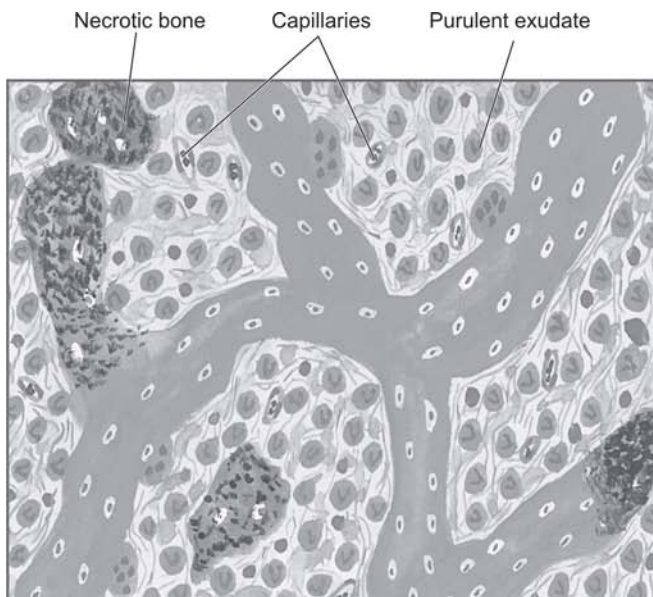


FIGURE 39.1: Chronic suppurative osteomyelitis. Histologic appearance shows necrotic bone and extensive purulent inflammatory exudates and inflammatory granulation tissue.

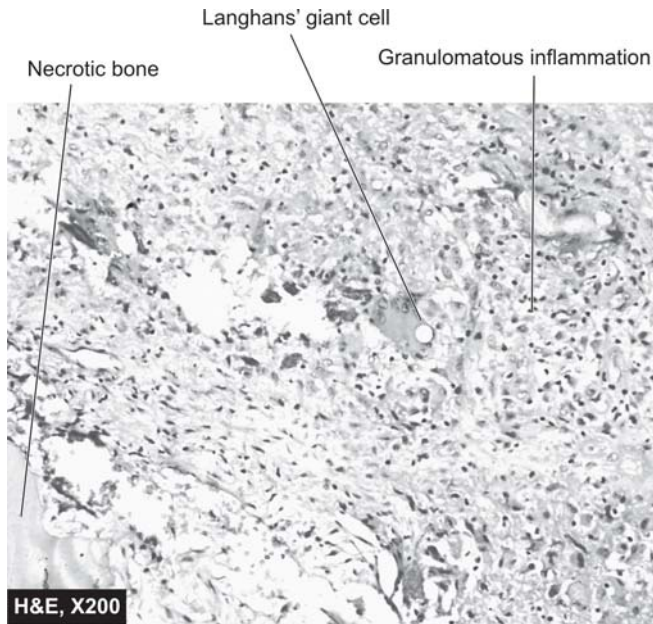


FIGURE 39.2: Tuberculous osteomyelitis consisting of epithelioid cell granulomas with minute areas of caseation necrosis and necrotic bone.

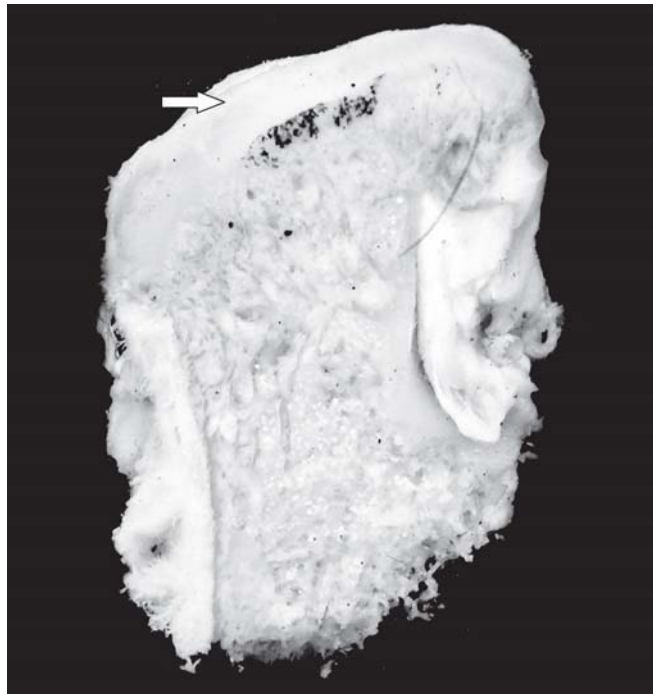


FIGURE 39.3: Osteochondromas. The amputated head of the long bone shows multiple mushroom-shaped elevated nodular areas. These nodules have cartilaginous caps (arrow) and inner osseous tissue.

chronic osteomyelitis but foci of caseation necrosis may at times be evident.

M/E

- Presence of epithelioid cell granulomas with Langhans' and foreign body giant cells.
- Admixture of acute and chronic inflammatory cells.
- Chips of necrotic bone.
- Formation of granulation tissue (Fig. 39.2).

OSTEOCHONDROMA

Osteochondromas or osteocartilaginous exostoses are common benign cartilage-forming lesions. They arise from metaphysis of long bones as exophytic lesions, most commonly lower femur or upper tibia (i.e. around knee joint) and upper humerus.

G/A

Osteochondromas have a broad or narrow base (i.e. either sessile or pedunculated) which is continuous with the cortex. They protrude exophytically as mushroom-shaped, cartilage-capped lesions enclosing well-formed cortical bone and marrow (Fig. 39.3).

M/E

- The outer part consists of mature cartilage resembling epiphyseal cartilage.

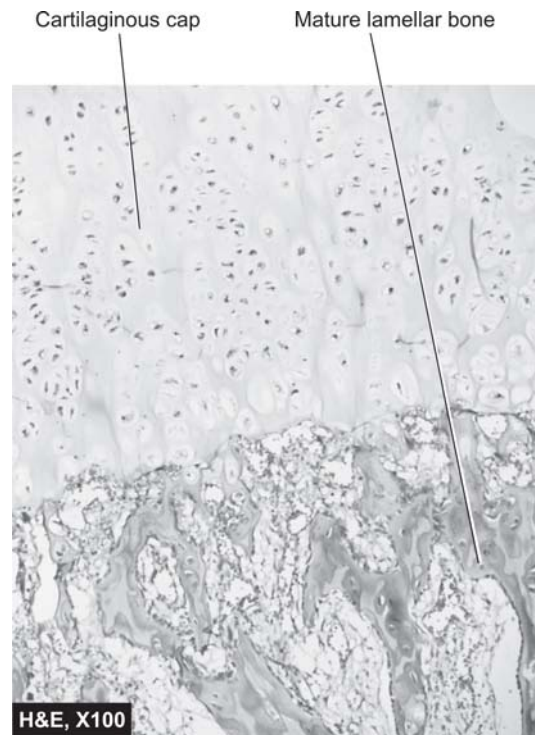


FIGURE 39.4: Osteochondroma. Microscopy reveals mature cartilage covering lamellar bone containing marrow spaces.

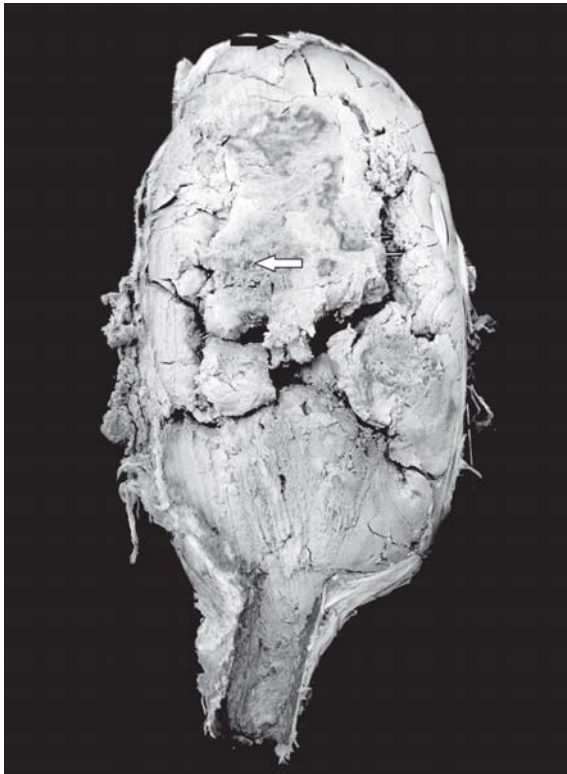


FIGURE 39.5: Giant cell tumour (osteoclastoma). The end of the long bone is expanded in the region of epiphysis (black arrow). Sectioned surface shows circumscribed, dark tan and necrotic tumour (white arrow).

- ii. The inner part is composed of mature lamellar bone enclosing marrow spaces (Fig. 39.4).

OSTEOCLASTOMA

Osteoclastoma or giant cell tumour is a tumour arising in the epiphysis of the long bones, more common in the age range of 20 to 40 years. Common sites of involvement are: lower end of femur and upper end of tibia (i.e. about the knee), lower end of radius, and upper end of fibula.

G/A Giant cell tumour is eccentrically located in the epiphyseal end of a long bone which is expanded. The tumour is well-circumscribed, dark-tan and covered by thin shell of subperiosteal bone. Cut surface of the tumour is characteristically haemorrhagic, necrotic and honey-combed (Fig. 39.5).

M/E

- i. Large number of osteoclast-like giant cells which are regularly scattered throughout the stroma.
- ii. Giant cells may contain as many as 100 benign nuclei and are similar to normal osteoclasts.
- iii. Stromal cells are mononuclear cells and are the real tumour cells and determine the behaviour of the tumour. They are uniform, plump, spindle-shaped or round to oval but may have varying degree of atypia and mitosis (Fig. 39.6).

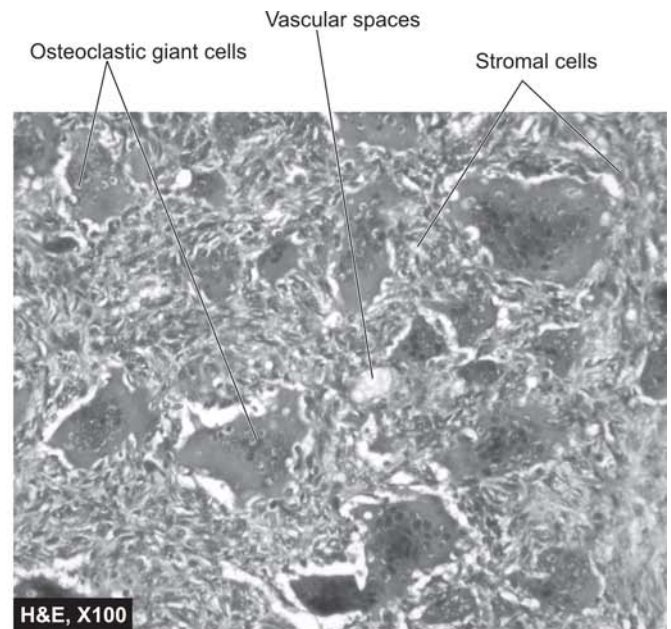
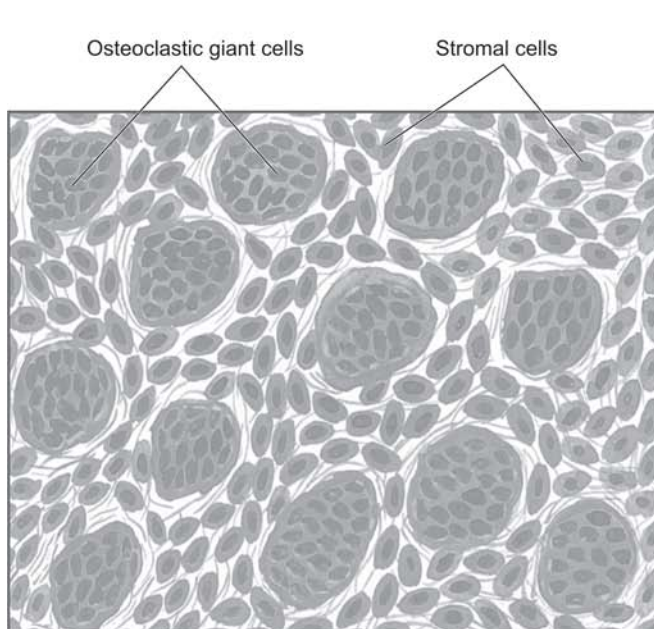


FIGURE 39.6: Osteoclastoma. The tumour shows spindle-shaped tumour cells, with uniformly distributed osteoclastic giant cells.



Bones and Joints II

- ◆ Ewing's Sarcoma
- ◆ Osteosarcoma
- ◆ Chondrosarcoma

Exercise

40

EWING'S SARCOMA

Ewing's sarcoma is a highly malignant round cell tumour arising in the medullary canal of diaphysis or metaphysis. The common sites are shafts of long bones, particularly femur, tibia, humerus and fibula. Common age for occurrence is between 5 to 20 years with predilection in females.

G/A Ewing's sarcoma is typically located in the medullary cavity and produces expansion of the affected diaphysis (shaft) or metaphysis. The tumour often extends into adjacent soft tissues. Cut surface of the tumour is characteristically grey-white, soft and friable (Fig. 40.1).

M/E

- The tumour is divided by fibrous septa into irregular lobules of closely-packed cells.
- The tumour cells comprising the lobules are small and uniform resembling lymphocytes and have round nuclei with frequent mitosis.
- The cytoplasm is scanty and contains glycogen positive for PAS.
- The tumour cells may be arranged around blood vessels forming pseudorosettes.
- Areas of acute inflammatory cell reaction and necrosis are often seen (Fig. 40.2).

OSTEOSARCOMA

Osteogenic sarcoma or osteosarcoma is the most common primary malignant bone tumour. Classically, the tumour occurs in young patients between the age of 10 to 20 years. The tumour arises in the metaphysis of long bones, most commonly in the lower end of femur and upper end of tibia (i.e. around knee joint).



FIGURE 40.1: Ewing's sarcoma. The specimen shows a tumour extending into soft tissues including the skeletal muscle (black arrow). Cut surface of the tumour is grey-white, cystic, soft and friable (white arrow).

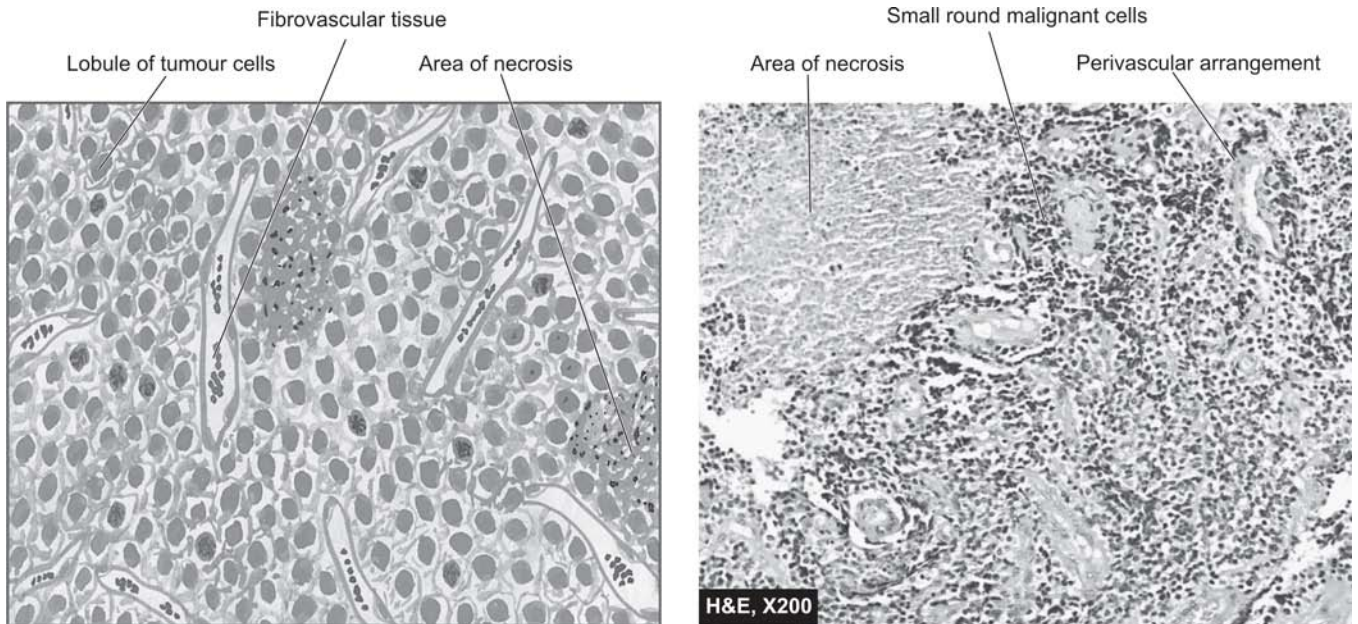


FIGURE 40.2: Ewing's sarcoma. Characteristic microscopic features are irregular lobules of uniform small tumour cells with indistinct cytoplasmic outlines which are separated by fibrous tissue septa having rich vascularity. Areas of necrosis and inflammatory infiltrate are also included.

G/A The tumour appears as a grey-white, bulky mass at the metaphyseal end of a long bone of the extremity, generally sparing the articular end of the bone. Codman's triangle formed by the angle between lifting of periosteum and underlying surface of the cortex may be grossly

identified. Cut surface of the tumour is grey-white with areas of haemorrhages and necrotic bone (Fig. 40.4).

M/E

- i. *Sarcoma cells:* The tumour cells are anaplastic mesenchymal stromal cells which show marked

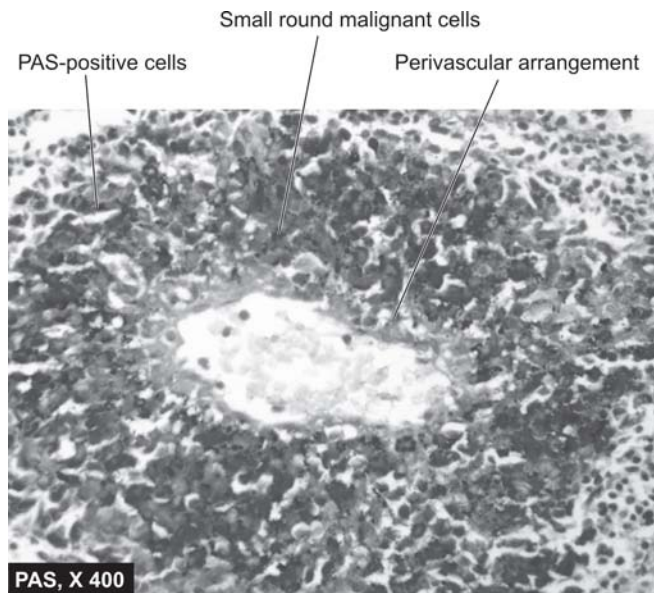


FIGURE 40.3: Ewing's sarcoma. The figure shows tumour cells containing scanty ill-defined cytoplasm having glycogen stained positive with PAS.

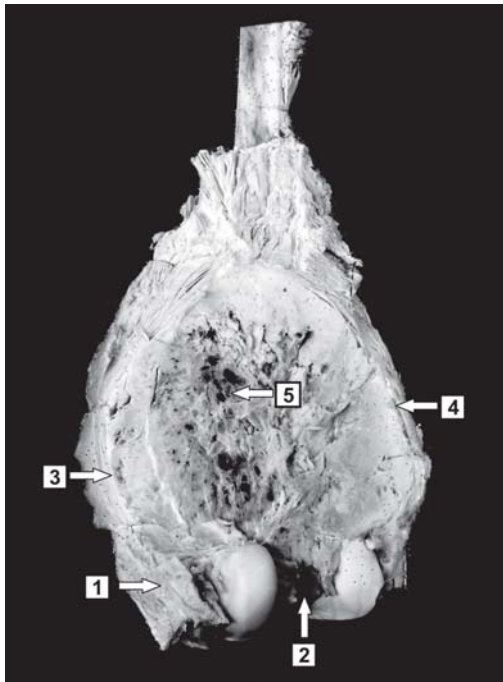


FIGURE 40.4: Osteosarcoma. The lower end of the femur shows a bulky expanded tumour in the region of metaphysis (1) sparing the epiphyseal cartilage (2). Sectioned surface of the tumour shows lifting of the periosteum by the tumour (3) and eroded cortical bone (4). Cut surface of the tumour is grey-white with areas of haemorrhage and necrosis (5).

pleomorphism and polymorphism i.e. variation in size as well as shape. The tumour cells may be spindle, round, oval, polygonal or bizarre tumour giant cells. They show hyperchromatism and atypical mitoses.

- ii. *Osteogenesis*: The anaplastic sarcoma cells form osteoid matrix and bone directly, which lies interspersed in the areas of tumour cells (Fig. 40.5).

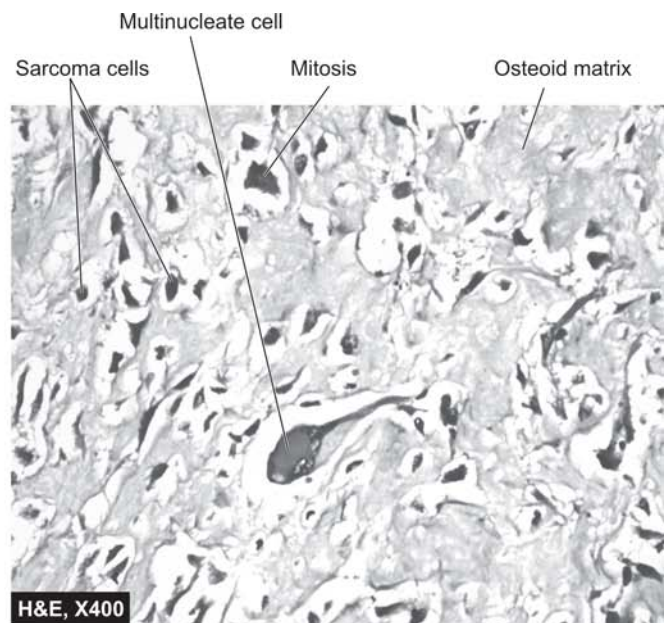
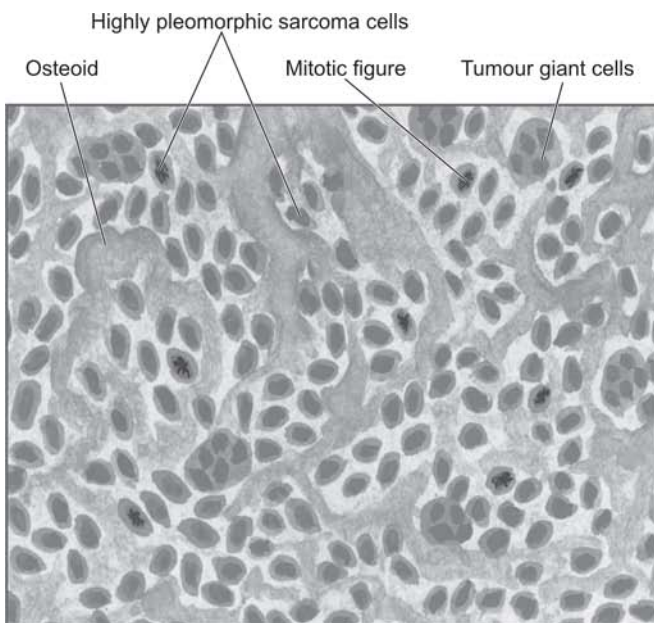


FIGURE 40.5: Osteosarcoma. The tumour cells show pleomorphic and polymorphic features with direct formation of osteoid by tumour cells.

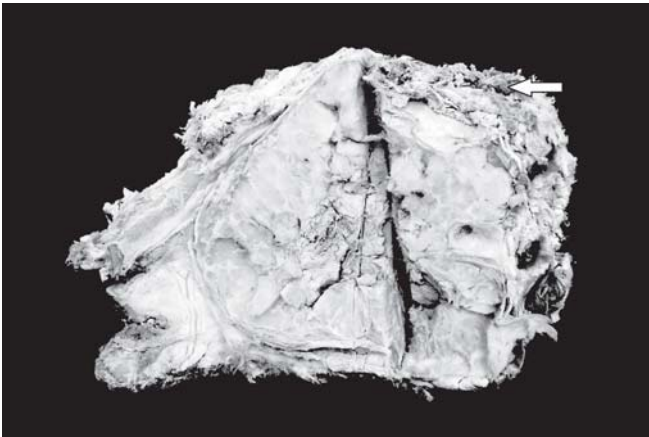


FIGURE 40.6: Chondrosarcoma. The scapula is expanded due to a gelatinous tumour. Sectioned surface shows lobulated mass with bluish cartilaginous hue infiltrating the soft tissues (arrow).

CHONDROSARCOMA

Chondrosarcoma is a malignant tumour of chondroblasts. However, it is much slow growing than osteosarcoma. The tumour usually occurs between 3rd and 6th decades of life. The tumour has 2 varieties—*central form* occurring in medullary cavity of the diaphysis or metaphysis (most commonly femur), and *peripheral form* that may be cortical or parosteal (most often in the vicinity of hip joint and shoulder).

G/A The tumour may vary in size from a few centimeters to extremely large and lobulated masses of firm

consistency. Cut section of the tumour shows translucent, bluish-white, gelatinous or myxoid appearance with foci of spotty calcification (Fig. 40.5).

M/E

- Lobular pattern of anaplastic cartilage cells.
- Cartilage cells have cytologic features of malignancy such as hyperchromatism, pleomorphism, two or more cells in the lacunae and tumour giant cells.
- Invasive character of the tumour into the adjoining soft tissues (Fig. 40.7).

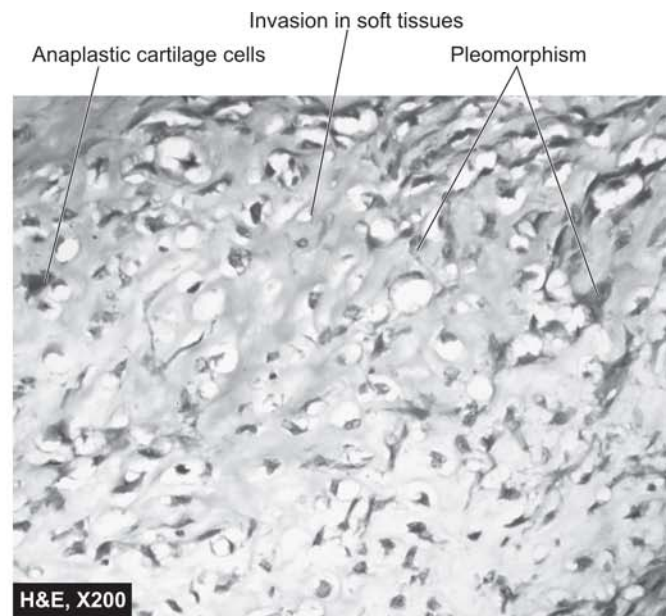
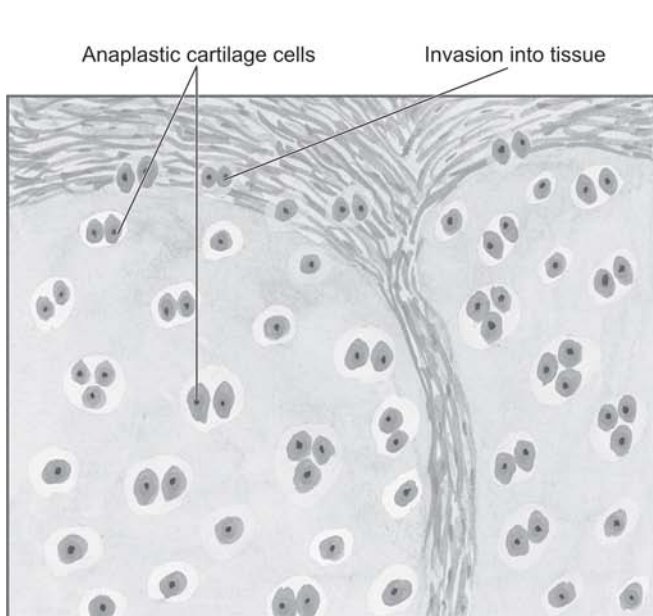


FIGURE 40.7: Chondrosarcoma. Histologic features include invasion of the tumour into adjacent soft tissues and cytologic characteristic of malignancy in the tumour cells.



Nervous System

- ◆ Acute Pyogenic Meningitis
- ◆ Meningioma
- ◆ Schwannoma
- ◆ Astrocytoma

Exercise

41

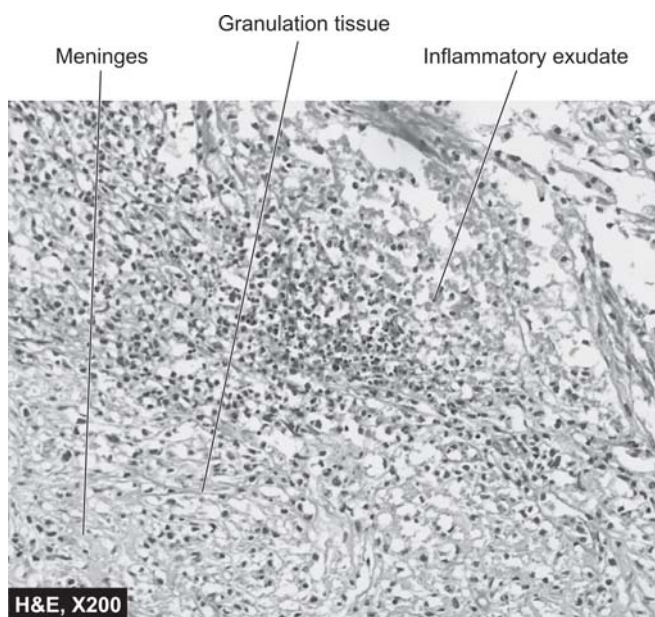
ACUTE PYOGENIC MENINGITIS

Acute pyogenic or purulent meningitis is acute infection of the pia-arachnoid and of the CSF.

G/A Pus accumulates in the subarachnoid space and the CSF becomes turbid or frankly purulent. The meninges are coated with exudate.

M/E

- The meninges, particularly around blood vessels, show numerous polymorphs and fibrinous material.
- There may be formation of granulation tissue of nonspecific type (Fig. 41.1).
- Bacteria may be identified in routine or Gram staining.



MENINGIOMA

The most common tumour arising from the pia-arachnoid is meningioma. The most common sites are in the front half of the head—lateral cerebral convexities, midline along the falx cerebri adjacent to the major venous sinuses parasagittally.

G/A Meningioma is well circumscribed, solid, spherical or hemispherical mass of variable size (1 to 10 cm in diameter). The tumour is generally firmly attached to the dura while the overlying bone may show hyperostosis. Cut surface of the tumour is firm and fibrous, sometimes with foci of calcification (Fig. 41.2).

M/E The most common microscopic type is mixed or transitional type. The features are as follows (Fig. 41.3):

- There is conspicuous whorled pattern of tumour cells, often around central capillary-sized blood vessels.
- There is combination of cells with syncytial and fibroblastic features. Syncytial cells resemble normal arachnoid cap cells and are polygonal cells with poorly-defined cell membranes (i.e. syncytial appearance).
- Many of the whorls may show psammoma bodies.
- Other secondary changes like xanthomatous and myxomatous degeneration may be seen.

SCHWANNOMA

Schwannoma or neurilemmoma arises from the cranial or spinal nerve roots. It may occur as an intraspinal tumour or a peripheral nerve sheath tumour. Multiple schwannomas are uncommon and occur in von Recklinghausen's disease.

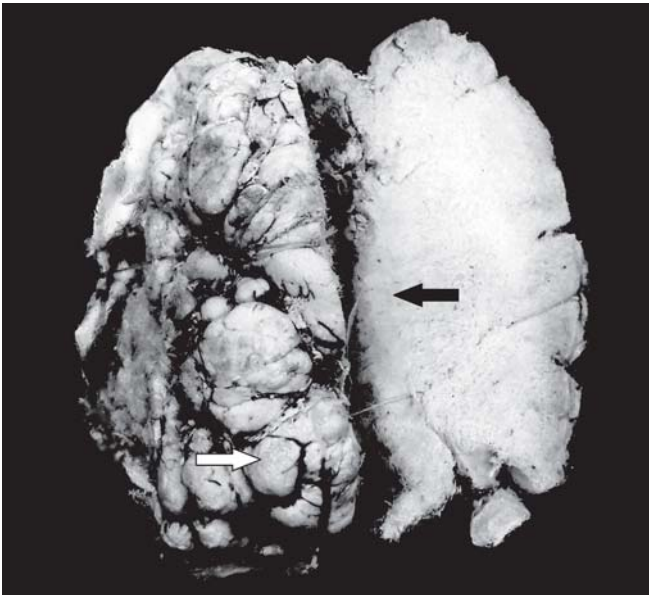


FIGURE 41.2: Meningioma. The tumour mass is circumscribed with irregular surface convolutions and prominent blood vessels (white arrow). It is firm in consistency with peripherally adherent thick firm dural tissue. Cut surface of the mass is firm and fibrous (black arrow).

G/A A schwannoma is an encapsulated, solid, sometimes cystic tumour, that produces eccentric enlargement of the nerve root from where it arises.

M/E

- The tumour is composed of fibrocellular bundles forming whorled pattern.
- There are areas of dense and compact cellularity (Antoni A pattern) alternating with loose acellular areas (Antoni B pattern).
- Areas of Antoni A show palisaded nuclei called Verocay bodies.
- Areas of degeneration containing haemosiderin and lipid-laden macrophages are often present (Fig. 41.4).

ASTROCYTOMA

Astrocytomas are the most common type of glioma. They are most frequent in late middle life and occur predominantly in the cerebral hemispheres. Depending upon the degree of anaplasia, they vary from low grade to high grade, the higher grades being labelled as anaplastic astrocytoma and glioblastoma multiforme.

G/A The tumour is poorly-defined, grey-white tumour of variable size. The tumour merges with the adjoining brain tissue. Cut section of higher grades of the tumour may show variegated appearance with areas of necrosis and haemorrhages.

M/E Microscopic features of higher grade of astrocytoma are as under:

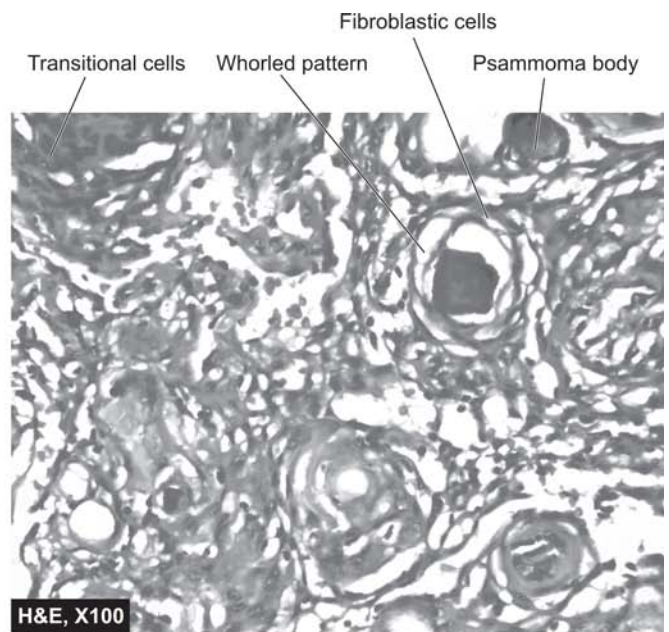
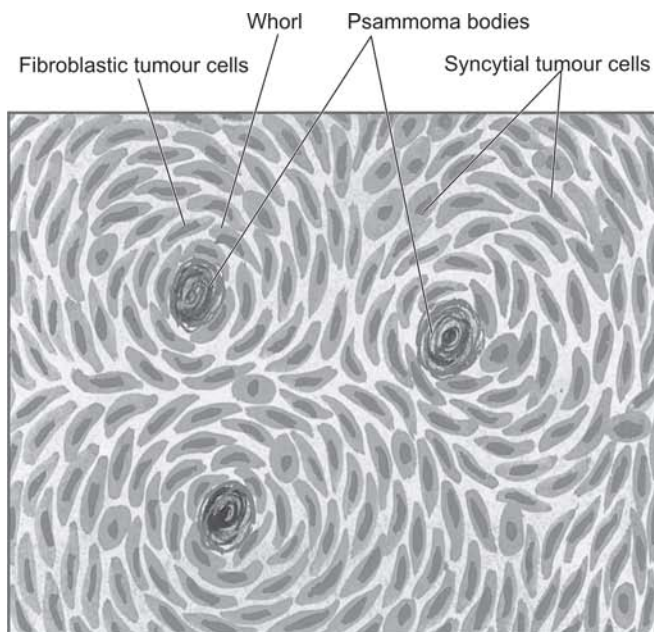


FIGURE 41.3: Meningioma. The tumour cells have features of both syncytial and fibroblastic type forming whorls which contain central laminated areas of calcification called psammoma bodies.

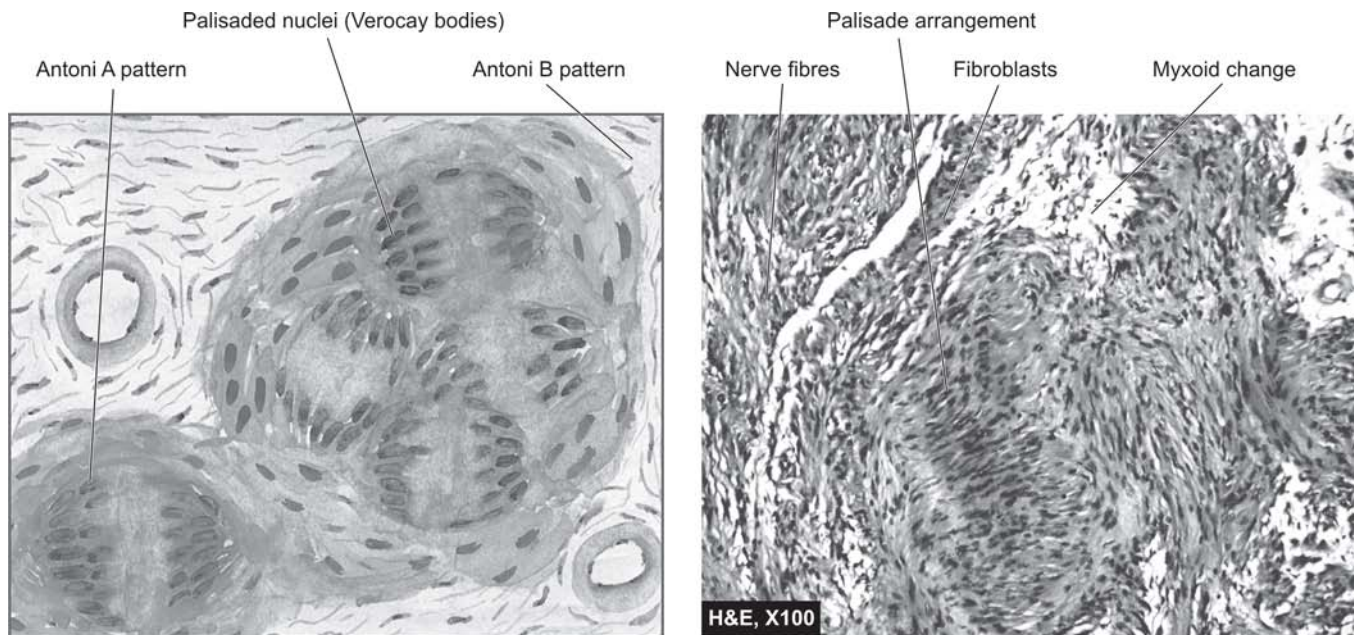


FIGURE 41.4: Schwannoma (neurilemmoma), showing whorls of densely cellular (Antoni A) and loosely cellular (Antoni B) areas with characteristic nuclear palisading (Verocay bodies).

- i. Highly cellular and anaplastic appearance.
- ii. The tumour cells have features of anaplasia such as pleomorphism, nuclear hyperchromatism and mitoses.
- iii. Proliferation of blood vessels and endothelial cells (Fig. 41.5).
- iv. Glioblastoma multiforme, in addition, shows pseudo-palisading of tumour cells around area of necrosis and multinucleate tumour giant cells.

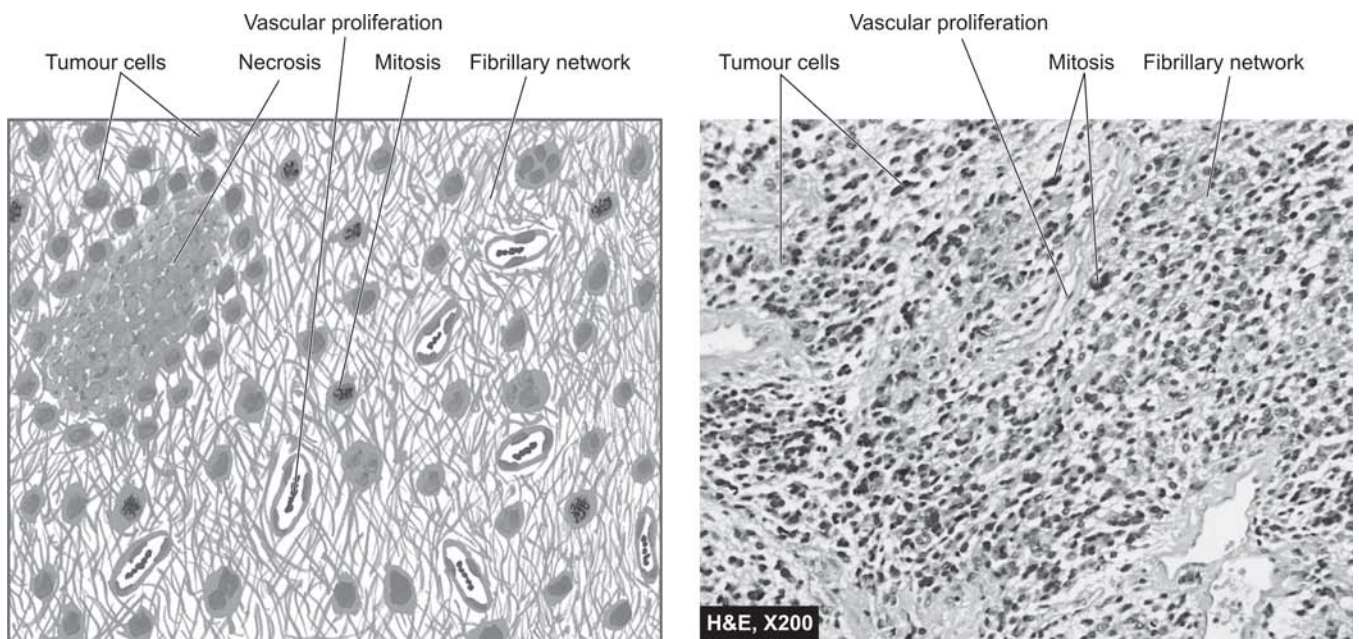


FIGURE 41.5: Astrocytoma, anaplastic. The tumour cells are anaplastic and there is proliferation of vascular endothelium.

SECTION FIVE

CYTOPATHOLOGY



GEORGE N PAPANICOLAOU (1883-1962)
'FATHER OF EXFOLIATIVE CYTOLOGY'

American pathologist, who developed Pap test
for detection and early diagnosis of uterine cervical cancer.

SECTION V: CYTOPATHOLOGY

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- * Learn the basic techniques and applications of exfoliative (Pap) and fine-needle aspiration cytology (FNAC).
- * Learn to describe, interpret and identify Pap smear for *Trichomonas* and cervical carcinoma, and fluid cytology for malignant cells.
- * Learn to describe, interpret and identify FNAC for tuberculous lymphadenitis, fibroadenoma and infiltrating duct carcinoma breast.

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Basic Cytopathologic Techniques

- ◆ Exfoliative Cytology
- ◆ Aspiration Cytology
- ◆ Imprint Cytology

Exercise

42

Cytology is the study of body cells that are either exfoliated spontaneously from epithelial surfaces or are obtained from various body tissues and organs by different techniques. Currently, cytology has following branches:

- A. Exfoliative cytology
- B. Aspiration cytology
- C. Imprint cytology

EXFOLIATIVE CYTOLOGY

This is the study of cells which are spontaneously shed off from epithelial surfaces into body cavities or fluid. The cells can also be obtained by scraping, brushing or wash of body surfaces. The principle of this technique is that in diseased states, rate of exfoliation of cells is increased.

Applications of Exfoliative Cytology

Exfoliative cytology is applied in diagnosing diseases of the following:

1. Female genital tract
2. Respiratory tract
3. Gastrointestinal tract
4. Urinary tract
5. Body fluids (pleural, peritoneal, pericardial, CSF and semen)
6. Buccal smears for sex chromatin

Female Genital Tract

Smears from female genital tract are known as 'Pap smears'. These smears are prepared by different methods depending upon the purpose for which they are intended:

- i. *Cervical smear* It is obtained by Ayre's spatula (Fig. 42.1) from portio of the cervix by rotating the spatula through 360° to sample the entire cervix. The scraped material is placed on a clean glass slide and smear prepared. It is ideal for detection of cervical carcinoma.
- ii. *Lateral vaginal smear (LVS)* is obtained by scraping upper third of lateral walls of the vagina and is ideal for cytohormonal assessment.

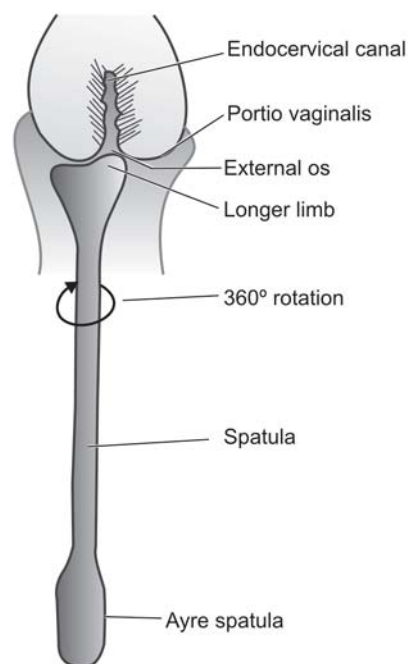


FIGURE 42.1: Method of obtaining cervical material for Fast smears.

- iii. *Vaginal pool smear* is obtained by aspirating material from posterior fornix of vagina and is done for detecting endometrial and ovarian carcinoma.

Respiratory Tract

Material from respiratory tract may be obtained during bronchoscopic procedures as expectorant (sputum), or by brushing (BB), washing (BW) and bronchioalveolar lavage (BAL). Sputum examination is advantageous as samples are easily obtained and cellular content is representative of entire respiratory tract. At least three samples of sputum, preferably early morning samples, should be examined.

Gastrointestinal Tract

Lesions in the oral cavity can be sampled by scraping the surface with a metallic or wooden spatula. Samples can be obtained from the oesophagus, stomach, small and large intestine either by brushing or lavage during fibreoptic endoscopy.

Urinary Tract

Samples from lesions in the urinary tract are either urinary sediment examined from voided urine/catheterised urine or washings of the urinary bladder obtained at cystoscopy.

Body Fluids

Fluid from pleural, peritoneal or pericardial cavity is obtained by paracentesis. At least 50-100 ml of fluid is aspirated. The sample is examined fresh but if delay is anticipated then fluid should be anticoagulated either in EDTA 1 mg/ml or 3.8% sodium citrate 1ml/10ml. Fluid should be centrifuged and smears are prepared from the sediment. If amount of fluid is less (less than 1 ml), then it can be subjected to cytospin centrifuged smear preparation (Fig. 42.2).

Buccal Smears for Sex Chromatin

Smears are prepared from the oral cavity after cleaning the area. Vaginal smears can also be used. In normal females, Barr bodies are present in 4-20% nuclei. In males their count is in less than 2% nuclei.

Fixation of Smears in Exfoliative Cytology

Methods of fixation depend upon type of staining employed. Pap smears are wet-fixed (i.e. smears are immersed in fixative without allowing them to dry).



FIGURE 42.2: Cytospin used for making smears in cases with small volume of fluid (Photograph courtesy of Thermo Shandon, UK through Towa Optics India Pvt. Ltd., Delhi).

Smears to be stained by Romanowsky stains are air-dried as fixation is affected during the staining procedure. Fixative used is either equal parts of ether and 95% ethanol, or 95% ethanol alone, 100% methanol, or 85% isopropyl alcohol. Fixation time of 10-15 minutes at room temperature is adequate. Smears may be left in fixative for 24 hours or more. Smears should be transported to the laboratory in fixative solution in coplin jars.

Staining of Smears in Exfoliative Cytology

Three staining procedures are commonly employed: Papanicolaou and H and E stains are used for *wet-fixed smears* while Romanowsky stains are used for *air-dried smears*.

Papanicolaou Stain

This is the best stain for routine cytodiagnostic studies. In this, haematoxylin gives nuclear stain while OG-6 and EA-50 are two cytoplasmic counterstains.

H & E Stain

This is the same as that used for histological sections. In this, haematoxylin is nuclear stain and eosin is cytoplasmic counterstain.

Romanowsky Stain

Leishman's stain, Giemsa and May-Grunwald-Giemsa (MGG) are usually used; the last one is most commonly used.

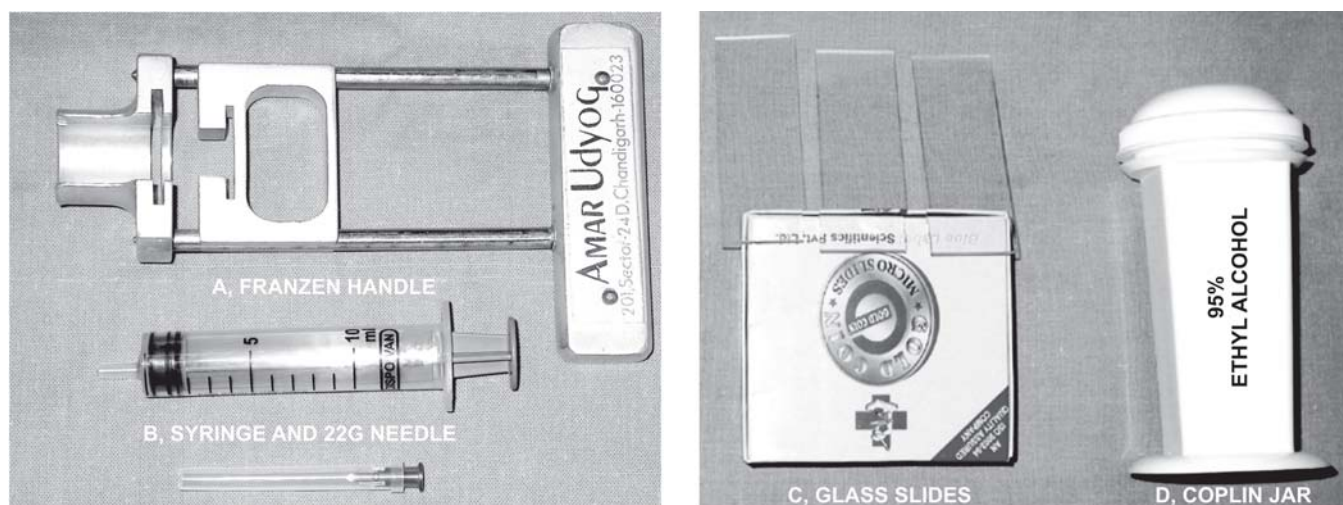


FIGURE 42.3: Equipment required for transcutaneous FNAC.

ASPIRATION CYTOLOGY

In this study, samples are obtained from diseased tissue by fine needle aspiration (FNA) or aspiration biopsy cytology (ABC).

Applications of FNA

FNA or ABC is applied for diagnosis of palpable as well as non-palpable lesions.

I. Palpable Mass Lesions in:

1. Lymph nodes
2. Breast
3. Thyroid
4. Salivary glands
5. Soft tissue masses
6. Bones

II. Non-Palpable Mass Lesions in:

1. Abdominal cavity
2. Thoracic cavity
3. Retroperitoneum

Procedure for FNA

Materials For performing FNA, a Franzen's handle, syringe with needles, clean glass slides and suitable fixative are required (Fig. 42.3).

Method

- No anaesthesia is required.

- Ask the patient to lie down in comfortable position exposing the target area.
- Palpate the target area.
- Clean the overlying skin with spirit.
- Fix 10/20 ml disposable syringe in Franzen's handle. Insert 20-25 gauge disposable needle into syringe (Fig. 42.3, A, B).
- Fix the mass by palpating hand and insert needle into target area (Fig. 42.4). Apply suction while moving

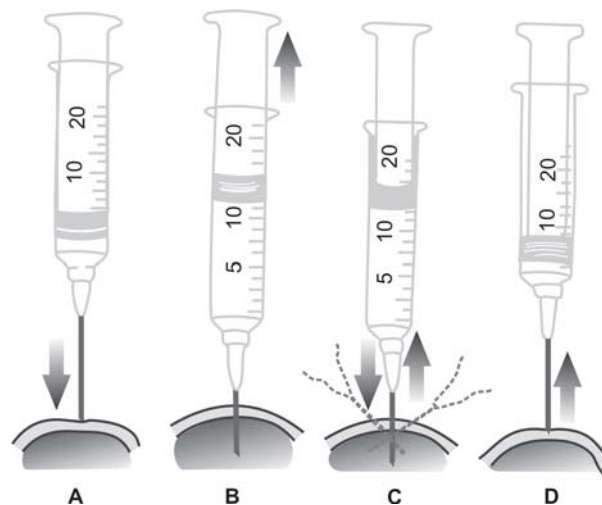


FIGURE 42.4: Procedure of FNAC of palpable masses. Needle is introduced into the mass (A). Plunger is retracted after needle enters the mass (B). Suction is maintained while needle is moved back and forth within the mass (C). Suction is released and plunger returned to original position before needle is withdrawn (D).



FIGURE 42.5: Preparation of smears. Semisolid aspirates are crush-smear by flat pressure with cover slip or glass slide (A). Fluid or blood droplet is collected along edge of spreader (B), and pulled as for peripheral blood films (C). Particles at the end of the smear are crush-smear (D).

needle back and forth within the lesion and change the direction of the needle.

- Terminate the aspiration when aspirated material or blood is visible at the base/hub of the needle.
- Release the suction before withdrawing the needle to equalise pressure within the syringe.
- After withdrawal of needle, apply pressure for 2-3 minutes at the site of puncture to arrest bleeding and prevent haematoma formation.
- Aspirated material from the needle is expressed on to clean glass slides by first detaching the needle and filling the syringe with air and expressing it with pressure.
- Smears are prepared as for blood smears. If the material is semi-solid, it is first crushed by gentle pressure with a glass slide and smears prepared (Fig. 42.5).
- Fixation and staining are the same as for exfoliative cytology.

Radiological Imaging Aids for FNA

Non-palpable lesions require some form of localisation by radiological aids for FNA to be carried out. Plain X-ray is usually adequate for lesions in bones and chest. Ultrasonography (USG) allows direct visualisation of needle in intra-abdominal and soft tissue masses. CT scan can be used for lesions in chest and abdomen.

Advantages of FNA over Surgical Biopsy

- i. Outdoor procedure
- ii. No anaesthesia required
- iii. Results obtained within hours
- iv. Procedure can be repeated
- v. Low cost procedure

IMPRINT CYTOLOGY

In imprint cytology touch preparations from cut surfaces of fresh unfixed surgically excised tissue are prepared on clean glass slides. These are fixed, stained and examined immediately. It is considered complementary to frozen section.



Exfoliative Cytology

- ◆ Pap Smear—Inflammatory
- ◆ Pap Smear—Carcinoma Cervix
- ◆ Fluid Cytology for Malignant Cells

Exercise

43

PAP SMEAR—INFLAMMATORY

A normal Pap smear shows the following cells:

- Superficial cells
- Intermediate cells
- Parabasal cells

The features of these cells are summarised in Table 43.1.

In inflammatory smear, the superficial and intermediate cells show features as under (Fig. 43.1):

- Cytoplasmic eosinophilia.
- Cytoplasmic vacuolisation.
- Large number of leucocytes in the background.
- Leucocytes into cytoplasm of epithelial cells.
- Perinuclear halos.
- Clumping of chromatin.
- Nuclear enlargement and pyknosis.
- Increase in the number of parabasal cells.





The specific inflammation could be by the following microbial infections:

- Trichomonas vaginalis*
- Candida albicans*
- Herpes simplex virus
- Human papilloma virus

The following morphological features are used to diagnose *Trichomonas vaginalis* vaginal infection (Fig. 43.2):

- Presence of trophozoites of the protozoa in the smear are seen as fuzzy grey-green, round or elliptical structure 8 to 20 μ in size and contain an eccentric, small round vesicular nucleus. Flagella are rarely seen.
- Other non-specific inflammatory changes as described above are seen.

Table 43.1: Squamous epithelial cells found in normal combined (Fast) smears.

Cell type	Size	Nuclei	Cytoplasm	Morphology
Superficial	30-60 μ m	< 6 μ m dark, pyknotic	Polyhedral, thin, broad, acidophilic or cyanophilic with keratohyaline granules.	
Intermediate	20-40 μ m	6-9 μ m vesicular	Polyhedral or elongated, thin, cyanophilic with folded edges.	
Parabasal	15-25 μ m	6-11 μ m vesicular	Round to oval, thick, well-defined, basophilic with occasional small vacuoles.	
Basal	13-20 μ m	Large, (> one-half of cell volume), hyperchromatic, may have small nucleoli	Round to oval, deeply basophilic.	

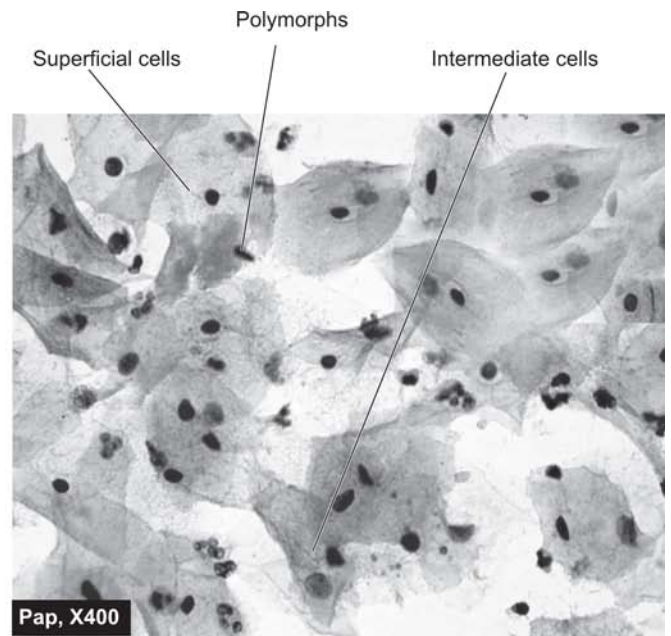
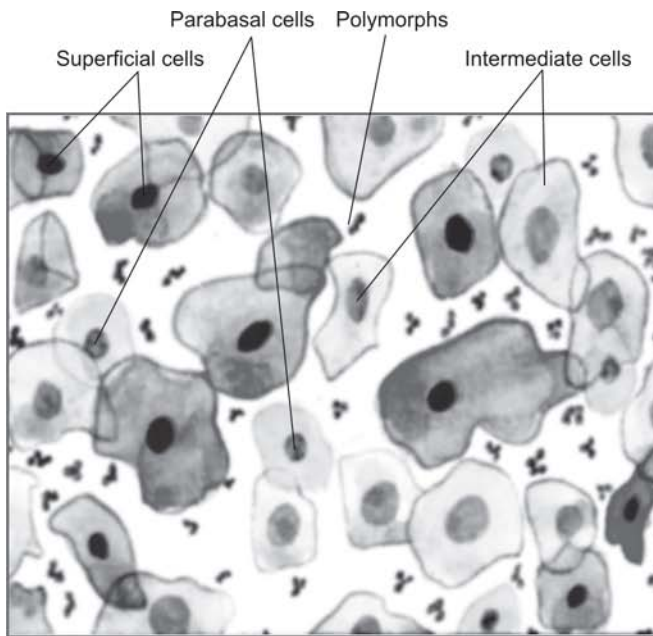


FIGURE 43.1: Pap smear, inflammatory. The field shows mainly superficial and some intermediate cells, and a few polymorphs.

PAP SMEAR—CARCINOMA CERVIX

Pap smear has become one of the key screening procedures for diagnosing precursor lesions as well as for carcinoma of the cervix. Compared to earlier system of categorizing Pap smears in to mild, moderate and severe dysplasias, currently the cytological diagnosis of

cervical lesions is based commonly on the *Bethesda system* which divides the precursor lesions into two tiers:

1. LSIL (low grade squamous intraepithelial lesions)
2. HSIL (high grade squamous intraepithelial lesions).

The characteristic cytological features of invasive squamous cell carcinoma are as follows (Fig.43.3):

- i. Cells present singly, in sheets and syncytium-like aggregates.
- ii. Marked variation in cell size and shape.
- iii. Altered nuclear-cytoplasmic ratio.
- iv. Nuclei having irregular membranes, prominent macronucleoli, irregularly distributed coarse chromatin and parachromatin clearing.
- v. Tadpole/caudate cells and spindled squamous cells are seen.
- vi. Associated tumour diathesis in the background (necrosis and old, degenerated blood).

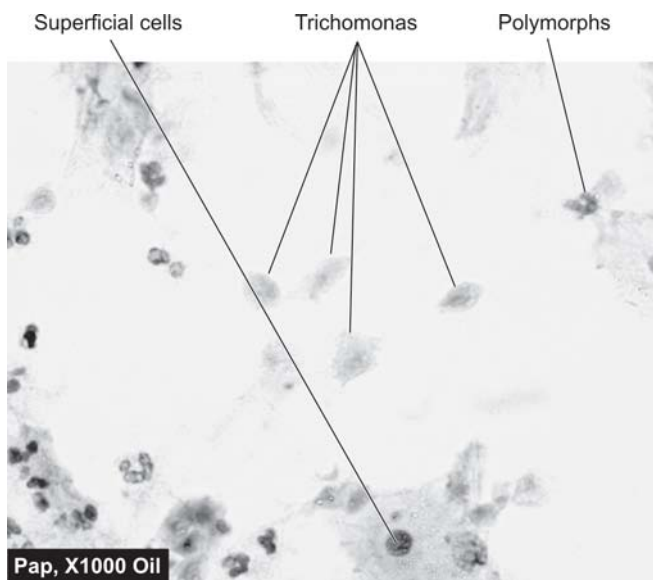


FIGURE 43.2: Pap smear showing *Trichomonas* vaginitis. Trophozoite of *Trichomonas* is shown by arrow.

FLUID CYTOLOGY FOR MALIGNANT CELLS

Accumulation of fluid in the body cavities may be due to:

- A. Benign conditions
- B. Malignant conditions

In benign conditions the fluid shows presence of mesothelial cells which can be confused with malignant cells. The characteristics of *mesothelial cells* are as under:

- i. Seen in small clusters, papillae, pseudoacini or singly.

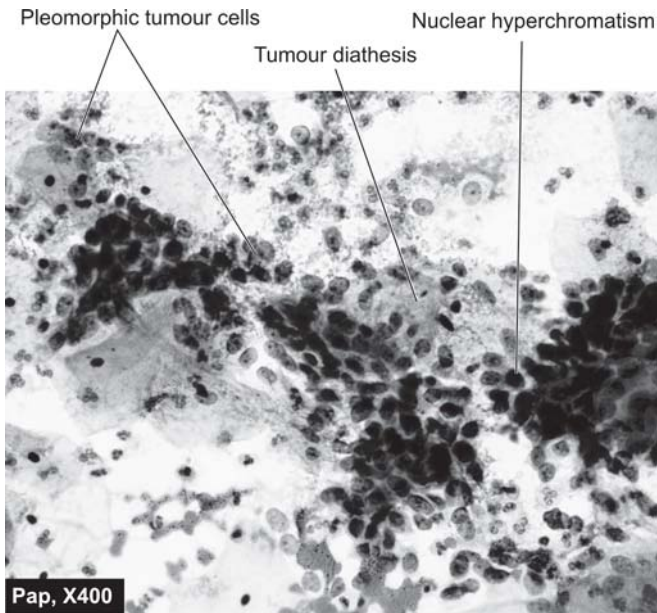


FIGURE 43.3: Pap smear in invasive carcinoma cervix. The field shows pleomorphic squamous cells in a sheet having coarse nuclear chromatin and some tumour diathesis in the background.

- ii. Round to oval 30 to 90 μm in size.
- iii. Cytoplasm is basophilic and is usually abundant.
- iv. Central nuclei with perinuclear halo.
- v. Clumped chromatin with 1-4 distinct nucleoli.

Malignant cells (adenocarcinoma) in effusion show following characteristics in smears (Fig. 43.4):

- i. seen in acini, papillae or singly.
- ii. Cells are variable-sized.

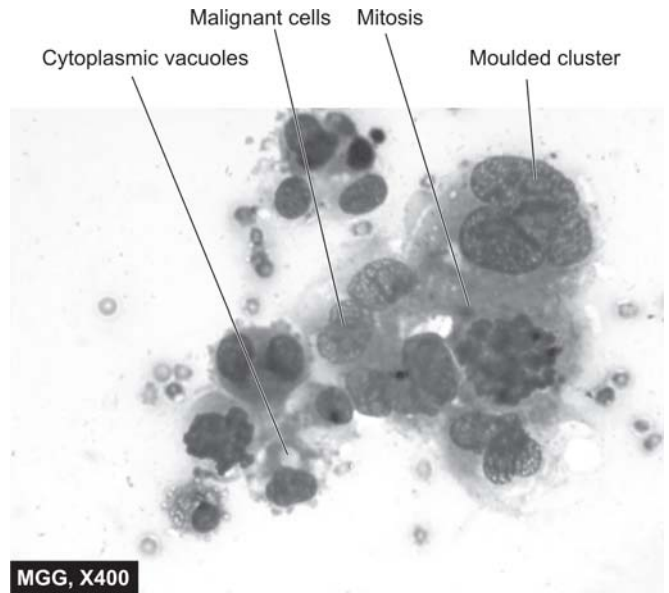


FIGURE 43.4: Adenocarcinoma in ascitic fluid. Moulded clusters of malignant cells seen with prominent nucleoli .

- iii. Cytoplasm is abundant deep purple.
- iv. Cytoplasmic vacuoles in some forming signet ring cells.
- v. Nuclei irregular shaped, well-defined nuclear border, hyperchromatic and nuclear overlapping.
- vi. Chromatin clumping with prominent nucleoli.
- vii. Frequent mitosis.



Fine Needle Aspiration Cytology

- ◆ FNA from Tuberculous Lymphadenitis
- ◆ FNA from Fibroadenoma Breast
- ◆ FNA from Duct Carcinoma Breast

Exercise

44

FNA FROM TUBERCULOUS LYMPHADENITIS

Fine needle aspiration of lymph nodes has become the primary tool in diagnosing tuberculosis. The characteristic findings are as under (Fig. 44.1):

- Multiple epithelioid cell granulomas are seen. Epithelioid cells have an elongated slipper-shaped vesicular nuclei and pale cytoplasm with indistinct cell borders.
- Variable number of Langhans' and foreign body type of giant cells.
- Background shows eosinophilic granular material (caseous necrosis).
- Ziehl-Neelsen stain for acid fast bacilli may be positive (Fig. 44.2).

- Not infrequently, the smears may show only polymorphs and necrotic debris especially in immunocompromised patients.

FNA FROM FIBROADENOMA BREAST

Fibroadenoma is a common benign biphasic tumour of the breast in young females presenting as a movable firm lump. The cytological smears show the following features (Fig. 44.3):

- Cellular smears.
- Monolayered sheets of uniform epithelial cells having antler horn configuration.
- Sheets show both epithelial and myoepithelial cells.

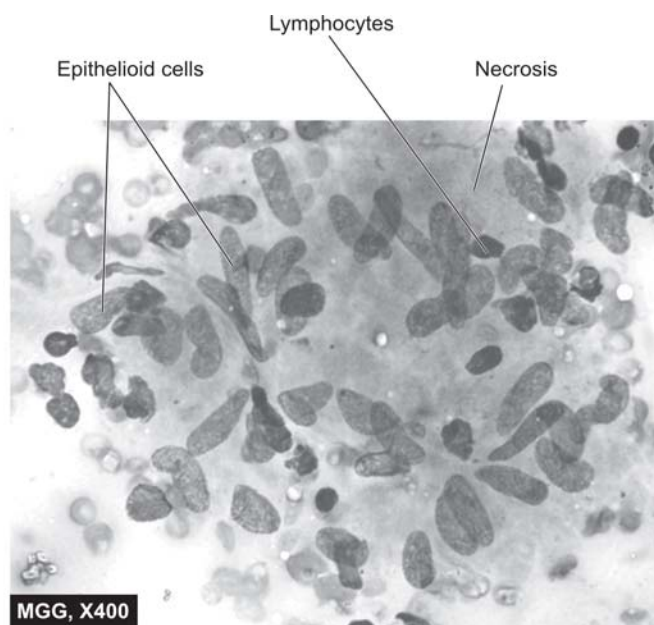
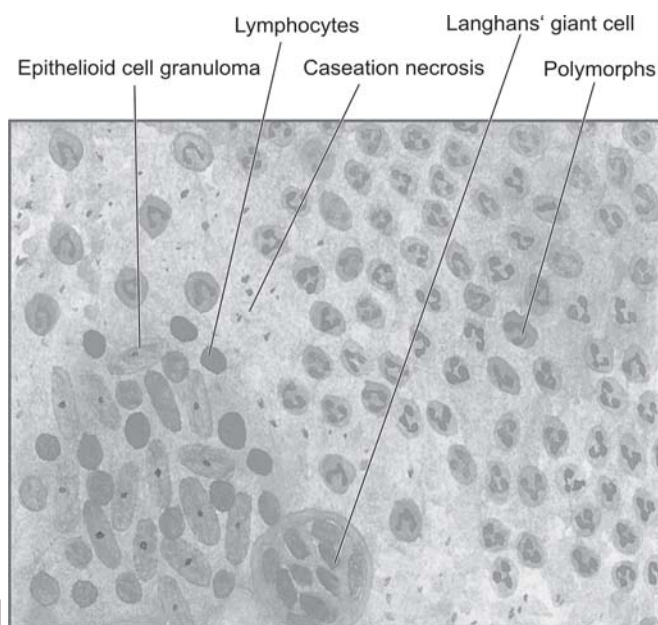


FIGURE 44.1: FNA smear from tuberculous lymphadenitis. There are epithelioid cell clusters and necrotic debris in the background.

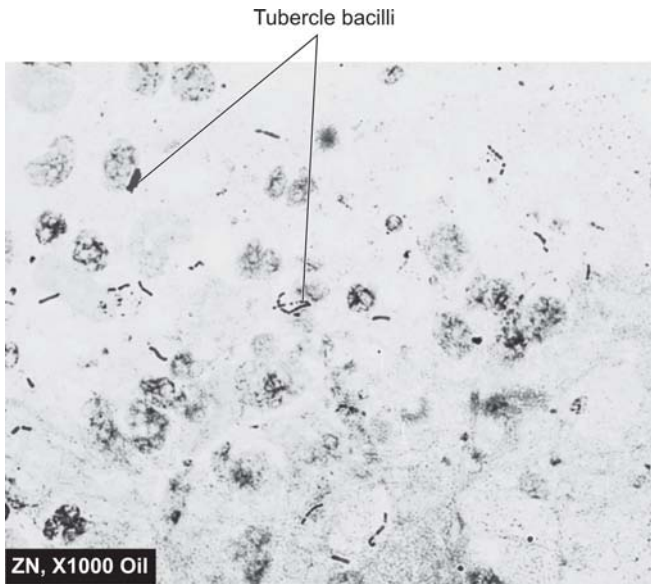


FIGURE 44.2: FNA smear from tuberculous lymphadenitis. The field shows acid fast bacilli.

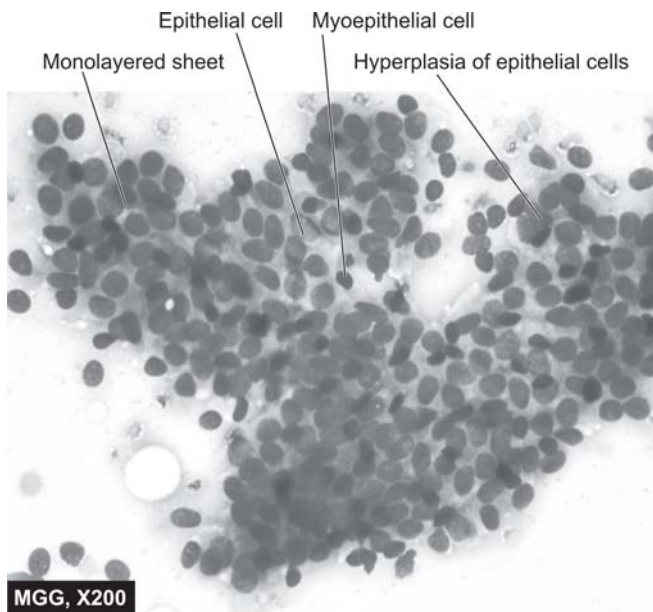


FIGURE 44.3: FNA breast from fibroadenoma breast. The field shows monolayered sheet of monomorphic cells and some fibromyxoid stromal fragment.

- iv. Numerous single, bipolar benign nuclei.
- v. Fragments of fibromyxoid stroma.

FNA FROM DUCT CARCINOMA BREAST

The characteristics of duct carcinoma breast in smear are as under (Fig. 44.4):

- i. Highly cellular smears.
- ii. Cells are less cohesive.
- iii. Cells disorderly arranged with overlapping.
- iv. Altered nuclear-cytoplasmic ratio.
- v. Cytoplasm is mild to moderate and well-defined.
- vi. Nuclei are large, irregular, hyperchromatic, some show prominent nucleoli.
- vii. Background shows necrotic debris.

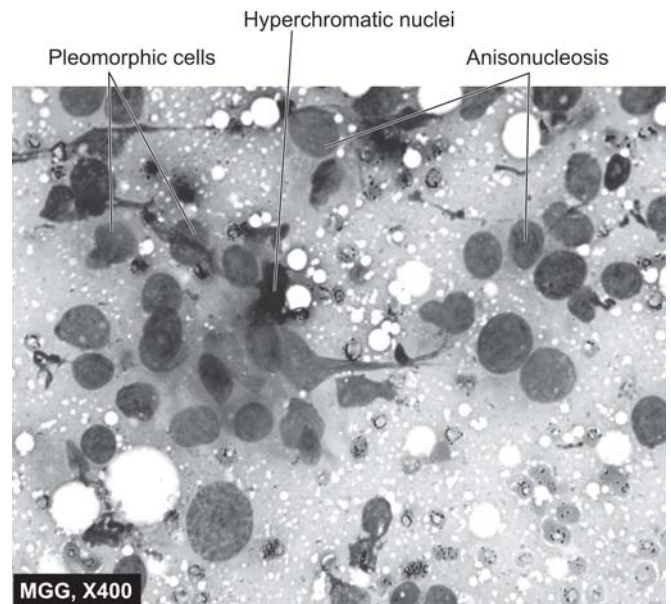


FIGURE 44.4: FNA breast, duct carcinoma. Scattered pleomorphic malignant cells with hyperchromatic nuclei.



SECTION SIX

HAEMATOLOGY



MM WINTROBE (1901-1986)

American Physician, who devised Wintrobe haematocrit tube for estimation of PCV and ESR and thus enabled measuring red cell indices.

Wintrobe was a pupil of William Loyd, a pioneering teacher and eminent author of last century, and regarded him as a very stimulating teacher.

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- * To perform and interpret the basic haematological techniques (haemoglobin estimation, counting of blood cells, reticulocyte preparation, preparation and staining of blood film, ESR, PCV, absolute values, screening tests for bleeding disorder).
- * Learn to perform and interpret blood grouping.
- * Learn to perform and interpret blood smear for common and main types of anaemias and leukaemias.
- * Learn to perform and interpret blood smear for common and main types of haemoparasites (malaria, microfilaria, leishmania).
- * Gain basic knowledge and learn to perform the bone marrow aspirate and trephine biopsy examination along with their applications.

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Haemoglobin Estimation— Various Methods

- ◆ Methods for Estimation of Haemoglobin
- ◆ Quality Control in Haemoglobin Estimation

Exercise

45

Haemoglobin (Hb) is the main component of red blood cells and is a conjugated protein. A molecule of Hb contains two pairs of polypeptide chains $\alpha_2 \beta_2$ and four haem groups each having an atom of ferrous iron. The oxygen-carrying capacity of Hb when fully saturated is 1.34 ml/g. Approximately 34% of the RBCs by weight is Hb. Iron content of Hb is 0.347 gm/100 g. The main function of Hb is to transport oxygen from lungs to the tissues. There are various forms of Hb as under :

- i. Oxyhaemoglobin (Hb O₂)
- ii. Carboxy haemoglobin (Hb CO)
- iii. Sulfhaemoglobin (SHb)
- iv. Methaemoglobin (Hi)

The measurement of concentration of Hb in the blood is known as haemoglobinometry.

Types of blood samples used for Hb estimation are as under:

- i. *Capillary blood* from finger prick.
- ii. *Intravenous sample*—It should be well anticoagulated, preferably in EDTA. Liquid anticoagulants should not be used at all as these dilute and decrease Hb concentration.

METHODS FOR ESTIMATION OF HAEMOGLOBIN

Various methods used for estimation of Hb are divided into 4 groups as under:

I. Colorimetric method: Colorimetric method is based on colorimetric measurement of the intensity of colour developed on addition of some substance to the blood. Colorimetric methods include the following:

1. Cyanmethaemoglobin method
2. Oxyhaemoglobin method
3. Electronic counter method
4. Direct reading electronic haemoglobinometer
5. Sahli's method

II. Measurement of O₂ carrying capacity of Hb: Measurement of O₂ carrying capacity of Hb can not be used for mass screening but is used in referral or research laboratories only.

III. Measurement of iron content of Hb: Measurement of iron content of Hb is used only for research purpose.

IV. Specific gravity method: It is a very rapid method and is useful for screening blood donors for anaemia in blood donation programme. Normal specific gravity of blood ranges from 1.048-1.066.

Some of the commonly used methods are discussed below.

Cyanmet Hb Method

This is the best method for Hb estimation and it has been recommended by International Committee for Standardization in Haematology (ICSH).

Principle Blood is diluted in a solution called Drabkin's fluid containing potassium ferricyanide and potassium cyanide (KCN). The oxy, carboxy and methHb are all converted into cyanmet Hb (HiCN) and there is development of pink colour. The intensity of pink colour can be measured in a spectrophotometer or photoelectric colorimeter at 540 nm and this is compared with that of a standard cyanmethaemoglobin solution.

Reagents Drabkin's fluid can be prepared as under:

Potassium ferricyanide	:	0.2 g
Potassium cyanide	:	0.05 g
Dihydrogen potassium phosphate	:	0.14 g
Distilled water	:	1000 ml

Drabkin's fluid should be clear and pale yellow having a pH of 7.0-7.4.



FIGURE 45.1: Photoelectric colorimeter used for taking reading of haemoglobin in cyanmet Hb method and oxyhaemoglobin method. (Photograph courtesy of Max Electronics India, Chandigarh).

Procedure

- Add 20 μl (0.02 ml) of blood to 5 ml of Drabkin's solution in a test tube (1:251 dilution).
- Mix well and allow it to stand for 3-5 minutes.
- Take reading of test and standard in a spectrophotometer or photoelectric colorimeter at 540 nm (Fig. 45.1).

Calculations

Hb concentration in test (g%) =

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Hb concentration of standard (mg/dl)} \times 251}{100 \text{ mg/g}}$$

Where 251 is the dilution factor.

Advantages

- i. There is no chance of visual error.
- ii. All forms of Hb except sulfhaemoglobin can be measured.
- iii. The standard is very stable.

Disadvantages

- i. We cannot take the reading immediately.
- ii. If blood is turbid due to plasma proteins, hyperlipidaemia, leukaemias, the absorbance is more and hence incorrect results may be obtained.
- iii. Results are affected due to hyperbilirubinemia.

Oxyhaemoglobin Method

This is a simple and quick method and results are not affected by hyperbilirubinaemia.

Principle Blood is diluted in a solution of ammonia. There is development of reddish pink-colour which is measured in a spectrophotometer or photoelectric colorimeter at 625 nm and compared with that of a standard oxyHb solution.

Procedure

- Add 20 μl (0.02 ml) of blood to 4 ml of 0.4 ml/l ammonia solution in a test tube.
- Use a tight fitting stopper and mix by inverting the tube several times.
- Take reading of test and standard in a spectrophotometer or photoelectric colorimeter with a yellow or green filter (625 nm).

Calculations As for cyanmet method.

Advantages

- i. The method is simple and quick.
- ii. Result is not affected by rise of plasma bilirubin.
- iii. Most forms of haemoglobins (i.e. HbO, Hi, and HbCO) are measured in this method.

Disadvantages

- i. It does not measure sulfhaemoglobin.
- ii. The standard is not stable.
- iii. Increased absorbance may be caused by turbidity due to hyperlipidaemia, leucocytosis ($> 30 \times 10^9/\text{L}$) and abnormal plasma proteins.

Electronic Counter Method

This is a multi-parameter determining electronic equipment.

Principle The method is based on electrical impedance principle. The blood is diluted with isoton and lysate which lyses the RBCs converting Hb into cyanmethaemoglobin and its concentration is measured in the spectrophotometer at 540 nm. In some instruments, cyanmethaemoglobin method is replaced with another method employing a non-toxic chemical, sodium lauryl sulphate.

Disadvantage

- i. High white cell count ($> 30,000/\mu\text{l}$) produces false elevation of Hb.

Direct Reading Electronic Haemoglobinometers

These have inbuilt filters. Reading of Hb in g/dl is visualised on the screen which may have light emitting diode (LED) display or analog meter. These equipments work on the principle of cyanmetHb, oxyHb method or colour comparators in which colour of blood is compared

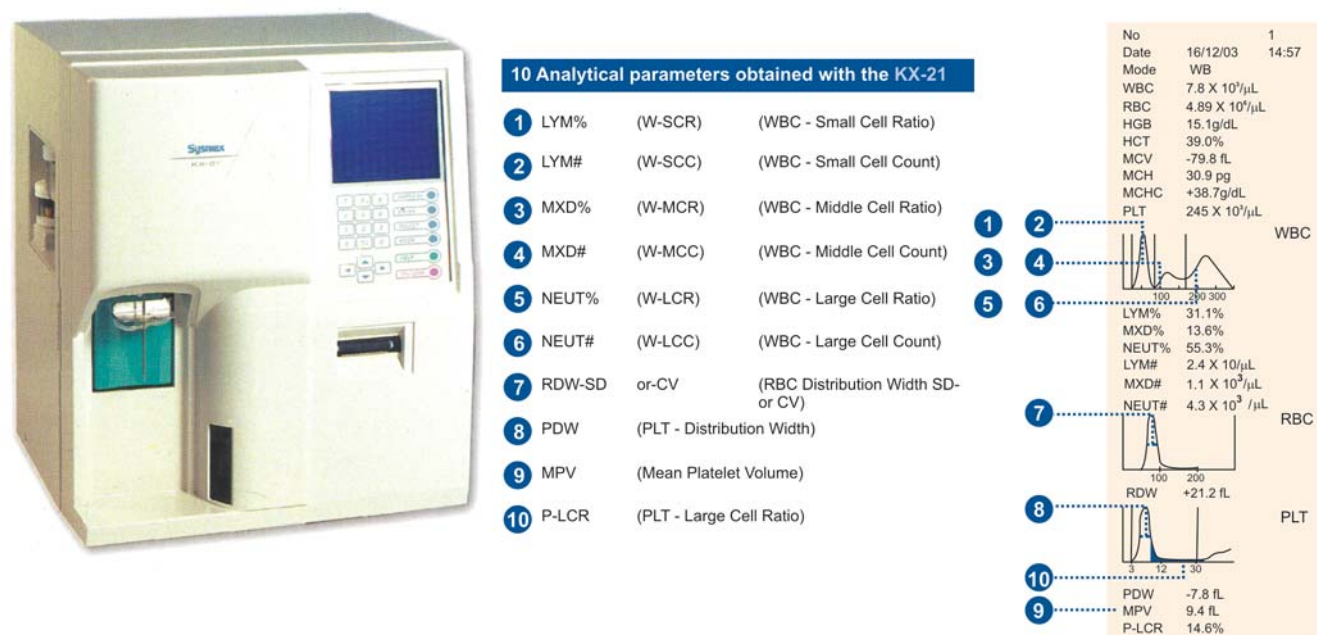


FIGURE 45.2: Electronic particle counter (Haematology Analyser) Model K X -21 (Photograph courtesy of Sysmex Corporation, Japan through Transasia Biomedicals Ltd., Mumbai).

without conversion to a derivative, against a range of colours which represent haemoglobin concentration (Fig. 45.2).

Disadvantage

- Calibration of the instrument can be faulty.

Sahli's Method

Principle Hb is converted into acid haematin with the action of dilute hydrochloric acid (N/10 HCl). The acid haematin is brown in colour and its intensity is matched with a standard brown glass comparator in a visual colorimeter called Sahli's colorimeter.

Procedure

- Fill Sahli's Hb tube upto mark 2 with N/10 HCl.
- Deliver 20 μL (0.02 ml) of blood from a Hb pipette into it.
- Stir with a stirrer and wait for 10 minutes.
- Add distilled water drop by drop and stir till colour matches with the comparator.
- Take the reading at upper meniscus (Fig. 45.3).

Advantages

- Simple bedside test.
- Reagents and apparatus are cheap.

Disadvantages

- There can be visual error.
- Carboxy, met and sulfhaemoglobins cannot be converted to acid haematin.
- Comparator can fade over the years.
- Colour of acid haematin also fades quickly.

Other methods like carboxy Hb and alkali haematin methods are not used these days.

Normal Values of Hb

Men	15 ± 2 g/dl
Women	13.5 ± 1.5 g/dl
Infants	16.5 ± 3 g/dl

Errors in Haemoglobinometry

- Sampling error:** Improper venipuncture technique e.g. more squeezing can alter the results, or the reading may be affected by type of anticoagulant used.
- Error in method:** Results are better with cyanmet and oxy Hb method. In Sahli's method chances of error are more.
- Error in equipment:** These could be due to quality of material of the equipment or calibration of the equipment.



FIGURE 45.3: Apparatus used for Sahli's haemoglobinometry.

4. *Operator's error:* This could be because of improper training, lack of familiarity with the equipment or overworked operator.

QUALITY CONTROL IN HAEMOGLOBIN ESTIMATION

For reliability of the results, quality assurance or quality control is a must. It includes proficiency in collection, labelling, storage and results of the test. Quality control has three components: *internal quality control*, *standardisation* and *external quality control*. Precision refers to reproducibility of a result but a test may be precise without being accurate. Inaccuracy occurs as a result of improper standard, reagents, calibration of equipment and poor technique. Accuracy is attained by use of reference material which has been assayed by different methods and in different laboratories.



Counting of Blood Cells

- ◆ WBC Count
- ◆ RBC Count
- ◆ Platelet Count

Exercise

46

WBC COUNT

This is determination of number of white blood cells per μl of blood.

Methods

There are two methods:

1. Visual haemocytometer method
2. Electronic method

Visual Haemocytometer Method

Principle This is counting of WBCs in a calibrated chamber by diluting of blood to 1:20 dilution with diluent which causes lysis of RBCs and staining of WBCs.

Diluting fluid Turk's fluid is used which has the following composition :

Glacial acetic acid	:	3.0 ml
1% Aqueous gentian violet	:	2.0 ml
Distilled water	:	195 ml

Procedure

- Suck anticoagulated blood or blood from finger prick upto mark 0.5 in WBC pipette (Fig. 46.1, A).
- Wipe tip and outside of the pipette.
- Draw diluting fluid upto mark 11 in the WBC pipette.
- Mix well by rotating the pipette for 2-3 minutes.
- Charge the Neubauer's chamber after discarding 1-2 drops of the mixture from the WBC pipette.
- Allow the cells to settle down for 2 minutes.
- Count the WBCs under low power (10x) in 4 large corner squares (Fig. 46.2). Count the cells lying on left and lower lines while ignoring those on its right and upper lines.

Calculations

Number of WBCs in volume $(1 \times 1 \times 0.1) \text{ mm}^3 = n$

Number of WBCs in $1 \text{ mm}^3 = n \times 10$

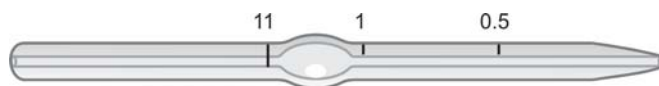
Dilution factor = 20

\therefore WBC count per $\text{mm}^3 (\mu\text{l}) = n \times 200$

Where n is the mean number of WBCs in 4 corner squares.

Precautions

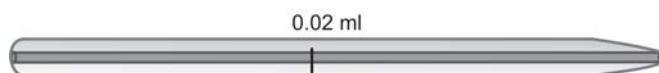
- i. The bench must be free of vibrations and chamber should not be exposed to heat.
- ii. The cover glass should be of special thickness and should have perfectly flat surface.
- iii. The chamber area should be completely filled leaving no air bubbles or debris in the chamber area.
- iv. The fluid should not overflow to the moat.



A, WBC PIPETTE



B, RBC PIPETTE



C, HAEMOGLOBIN PIPETTE

FIGURE 46.1: Pipettes for WBC (A) and RBC counting (B) contrasted with haemoglobin pipette (C).

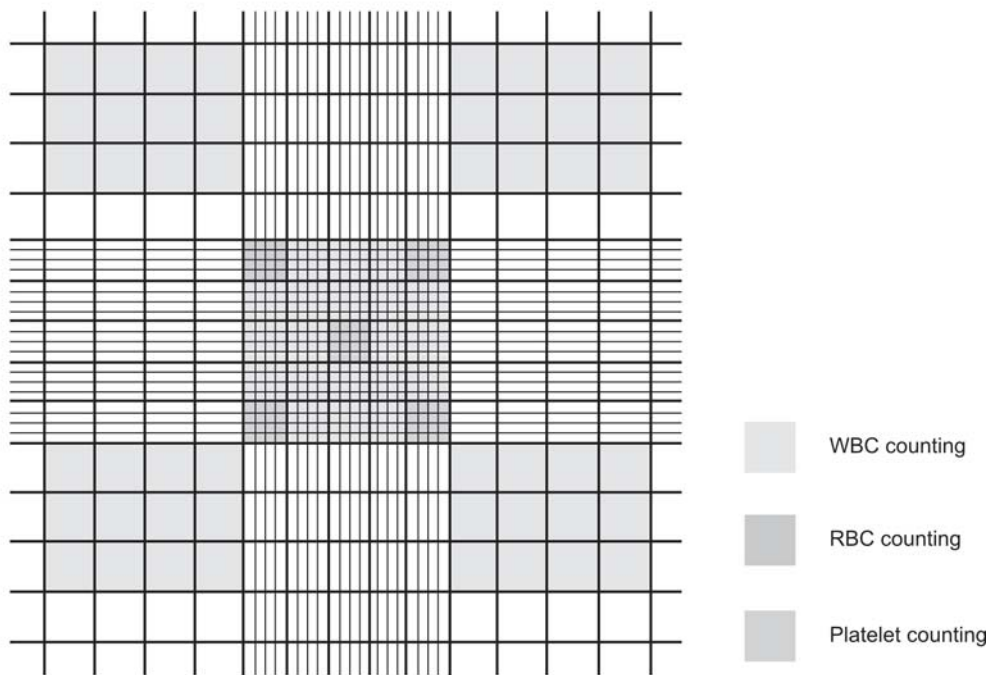


FIGURE 46.2: Improved Neubauer's chamber. Counting areas for WBCs, RBCs and platelets are depicted by different colours diagrammatically though the improved Neubauer's chamber does not have any such colours.

Electronic Method

Electronic counter is based on the principle of aperture impedance method, or light scattering technology, or both. In this, particles passing through a chamber in single file scatter the light and convert by a detector into pulses proportionate to the size of the cells, which are then counted electronically. A lysate is used to lyse red cells so as to count WBCs.

Advantages

- i. Easy and rapid method.
- ii. Time saving method.
- iii. Very large number of cells are counted rapidly.
- iv. There is high level of precision.

Disadvantages

- i. Costly equipment.
- ii. Calibration error.
- iii. Nucleated RBCs are counted as leucocytes.
- iv. Platelet clumps counted as leucocytes.

Normal Range for WBC count

Adults	: 4,000 – 11,000/ μ l
Infants at birth	: 10,000- 26,000/ μ l
Children under 1 year	: 6,000 – 18,000/ μ l

Causes of Abnormal Leucocyte Count

Increased leucocyte count – Leucocytosis

Decreased leucocyte count – Leucopenia

The conditions causing *leucocytosis* and *leucopenia* are given in Exercise 49.

RBC COUNT

This is defined as determination of the number of RBCs per μ l of blood.

Methods for RBC Counts

1. Visual haemocytometer method
2. Electronic method

Visual Haemocytometer Method

Principle This is counting of RBCs in a calibrated chamber by dilution of blood to 1 in 200 dilution with a diluent which is isotonic to blood. The diluent used prevents clotting, clumping and rouleaux formation and does not destroy WBCs.

Diluting fluids

Two types of diluting fluids are used for RBC counting: Hayem's fluid and Dacie's fluid.

Composition of Hayem's fluid

Mercuric chloride	:	0.25 g
Sodium chloride	:	0.5 g
Sodium sulphate	:	2.5 g
Distilled water	:	100 ml

Composition of Dacie's fluid

40% Formaldehyde	:	5 ml
3% Trisodium citrate	:	495 ml

Procedure

- Draw anticoagulated blood or blood from finger prick upto mark 0.5 in RBC pipette (Fig. 46.1, B).
- Wipe tip and outside of the pipette.
- Draw diluting fluid upto mark 101 in the RBC pipette.
- Mix well by rotating the pipette for 2-3 minutes.
- Charge the Neubauer's chamber after discarding 1-2 drops of mixture from the RBC pipette.
- Allow the cells to settle down for 2 minutes.
- Count RBCs under high power 40X in 80 tiny squares (5 × 16 tiny squares) in the centre of the chamber as shown in Fig. 46.2.

Calculations

Volume of area in which

$$\text{RBCs counted in 5 squares} = \left(\frac{1}{5} \times \frac{1}{5} \times 0.1\right) \times 5$$

$$\therefore \text{Number of RBCs in volume} \\ \left(\frac{1 \times 1 \times 0.1}{5}\right) \text{ mm}^3 = n$$

$$\text{Number of RBCs in 1 mm}^3 = n \times 10 \times 5$$

$$\text{Dilution factor} = 200$$

$$\therefore \text{RBC count per mm}^3 (\mu\text{l}) = n \times 50 \times 200 = n \times 10,000$$

Where n is the number of RBCs counted in 5 small squares.

Electronic Method

Principle Principle of electronic method for counting RBCs is the same as for WBCs. But unlike WBC counting, no lysate is used; instead anticoagulated blood is diluted with particle-free diluting fluid such as physiological saline or phosphate buffer saline.

Advantages

- i. Easy and rapid method.
- ii. Many thousands of cells are counted compared to fewer cells counted in manual method.

Disadvantages

- i. Costly equipment.
- ii. Calibration error.

- iii. Altered composition of diluent causes erroneous results.
- iv. Giant platelets are counted as RBCs.
- v. High WBC count alters results.

Normal Range for RBC Count

Males 5.0 – 6.0 million/ μl (5.5 + 0.5 million/ μl)

Females 4.5 – 5.5 million/ μl (5 + 0.5 million/ μl)

Children 4.0 – 5.0 million/ μl (4.5 + 0.5 million/ μl)

Cause of Decreased RBC Count

- i. Anaemia

Cause of Increased RBC Count

- i. Polycythemia

PLATELET COUNT

Platelets are thin discs 2-4 μm in diameter. They function in hemostasis, in maintaining vascular integrity and in the process of blood coagulation. Their life span is 7-10 days.

Methods for Counting Platelets

1. Visual method
2. Electronic method

Visual Method

Type of blood used Use only venous blood as the blood obtained from finger prick causes clumping of platelets.

Diluting fluid 1% ammonium oxalate is prepared as under:

Ammonium oxalate	:	1g
Distilled water	:	100 ml

Filter it and keep in a refrigerator at 4°C.

Procedure

- Using an RBC pipette, prepare a 1:200 dilution as for RBC method (see Fig. 46.1, B).
- Mix for 2 minutes, charge the Neubauer's counting chamber.
- Place the charged Neubauer's chamber into a petridish having a moist filter paper at bottom for allowing the platelets to settle down.
- Count the platelets as for red cell count using 40x objective with reduced condenser aperture.
- If platelet count is low, a WBC pipette can be used for charging the Neubauer's chamber.

Calculations

Number of platelets in 5 squares

$$= \left(\frac{1}{5} \times \frac{1}{5} \times 0.1\right) \times 5$$

∴ Number of platelets in volume

$$\frac{(1 \times 1 \times 0.1) \text{ mm}^3}{5} = n$$

Number of platelets per mm³ = n × 50

Dilution factor = 200

∴ Platelet count per mm³ (μl) = n × 50 × 200

$$= n \times 10,000$$

A phase contrast microscope can be used for platelet counting which gives better results.

Rough Visual Method for Platelet Counting

Prepare a thin peripheral blood film, stain it with any of the Romanowsky stain. Dry it and examine under high power. If you find one clump of platelet per high power field, then number of platelets is adequate; roughly each platelet under high power represents count of 25,000 platelets per mm³.

Electronic Method

Platelets can be counted by electronic particle counter method which implies electrical impedance principle as for counting RBCs.

Disadvantages

- i. Equipment is costly.
- ii. Calibration error.
- iii. Debris counted as platelets.
- iv. Heinz bodies and Howell-Jolly bodies can be counted as platelets.

Normal Platelet Count

1,50,000-400,000/μl

Conditions causing abnormal platelet counts

Decreased count is termed thrombocytopenia

Increased count is termed thrombocytosis.

Conditions causing Thrombocytopenia**1. Impaired platelet production:**

- i. Aplastic anaemia
- ii. Acute leukaemias
- iii. Myelofibrosis
- iv. Marrow infiltration by malignancy
- v. Drugs (e.g. chloramphenicol, thiazides, anticancer drugs)
- vi. Chronic alcoholism

2. Accelerated platelet destruction:

- i. ITP
- ii. SLE
- iii. AIDS
- iv. CLL
- v. DIC
- vi. Giant haemangioma
- vii. Drug-induced (e.g. sulfonamides, quinine, gold)
- viii. Microangiopathic haemolytic anaemia
- ix. Splenomegaly
- x. Massive transfusion of blood

Conditions Causing Thrombocytosis

- i. Essential thrombocytosis
- ii. Chronic infection
- iii. Haemorrhage
- iv. Postoperative state
- v. Malignancy
- vi. Post-splenectomy.



Reticulocyte Count

- ◆ Reticulocytes
- ◆ Methods for Counting of Reticulocytes

Exercise

47

RETICULOCYTES

Reticulocytes are juvenile or immature non-nucleated red cells which still contain the remains of RNA protein and continue to synthesise haemoglobin after loss of nucleus. Their number in the peripheral blood is an accurate reflection of erythropoietic activity in the bone marrow. An increased erythropoietic stimulus leads to premature release of juvenile RBCs (reticulocytes) into the circulation. Time taken by reticulocytes to mature into RBCs is approximately 1-2 days.

METHODS FOR COUNTING OF RETICULOCYTES

1. Visual method
2. Automated method

Visual Method

Principle When blood is briefly incubated in a solution of new methylene blue or brilliant cresyl blue, the RNA is precipitated as dye-RNA protein complex. This reaction takes place only in vitally stained unfixed preparations i.e. *supravital staining* done on living cells rather than on air-dried blood smears.

Preparation of staining solution 1% New methylene blue is prepared as under:

New methylene blue : 1.0 g
Iso-osmotic phosphate buffer (pH 7.4) : 100 ml
Dissolve in a dark bottle and filter before use.

Procedure

- Take 2-3 drops of new methylene blue in a small tube.
- Add 4-8 drops of patient's EDTA blood and mix.
- Incubate at 37°C for 15-20 minutes.

- Remix the tube contents.
- Place a drop of stained blood on a clean glass slide and make a thin blood film.
- Dry and examine under oil immersion.

Counting of Reticulocytes

Microscopically, reticulocytes have dark blue network or dark blue granules while RBCs appear pale blue or blue green (Fig. 47.1). Select a well spread portion of the smear in which the red cells are just overlapping. Count at least 100 reticulocytes. For counting of reticulocytes present per 100 RBCs, the visual field is restricted to manageable counts. This is achieved by either using

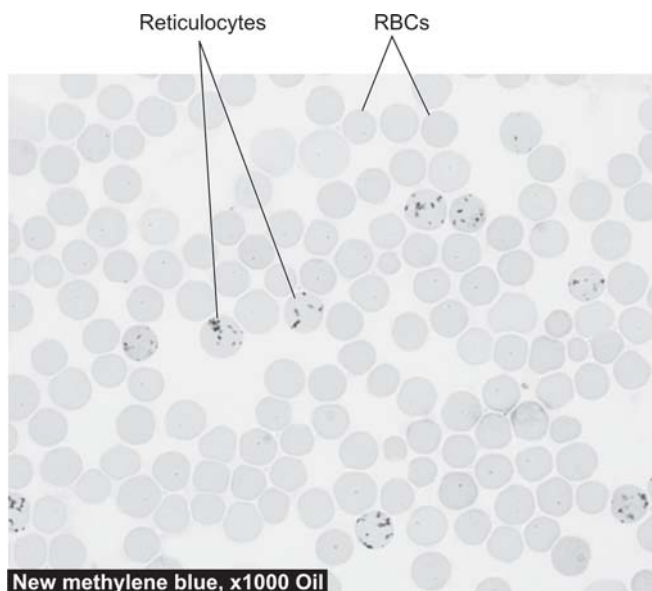


FIGURE 47.1: Reticulocytes contrasted with mature RBCs (New methylene blue, x 1000 oil).

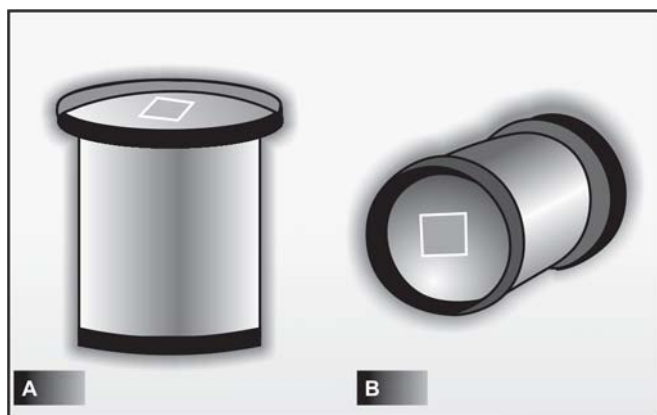


FIGURE 47.2: Ehrlich's eyepiece as viewed from top (A) and as viewed from below (B).

Ehrlich's eye piece having adjustable square window in it, or alternatively a round piece of thick paper of the diameter of eyepiece with a small square window of 4 mm diameter in it can be used (Fig. 47.2).

Calculations

Number of reticulocytes in n fields = x

Average number of cells/field = y

Total number of cells in n fields = $n \times y$

Reticulocyte count (%) = $\frac{x}{n \times y} \times 100$

Absolute number of reticulocytes per μl =

$$\frac{\text{Reticulocyte count}}{100} \times \text{RBC count}$$

Automated Method

Reticulocytes can be counted by automated reticulocyte analyser or by flow cytometry by using fluorescent dyes (e.g. auramine O, thiazole orange, acridine orange and thioflavine T) or non-fluorescent dyes (e.g. oxazine). Advantage of automatic equipment is that it is superior to that of manual counts since many more cells are counted and the subjectivity in recognition of reticulocytes is eliminated. However, automated equipment is costly and Howell-Jolly bodies, Heinz bodies, Pappenheimer bodies and giant platelets are counted as reticulocytes.

Normal Values

Infants	: 2-6%
Adults and children	: 0.5 – 2.5%
Absolute reticulocyte count	: 25,000-75,000/ μl

Abnormal Counts

Increased count (Reticulocytosis)

- After haemorrhage
- Haemolysis
- Haematopoietic response of anaemia to treatment

Decreased counts (Reticulocytopenia)

- Ineffective erythropoiesis.
- Aplastic anaemia.
- Thalassemia.
- Myelosclerosis.



Preparation of Peripheral Blood Film, Staining and DLC

- ◆ Thin Blood Film
- ◆ Thick Blood Film
- ◆ Various Stains for PBF
- ◆ Examination of PBF for DLC
- ◆ Morphologic Identification of Mature Leucocytes

Exercise

48

The peripheral blood film (PBF) is of two types:

1. Thin blood film
2. Thick blood film

THIN BLOOD FILM

Thin PBF can be prepared from anticoagulated blood obtained by venepuncture or from free flowing finger prick blood by any of the following three techniques :

1. Slide method
2. Coverglass method
3. Spin method

Slide Method

Procedure

- Place a drop of blood in the centre of a clean glass slide 1 to 2 cm from one end.
- Place another slide (spreader) with smooth edge at an angle of 30-45° near the drop of blood.

- Move the spreader backward so that it makes contact with drop of blood.
- Then move the spreader forward rapidly over the slide.
- A thin peripheral blood film is thus prepared (Fig. 48.1).
- Dry it and stain it.

Qualities of a Good Blood Film

- i. It should not cover the entire surface of slide.
- ii. It should have smooth and even appearance.
- iii. It should be free from waves and holes.
- iv. It should not have irregular tail.

Parts of a Thin Blood Film

A PBF consists of 3 parts (Fig. 48.2):

1. *Head* i.e. the portion of blood film near the drop of blood.
2. *Body* i.e. the main part of the blood film.
3. *Tail* i.e. the tapering end of the blood film.

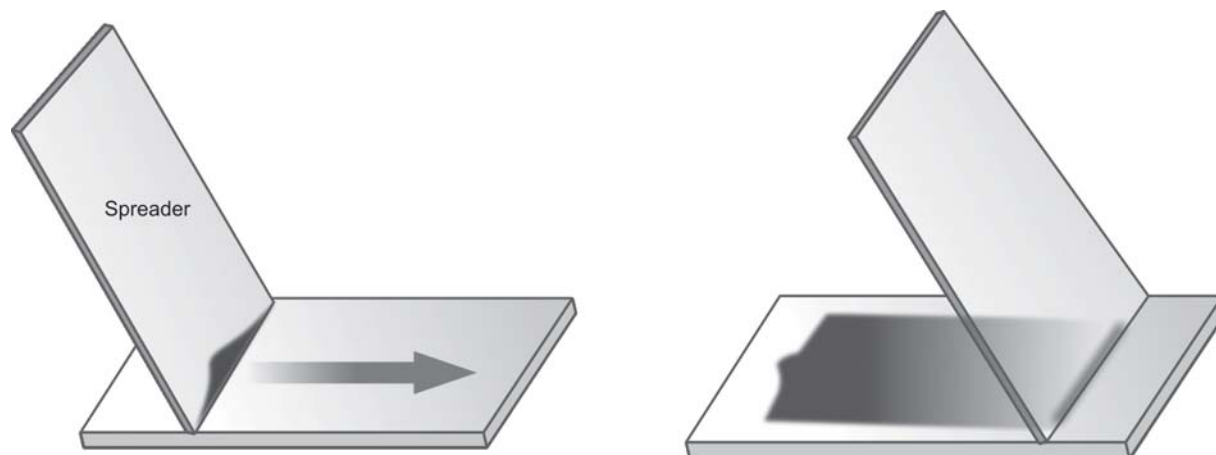


FIGURE 48.1: Method of making thin PBF by slide method.

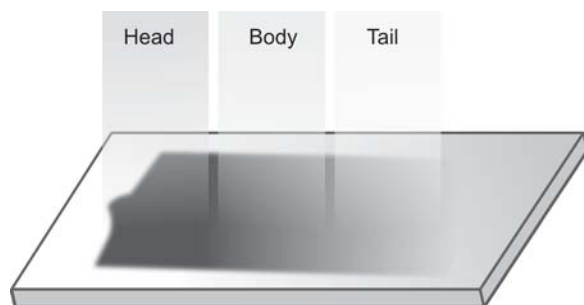


FIGURE 48.2: Parts of a thin blood film.

Cover Glass Method

Procedure

- Take a No.1 (22 mm square) clean cover glass.
- Touch it on to the drop of a blood.
- Place it on another similar coverglass in crosswise direction with side containing drop of blood facing down.
- Pull the coverglass quickly.
- Dry it and stain it.
- Mount it with a mountant, film side down on a clean glass slide.

Spin Method

This is an automated method.

Procedure

- Place a drop of blood in the centre of a glass slide.
- Spin at a high speed in a special centrifuge, cytospin.
- Blood spreads uniformly.
- Dry it and stain it.

THICK BLOOD FILM

This is prepared for detecting blood parasites such as malaria and microfilaria.

Procedure

- Place a large drop of blood in the centre of a clean glass slide.
- Spread it in a circular area of 1.5 cm with the help of a stick or end of another glass slide.
- Dry it and you should be able to just see the printed matter through the smear, when kept on printed paper.

VARIOUS STAINS FOR PBF

Romanowsky stains are universally employed for staining of blood films. All Romanowsky combinations have two

essential ingredients i.e. methylene blue and eosin or azure. Methylene blue is the basic dye and has affinity for acidic component of the cell (i.e. nucleus) and eosin/azure is the acidic dye and has affinity for basic component of cell (i.e. cytoplasm).

Most Romanowsky stains are prepared in methyl alcohol so that they combine fixation and staining.

Various stains included under Romanowsky stain are as under :

- i. Leishman stain
- ii. Giemsa stain
- iii. Wright stain
- iv. Field stain
- v. Jenner stain
- vi. JSB stain

Staining of Thin Blood Film

Leishman Stain

Preparation Dissolve 0.2 g of powdered Leishman's dye in 100 ml of acetone-free methyl alcohol in a conical flask. Warm it to 50°C for half an hour with occasional shaking. Cool it and filter it.

Procedure for staining

- Pour Leishman's stain dropwise (counting the drops) on the slide and wait for 2 minutes. This allows fixation of the PBF in methyl alcohol.
- Add double the quantity of buffered water dropwise over the slide (i.e. double the number of drops).
- Mix by rocking for 8 minutes.
- Wash in water for 1 to 2 minutes.
- Dry in air and examine under oil immersion lens of the microscope.

Giemsa Stain

Preparation Stock solution of Giemsa stain is prepared by mixing 0.15 g of Giemsa powder in 12.5 ml of glycerine and 12.5 ml of methyl alcohol. Before use dissolve one volume of stock solution in nine volumes of buffered water (dilution 1:9).

Procedure

- Pour diluted stain over slide.
- Wait for 15-60 minutes.
- Wash in water.
- Dry it and examine under oil immersion lens of the microscope.

Staining of Thick Smear

It can be stained with any of the Romanowsky stains listed above except that before staining, the smear is

dehaemoglobinised by putting it in distilled water for 10 minutes.

Autostainers

Currently, automatic staining machines are available which enable a large batch of slides to be stained with a uniform quality.

Precautions in Staining of PBF

1. *Dark blue blood film:* It can be due to overstaining, inadequate washing or improper pH of the buffer. In this RBCs are blue, nuclear chromatin is black, granules of the neutrophils are overstained and granules of the eosinophils are blue or grey.
2. *Light pink blood film:* In this RBCs are bright red, the nuclear chromatin is pale blue and granules of the eosinophils are dark red. It can be due to understaining, prolonged washing, mounting the film before drying and improper pH of the buffer.
3. *Precipitate on the blood film:* This could be due to inadequate filtration of the stain, dust on the slide, drying during staining and inadequate washing.

EXAMINATION OF PBF FOR DLC

Choose an area near the junction of body with the tail of the smear where there is slight overlapping of RBCs i.e. neither rouleaux formation which occurs in head and body, nor totally scattered RBCs as occurs at the tail. By moving the slide in horizontal directions under oil immersion (Fig. 48.3), start counting the types of WBCs and go on entering P, L, M, E, B in a box having 100 cubes as shown in Figure 48.4. Alternatively 100 leucocytes can be counted by pressing the keys of the automated DLC counter (Fig. 48.5). Zigzag counting of WBCs is discouraged. WBCs are then expressed as percent in the following sequence: polymorphonuclear leucocytes (P), lymphocytes (L), monocytes (M), eosinophils (E), basophils (B) i.e. P, L, M, E, B. Invariably, normal range is expressed alongside the results (Table 48.1).

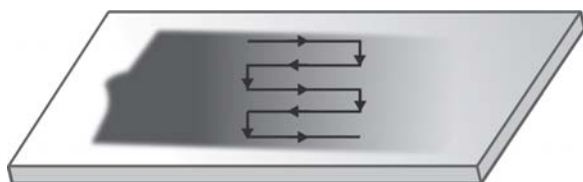


FIGURE 48.3: Counting of cells in PBF by moving in horizontal direction at the junction of the body with the tail of PBF.

Table 48.1: Normal values for leucocytes in health in adults

	Normal range	Absolute value
Polymorphs(P)	40-75%	2,000-7,500/ μ l
Lymphocytes(L)	20-40%	1,500-4,000/ μ l
Monocytes(M)	2-10%	200-800/ μ l
Eosinophils(E)	1-6%	40-400/ μ l
Basophils(B)	0-1%	10-100/ μ l

MORPHOLOGIC IDENTIFICATION OF MATURE LEUCOCYTES

Polymorph (Neutrophil)

A polymorphonuclear neutrophil (PMN), commonly called polymorph or neutrophil, is 12-15 μ m in diameter. It consists of a characteristic dense nucleus, having 2-5 lobes and pale cytoplasm containing numerous fine violet-pink granules.

L	P	P	L	P	L	P	P	L	P
P	L	P	P	L	P	P	L	P	P
P	P	P	L	P	P	L	P	P	L
P	P	P	L	P	P	P	L	P	P
L	P	L	P	M	L	L	P	M	P
P	B	E	P	P	L	P	E	P	P
P	M	P	L	L	P	L	P	L	L
L	P	E	P	P	P	L	L	P	P
P	P	P	L	P	L	P	P	L	P
P	L	L	P	P	M	P	L	P	L

Result of DLC

P	L	M	E	B
60%	32%	4%	1%	3%

FIGURE 48.4: Counting of WBCs for DLC in squares and expressing result of DLC.

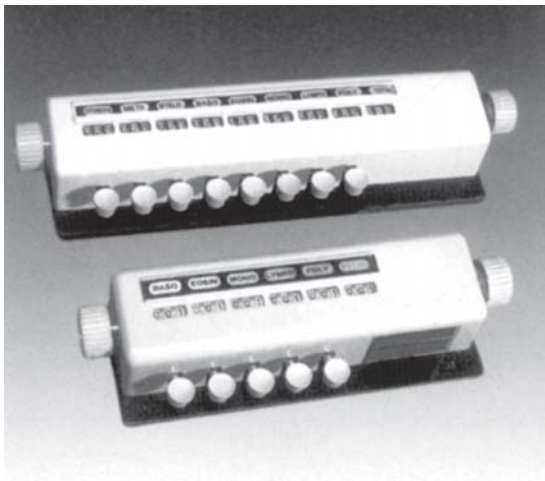


FIGURE 48.5: Counting of WBCs for DLC in DLC counters with pressing keys (Photography by courtesy of Yorco Sales Pvt. Ltd., Delhi).

Lymphocyte

Majority of lymphocytes in the peripheral blood are small (9-12 μm in diameter) but large lymphocytes (12-16 μm in diameter) are also found. Both small and large lymphocytes have round or slightly indented nucleus

with coarsely clumped chromatin and scanty basophilic and agranular cytoplasm.

Monocyte

The monocyte is the largest mature leucocyte in the peripheral blood measuring 12-20 μm in diameter. It possesses a large, central, oval, notched or indented or horseshoe-shaped nucleus which has characteristically fine reticulated chromatin network. The cytoplasm is abundant, pale blue and contains many fine granules and vacuoles.

Eosinophil

Eosinophil is similar to segmented neutrophil in size (12-15 μm in diameter) but has coarse, deep red staining granules in the cytoplasm and has usually two nuclear lobes in the form of a spectacle.

Basophil

Basophil resembles the other mature granulocytes but is distinguished by coarse, intensely basophilic granules which usually fill the cytoplasm and often overlap and obscure the nucleus.

Morphological features of different leucocytes are summarised in Table 48.2.

TABLE 48.2: Morphology of mature leucocytes.					
Feature	Neutrophil	Lymphocytes (small and large)	Monocyte	Eosinophil	Basophil
Morphology					
Cell diameter	12-15 μm	SL: 9-12 μm LL: 12-16 μm	12-20 μm	12-15 μm	12-15 μm
Nucleus	2-5 lobed, clumped chromatin	Large nucleus, round to indented, fills the cell, clumped chromatin	Large, lobulated, indented, with fine chromatin	Bilobed, clumped chromatin	Bilobed, clumped chromatin
Cytoplasm	Pink or violet granules	Peripheral rim of basophilic cytoplasm, no granules	Light basophilic, may contain fine granules or vacuoles	Coarse crimson red granules	Large coarse purplish granules obscuring the nucleus
Normal %	40-75	20-40	2-10	1-6	0-1
Absolute count per μl	2,000-7,500	1,500-4,000	200-800	40-400	10-100



DLC in Cases with Leucocytosis

- ◆ Visual Counting
- ◆ Automated Counting
- ◆ Pathologic Variations in DLC

Exercise

49

Differential leucocyte count (DLC) can be performed by two methods:

1. Visual counting
2. Automated counting

VISUAL COUNTING

This is counting of WBCs after identifying them by their morphologic features as discussed in the preceding exercise.

AUTOMATED COUNTING

It is done by electronic counting method. There are three types of electronic methods—by cell size analysis, by flow cytometry, and by high resolution pattern recognition. In addition to counting, these methods also provide additional information on cell size, shape, nuclear size and density. Automated DLC counters have a differential counting capacity of counting either 3-part DLC (granulocytes, lymphocytes and monocytes) or 5-part DLC (P, L, M, E, B). However, automated method of DLC suffers from the disadvantage that normoblasts are counted as lymphocytes and these counters are quite expensive.

PATHOLOGIC VARIATIONS IN DLC

Neutrophils

Increase in neutrophil count above 7,500/ μ l is called *neutrophilia* (Fig. 49.1) while fall in neutrophil count below 2,000/ μ l is termed neutropenia. The causes for neutrophilia and neutropenia are given in Table 49.1.

Table 49.1: Causes of neutrophilia and neutropenia.

<i>Neutrophilia</i>	<i>Neutropenia</i>
<ol style="list-style-type: none"> 1. <i>Acute infections</i> (By bacteria, fungi, parasites and some viruses) <ol style="list-style-type: none"> i. Pneumonia ii. Acute appendicitis iii. Acute cholecystitis iv. Salpingitis v. Peritonitis vi. Abscess vii. Acute tonsillitis viii. Actinomycosis ix. Poliomyelitis x. Furuncle xi. Carbuncle 2. <i>Intoxication</i> <ol style="list-style-type: none"> i. Uraemia ii. Diabetic ketosis iii. Poisoning by chemicals iv. Eclampsia 3. <i>Inflammation from tissue damage</i> <ol style="list-style-type: none"> i. Burns ii. Ischaemic necrosis iii. Gout iv. Hypersensitivity reaction 4. <i>Acute haemorrhage</i> <ol style="list-style-type: none"> i. Acute haemolysis 5. <i>Neoplastic conditions</i> <ol style="list-style-type: none"> i. Myeloid leukaemia (CML) ii. Polycythaemia vera iii. Myelofibrosis iv. Disseminated cancers 6. <i>Miscellaneous conditions</i> <ol style="list-style-type: none"> i. Administration of corticosteroids ii. Idiopathic neutrophilia 	<ol style="list-style-type: none"> 1. <i>Infections</i> <ol style="list-style-type: none"> i. Typhoid ii. Brucellosis iii. Measles iv. Malaria v. Kala azar vi. Miliary tuberculosis 2. <i>Drugs and chemicals and physical agents</i> <ol style="list-style-type: none"> i. Antimetabolites ii. Benzene iii. Nitrogen mustard iv. Irradiation 3. <i>Haematological and other diseases</i> <ol style="list-style-type: none"> i. Aplastic anaemia ii. Pernicious anaemia iii. SLE iv. Gaucher's disease v. Cachexia vi. Anaphylactic shock

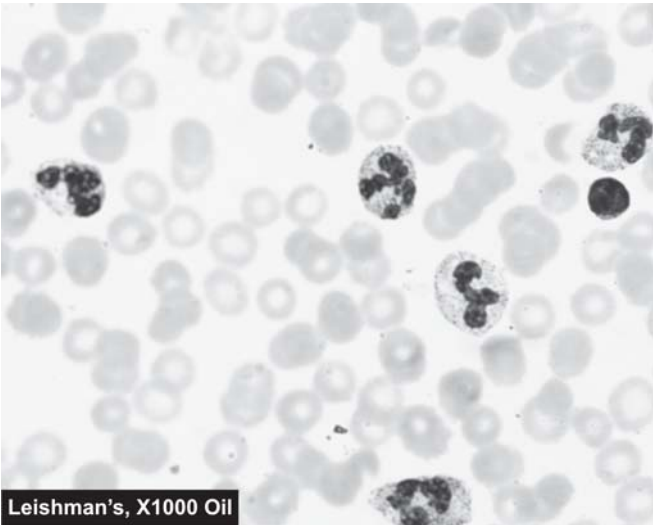


FIGURE 49.1: Neutrophilia in PBF.

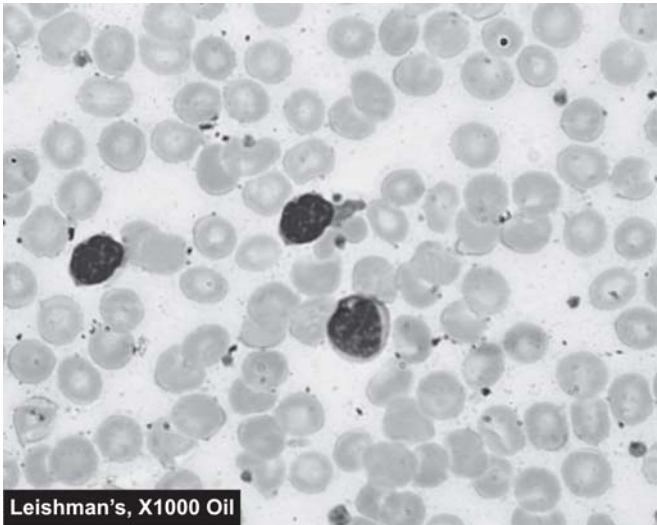


FIGURE 49.2: Lymphocytosis in PBF.

Lymphocytes

When the absolute lymphocyte count increases to more than 4,000/ μ l it is termed *lymphocytosis* (Fig. 49.2) while absolute lymphocyte count below 1,500/ μ l is called *lymphopenia*; causes for these are given in Table 49.2.

Table 49.2: Causes of lymphocytosis and lymphopenia.	
Lymphocytosis	Lymphopenia
1. <i>Acute Infections</i> <ul style="list-style-type: none">i. Pertussisii. Infectious mononucleosisiii. Viral hepatitis	i. Aplastic anaemia
2. <i>Chronic Infections</i> <ul style="list-style-type: none">i. Tuberculosisii. Brucellosisiii. Secondary syphilis	ii. High dose of steroid administration
3. <i>Haematopoietic Disorders</i> <ul style="list-style-type: none">i. CLLii. NHL	iii. AIDS
	iv. Hodgkin's disease
	v. Irradiation

Monocytes

A rise in absolute monocyte count above 800/ μ l is called *monocytosis* (Fig. 49.3). Causes of monocytosis are given in Table 49.3.

Eosinophils

Increase in the absolute eosinophil count above 400/ μ l is termed *eosinophilia* (Fig. 49.4) while the fall in number

is called *eosinopenia*; the causes for abnormal eosinophil count are given in Table 49.4.

Basophil

Basophilia refers to an increase in the absolute basophil count above 100/ μ l (Fig. 49.5). Causes of basophilia are given in Table 49.5.

Table 49.3: Monocytosis
1. <i>Bacterial infections</i> <ul style="list-style-type: none">i. Tuberculosisii. SABAiii. Syphilis
2. <i>Protozoal infections</i> <ul style="list-style-type: none">i. Malariaii Kala azariii. Trypanosomiasis
3. <i>Haematopoietic disorders</i> <ul style="list-style-type: none">i. Monocytic leukaemiaii. Hodgkin's diseaseiii. Multiple myelomaiv. Myeloproliferative disorders
4. <i>Miscellaneous conditions</i> <ul style="list-style-type: none">i. Sarcoidosisii. Cancer of ovary, breast, stomach

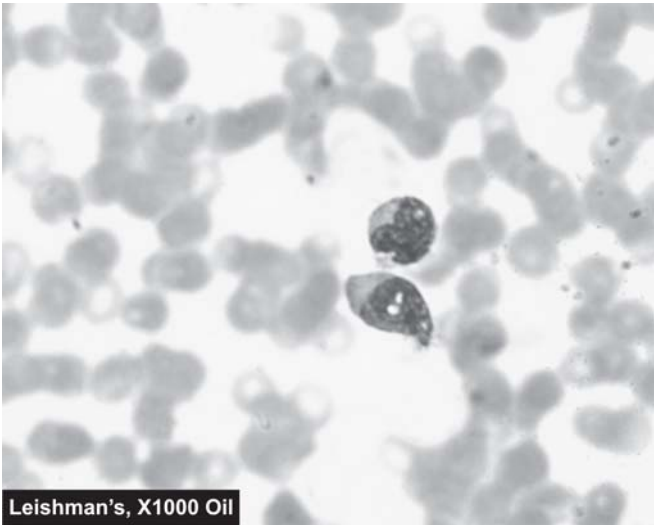


FIGURE 49.3: Monocytes in PBF.

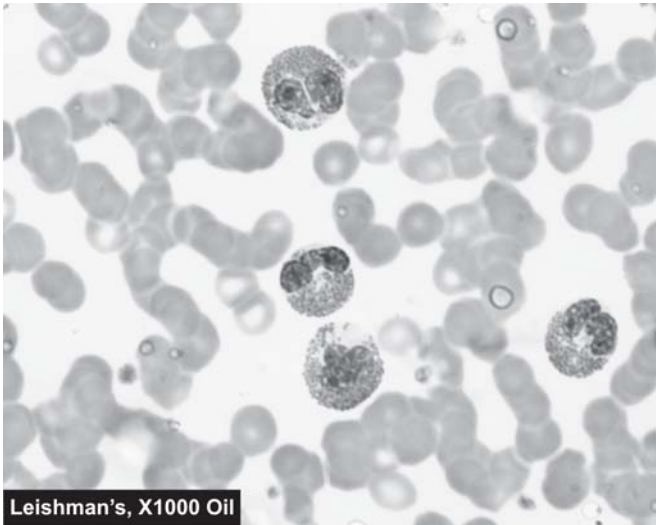


FIGURE 49.4: Eosinophilia in PBF.

Table 49.4: Causes of eosinophilia and eosinopenia.	
Eosinophilia	Eosinopenia
1. Allergic disorders	Steroid administration
i. Bronchial asthma	
ii. Urticaria	
iii. Hay fever	
iv. Drug hypersensitivity	
2. Parasitic infestations	
i. Round worm	
ii. Hookworm	
iii. Tape worm	
iv. Echinococcosis	
3. Skin diseases	
i. Pemphigus	
ii. Dermatitis herpetiformis	
iii. Erythema multiforme	
4. Pulmonary diseases	
i. Loeffler's syndrome	
ii. Tropical eosinophilia	
5. Haematopoietic diseases	
i. Chronic myeloid leukaemia	
ii. Polycythaemia vera	
iii. Hodgkin's disease	
iv. Pernicious anaemia	
6. Miscellaneous conditions	
i. Rheumatoid arthritis	
ii. Polyarteritis nodosa	
iii. Sarcoidosis	
iv. Irradiation	

Table 49.5: Basophilia
i. Chronic myeloid leukemia
ii. Polycythaemia vera
iii. Myxoedema
iv. Ulcerative colitis
v. Hodgkin's disease
vi. Urticaria pigmentosa



FIGURE 49.5: Basophil in PBF.



ESR, PCV (Haematocrit) and Absolute Values

- ◆ Erythrocyte Sedimentation Rate (ESR)
- ◆ Packed Cell Volume (PCV) or Haematocrit
- ◆ Absolute Values

Exercise

50

ERYTHROCYTE SEDIMENTATION RATE (ESR)

ESR is used as an index for presence of an active disease which could be due to many causes.

Principle

When well mixed anticoagulated blood is placed in a vertical tube, the erythrocytes tend to fall towards the bottom of the tube/pipette till they form a packed column in the lower part of the tube in a given time.

Mechanism of ESR

Fall of RBCs depends upon following factors:

- Rouleaux formation
- Concentration of fibrinogen in plasma
- Concentration of α and β globulins
- Length of the tube
- Ratio of red cells to plasma
- Bore of the tube
- Position of the tube

i) Rouleaux formation The erythrocytes sediment in the tube/pipette because their density is greater than that of plasma. When a number of erythrocytes aggregate in the form of rouleaux and settle down, their area is much less than that of the sum of the area of constituent corpuscles. The rouleaux formation is very important factor which increases the ESR.

ii) Concentration of fibrinogen It leads to colloidal changes in plasma which cause increased viscosity of plasma. Concentration of fibrinogen parallels ESR. If concentration of fibrinogen is raised, ESR is increased. In defibrinated blood, ESR is very low.

iii) Concentration of α and β globulins These protein molecules have a greater effect than other proteins in

decreasing the negative charge of the RBCs that tends to keep them apart thus promoting rouleaux formation. Albumin retards the ESR; thus conditions where albumin is low ESR is more.

iv) Ratio of red cells to plasma The change in the ratio of RBCs to plasma affects ESR. When plasma is more, ESR will be increased, and vice versa.

v) Length of the tube If length of the tube/pipette is more, RBCs will have to travel a longer distance and thus ESR is low than when length of the tube is short, and vice versa.

vi) Bore of the tube If bore of the tube is more the negative charge which keeps the RBCs apart will be less and ESR will be more, and vice versa.

vii) Position of the tube If the tube/pipette is not vertical, the RBCs will have to travel less distance and ESR will be more.

Phases in ESR

ESR takes place in the following 3 phases which are carried out in one hour:

- *Phase of rouleaux formation:* In the initial period of 10 minutes, the process of rouleaux formation occurs and there is little sedimentation.
- *Phase of settling:* In the next 40 minutes, settling of RBCs occurs at a constant rate.
- *Phase of packing:* In the last 10 minutes sedimentation slows and packing of the RBCs to the bottom occurs.

That is why ESR by all methods is expressed as mm first hour rather than per hour.

Methods of ESR

1. Westergren's method
2. Wintrobe's method
3. Micro ESR method
4. Automated methods

1. Westergren's Method

Owing to its simplicity this method used to be the most commonly employed standard method prior to the AIDS-era. Westergren's pipette is a straight pipette 30 cm long open at both ends with internal bore diameter of 2.5 mm and is calibrated from 0-200 mm from top to bottom (Fig. 50.1,A).

Anticoagulant Trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) as 3.8 g/dl liquid anticoagulant is used. It is used in the concentration of 1:4 i.e. four parts of blood are added to one part of anticoagulant.

Procedure

- The patient is advised to come in the morning fasting (as heavy protein diet affects concentration of plasma proteins).
- Take 1.6 ml of patient's blood and mix it with 0.4 ml of citrate anticoagulant already put in a tube. The test should be done within two hours of taking blood.
- Fill the pipette upto mark O with citrated blood with the help of rubber teat by vacuum filling and fix it in a rack vertically away from sun light or vibrations.
- Let it stand for one hour after which reading is taken at the upper meniscus of the RBCs.

Normal values

Males 3-5 mm 1st hour
Females 4-7 mm 1st hour

Advantages

- i. It is a more sensitive method.
- ii. It is easy to fill and clean the Westergren's pipette.

Disadvantages

- i. Requires more amount of blood.
- ii. Dilution of blood in anticoagulant affects ESR.
- iii. Filling of blood by mouth pipetting should be strictly discouraged.

2. Wintrobe's Method

The Wintrobe tube is a glass tube closed at one end. The tube is 110 mm long and has an internal bore

diameter of 2.5 mm. The tube is graduated on both sides : from 0 to 10 on one side and 10 to 0 cm on the other (Fig. 50.1,B).

Anticoagulants Either of the following 2 anticoagulants can be used:

- i. Ethylene diamine tetraacetic acid (EDTA) solid crystals 1-2 mg/ml.
- ii. Double oxalate (solid) 2-3 mg/ml (ammonium oxalate and sodium or potassium oxalate in the ratio of 3:2; the former causes swelling and the latter causes shrinkage of RBCs and hence RBC shape is retained).

Procedure

- The patient is called in the morning fasting.
- Draw 1 ml of blood into the anticoagulant.
- Fill the Wintrobe tube upto mark 0 with anticoagulated blood with the help of a Pasteur pipette having a long stem (15 cm) so as to fill the tube free of air bubbles (Fig. 50.1,B).
- Place the tube vertically in a stand and note the ESR after one hour.

Normal values

Males 0-7 mm 1st hour
Females 0-15 mm 1st hour

Advantages

- i. It is simple method and requires small amount of blood.
- ii. There is no dilution with anticoagulant.
- iii. Packed cell volume (PCV) can also be done by the same tube.
- iv. Filling of tube with Pasteur pipette eliminates chance of any infection due to handling of blood.

Disadvantages

- i. Because of short column and choice of anticoagulant, it is not as sensitive index of diseases.
- ii. Addition of more anticoagulant can lower ESR.
- iii. ESR of more than 100 mm can not be measured.

3. Micro ESR Method

This method is used in pediatric patients or in patients where venepuncture is not possible. In this method a capillary 160 mm long with an internal bore diameter of 1 mm is used. The capillary is graduated 1 mm apart for 50 mm, with two red lines on it. Alternatively, non-graduated heparinised capillary may be used and the reading is taken by measurement of length of column (Fig. 50.1,C).

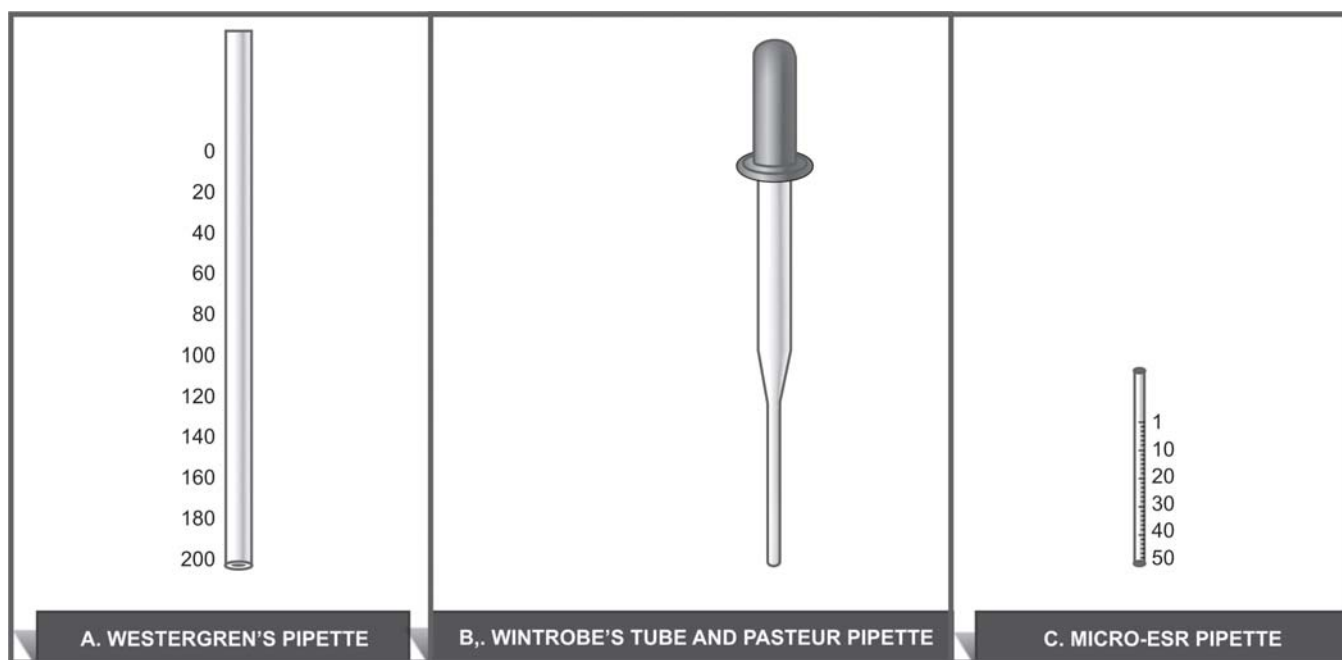


FIGURE 50.1: Westergren's pipette, Wintrobe's tube with Pasteur pipette and micro ESR pipette.

Anticoagulant Mixture of sodium citrate and EDTA is used.

Procedure

- Fill the microsedimentation pipette upto first red mark with anticoagulant.
- Fill the pipette with free flowing capillary blood upto second red mark.
- Invert it several times and allow it to stand for one hour in the sedimentation rack.
- Take the reading and results are given as that for Westergren's method.

4. Automated ESR Method

Automated closed systems use either blood collected in special evacuated tubes containing citrate or EDTA. It is taken up through a pierceable cap and then automatically diluted in the system.

Clinical Significance of ESR

ESR is a non-specific method of evaluating diseases. It is seldom used for diagnostic purpose but its use is limited to monitoring the prognosis of disease process.

Diagnostic Uses

- i. Rheumatoid arthritis
- ii. Chronic infections
- iii. Collagen diseases

- iv. Multiple myeloma
- v. Macroglobulinaemia

Monitoring Prognosis of Diseases

To see the response to treatment in:

- i. Tuberculosis
- ii. Temporal arteritis
- iii. Polymyalgia rheumatica
- iv. In patients of Hodgkin's disease, ESR of < 10 mm 1st hour indicates good prognosis while ESR of > 60 mm 1st hour indicates poor prognosis.

Table 50.1 sums up the list of conditions causing raised and lowered ESR.

Table 50.1: Causes of abnormal ESR	
Diseases causing raised ESR	Diseases causing low ESR
i. Tuberculosis	i. Polycythaemia
ii. SABE	ii. Spherocytosis
iii. Acute myocardial infarction	iii. Sickle cell anaemia
iv. Rheumatoid arthritis	iv. Congestive heart failure
v. Shock	v. Newborn infant
vi. Anaemias	vi. Hypofibrinogenaemia
vii. Liver disease	
viii. Multiple myeloma	
ix. Pregnancy	
x. Ankylosing spondylitis	

PACKED CELL VOLUME (PCV) OR HAEMATOCRIT

PCV is defined as ratio of volume of RBCs to that of whole blood and is expressed as percentage.

Methods for Estimation of PCV

1. Macro method (Wintrobe's method)
2. Micro haematocrit method
3. Electronic method

1. Macro (Wintrobe's) Method

In this method PCV is measured by Wintrobe tube which has a length of 110 mm and internal bore of 2.5 mm and graduated from 0–10 cm on both directions (as discussed in preceding section of this exercise).

Procedure

- Fill the Wintrobe tube upto mark 10 with well mixed anticoagulated blood (EDTA) by Pasteur's pipette free of air bubbles.
- Centrifuge the tube at 2000-2300 g for 30 minutes.
- After centrifugation layers are noted in the wintrobe tube as under (Fig. 50.2):
 - i. Uppermost layer of *plasma*.
 - ii. Thin white layer of *platelets*.
 - iii. Greyish-pink layer of *leucocytes*.
 - iv. Lowermost is the layer of *RBCs*.
 - v. Grey-white layer of leucocytes and platelets interposed between plasma above and packed RBCs below is called *buffy coat*.
- Note the lowermost height of column of packed RBC layer and express it as percentage.

Advantages of Macro Method

- i. PCV and ESR can be measured simultaneously.
- ii. Buffy coat can be prepared for other tests.
- iii. By seeing the colour of plasma we can know about some of the pathological conditions e.g. in jaundice it is yellow, in haemolysis it is pink, in hyperlipidaemia it is milky.

2. Microhaematocrit Method

In this method a capillary tube 70 mm long with an internal bore of 1 mm is used and blood from skin puncture is directly taken into heparinised capillary tube.

Procedure

- Take a heparinised capillary tube.
- Fill it with blood by capillary action leaving 10 mm unfilled.
- Seal the empty end by plastic seal or by heating on flame.
- Centrifuge it in microhaematocrit centrifuge at 10,000 g for 5 minutes (Fig. 50.3).

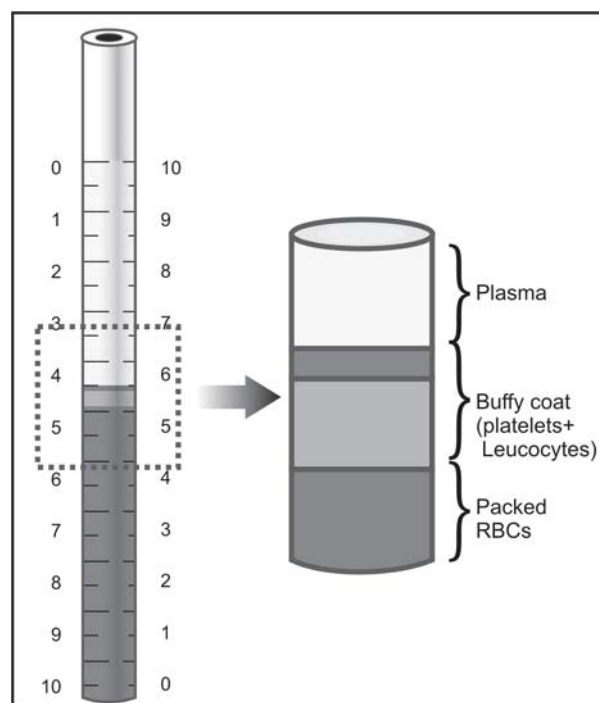


FIGURE 50.2: Haematocrit by Wintrobe's tube method.



FIGURE 50.3: Microhaematocrit method for PCV. Haematocrit 20 model (Photograph courtesy of Hetlich, Germany through Global Medical System, Delhi).

- Measure the blood column by using a reading device which is usually a part of centrifuge.

Advantages of Micro Method

- Less amount of blood is required.
- Results are available within 5 minutes.
- Method is more accurate, trapping of plasma is less.

Sources of Errors in Macro and Micro Methods

- Improper handling of sample.
- Calibration error.
- Unclean and contaminated tube.
- Improper centrifugation time.

3. Electronic Method

Electronic methods employ automated counters where derivation of RBC count, PCV and MCV are closely interrelated.

Clinical Significance of PCV

PCV reflects the concentration of red cells and not the total red cell mass. Table 50.2 lists the conditions causing raised and lowered PCV.

ABSOLUTE VALUES

Based on normal values of RBC count, haemoglobin and PCV, a series of absolute values or red cell indices can be derived which have diagnostic importance in various haematologic disorders. These are as under:

1. Mean corpuscular volume (MCV)

$$= \frac{\text{PCV in L/L}}{\text{RBC count/L}}$$

The normal value is 85 + 8 fl (77-93 fl).

Table 50.2: Causes of abnormal PCV

<i>Diseases causing raised PCV</i>	<i>Diseases causing low PCV</i>
i. Polycythaemia	i. Anaemia
ii. Dehydration due to severe vomitings, diarrhoea profuse sweating	ii. Pregnancy
iii. Burns	
iv. Shock	

2. Mean corpuscular haemoglobin (MCH)

$$= \frac{\text{Hb/L}}{\text{RBC count/L}}$$

The normal range is 29.5 + 2.5 pg (27-32 pg).

3. Mean corpuscular haemoglobin concentration (MCHC)

$$= \frac{\text{Hb/dl}}{\text{PCV in L/L}}$$

The normal value is 32.5 + 2.5 g/dl (30-35 g/dl).

Since MCHC is independent of red cell count and size, it is considered to be of greater clinical significance as compared with other absolute values. It is low in iron deficiency anaemia but is usually normal in macrocytic anaemia.

Significance

- In iron deficiency and thalassaemia, MCV, MCH and MCHC are reduced.
- In anaemia due to acute blood loss and haemolytic anaemias, MCV, MCH and MCHC are all within normal limits.
- In megaloblastic anaemias, MCV is raised above the normal range.



Screening Tests for Bleeding Disorders

- ◆ Bleeding Time
- ◆ Clotting Time

Exercise

51

For the investigation of a case of bleeding disorder, clinical history is very important. An accurate clinical and family history will often give an important time-saving clue to the nature of underlying bleeding disorder.

- Appearance of purpuric spots or bruises over minimally injured surface suggests an abnormality of the vascular wall.
- Continuous bleeding from mucous membranes, cuts, wounds, deeper haematomas or bleeding into the joints will suggest a defect in the blood coagulation system.
- Excessive bleeding following delivery, or retention of dead foetus, or oozing following extensive surgery, suggest the possibility of fibrinogen depletion or presence of circulating anticoagulant agents.
- History should also be taken about the ingestion of antiplatelet drugs such as aspirin.

Two of the commonly used screening tests, bleeding time and whole blood clotting time, are discussed below.

BLEEDING TIME

Bleeding time is duration of bleeding from a standard puncture wound on the skin which is a *measure of the function of the platelets as well as integrity of the vessel wall*. This is one of the most important preliminary indicators for detection of bleeding disorders. This is also the most commonly done preoperative investigation in patients scheduled for surgery.

Principle

A small puncture is made on the skin and the time for which it bleeds is noted. Bleeding stops when platelet plug forms and breach in the vessel wall has sealed.

Methods for Bleeding Time

1. Finger tip method
2. Duke's method
3. Ivy's method

1. Finger Tip Method

Procedure

- Clean the tip of a finger with spirit.
- Prick with a disposable needle or lancet.
- Start the stop-watch immediately.
- Start gently touching the pricked finger with a filter paper till blood spots continue to be made on the filter paper.
- Stop the watch when no more blood spot comes on the filter paper and note the time.

Disadvantages

- i. It is a crude method.
- ii. Bleeding time is low by this method.

Normal bleeding time 1-3 minutes.

2. Duke's Method

Procedure

- Clean the lobe of a ear with a spirit swab.
- Using a disposable lancet/needle, puncture the lower edge of the ear lobe to a depth of approximately 3 mm.
- Start the stop-watch immediately.
- Allow the drops of blood to fall on a filter paper without touching the ear lobe and then slowly touching the blood drop gently on a new area on the filter paper.
- Stop the watch when no more blood comes over the filter paper and note the time.

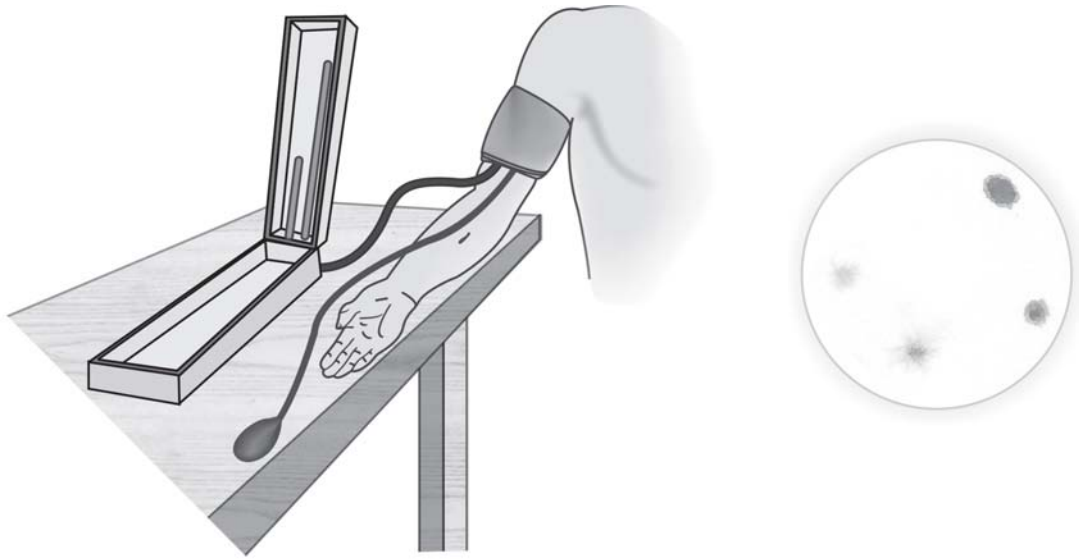


FIGURE 51.1: Ivy's method for bleeding time.

Advantages of the method

- The ear lobule has abundant subcutaneous tissue and is vascular.
- Flow of blood is quite good.

Normal bleeding time 3-5 minutes

3. Ivy's Method

Procedure

- Tie the BP apparatus cuff around the patient's upper arm and inflate it upto 40 mmHg which is maintained throughout the test.
- Clean an area with spirit over the flexor surface of forearm and let it dry.
- Using a disposable lancet or surgical blade make 2 punctures 3 mm deep 5-10 cm from each other taking care not to puncture the superficial veins.
- Start the stop-watch immediately.
- Go on blotting each puncture with a filter paper as in Duke's method.
- Stop the watch, note the time in each puncture and calculate average bleeding time (Fig. 51.1).

Advantages of the method

- This is the method of choice.
- It is a standardized method.
- Bleeding time is more accurate.

Normal bleeding time 3-8 minutes.

Clinical Application of Bleeding Time

The bleeding time is *prolonged* in:

- Thrombocytopenia
- Disorders of platelet functions
- Acute leukaemias
- Aplastic anaemias
- Liver disease
- von Willebrand's disease
- DIC
- Abnormality in the wall of blood vessels
- Administration of drugs prior to test e.g. aspirin

CLOTTING TIME

This is also known as whole blood clotting time and is a *measure of the plasma clotting factors*. It is a screening test for coagulation disorders.

Various other tests for coagulation disorders include: prothrombin time (PT), partial thromboplastin time (PTTK) or activated partial thromboplastin time with kaolin (APTTK), and measurement of fibrinogen.

Whole Blood Clotting Time

Methods

- Capillary tube method
- Lee and White method

1. Capillary Tube Method

Procedure

- Clean the tip of a finger with spirit.
- Puncture it upto 3 mm deep with a disposable needle.
- Start the stop-watch.
- Fill two capillary tubes with free flowing blood from the puncture after wiping the first drop of blood.
- Keep these tubes at body temperature.
- After 2 minutes start breaking the capillary tube at 1 cm distance to see whether a thin fibrin strand is formed between the two broken ends.
- Stop the watch and calculate the time from average of the two capillary tubes.

Disadvantages

- i. Method is insensitive.
- ii. Method is unreliable.

Advantages

Can be performed when venous blood can not be obtained.

Normal clotting time 1-5 minutes.

2. Lee and White Method

Procedure

- After cleaning the forearm, make a venepuncture and draw 3 ml of blood in a siliconised glass syringe or plastic syringe.
- Start the stop-watch.
- Transfer 1 ml of blood each into 3 glass tubes which are kept at 37°C in a waterbath (Fig. 51.2).
- After 3 minutes tilt the tubes one by one every 30 seconds.
- The clotting time is taken when the tubes can be tilted without spilling of their contents.
- Calculate the clotting time by average of 3 tubes.

Advantages

- i. More accurate and standard method.
- ii. Test can be run with control.

Disadvantages

- i. It is also a rough method.
- ii. There can be contamination of syringe or tubes.

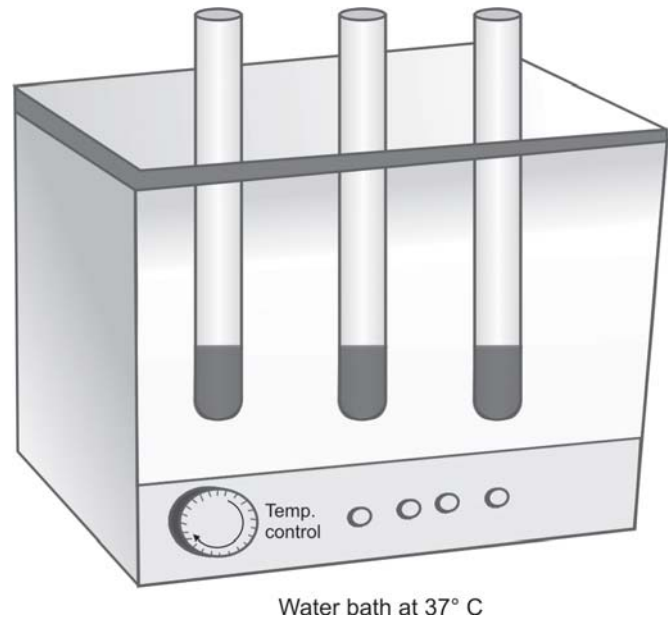


FIGURE 51.2: Lee and White method for clotting time.

Normal clotting time 5-10 minutes.

Sources of Error

- i. The temperature should be maintained because higher temperature accelerates clotting.
- ii. The diameter of the glass tubes should be uniform because clotting is accelerated in narrow tubes.
- iii. Vigorous agitation of the tubes should be avoided as it shortens the clotting time.

Clinical Applications

Clotting time is prolonged in:

- i. Severe deficiency of coagulation factors.
- ii. Afibrinogenaemia.
- iii. Administration of heparin.
- iv. Disseminated intravascular coagulation (DIC).
- v. Administration of drugs such as anticoagulants.



Blood Grouping

- ◆ ABO System
- ◆ Rhesus (Rh) System

Exercise

52

There are more than 300 blood group systems but ABO and rhesus (Rh) systems are of importance from clinical point of view. These are inherited characters which give rise to antigen-antibody reactions.

ABO SYSTEM

Principle

ABO system was discovered by Landsteiner in 1900. The red cells contain different types of antigens (agglutinogen), while plasma contains antibodies (agglutinins). In order to determine the blood group of a subject, the red cells are allowed to react with a sera containing known antibody (agglutinin).

Methods for ABO Grouping

1. Slide method
2. Tube method

1. Slide Method

- Take a clean glass slide (Fig. 52.1).
- Divide one slide into two halves with a glass marking pencil and mark these areas as A and B.
- Place a drop of serum anti-A (blue) on the slide in area marked A and a drop of serum anti-B (yellow) in the area marked B.
- Make a finger prick with a disposable needle after cleaning the area.
- Place a drop of blood near anti-A and anti-B serum and mix them with a stick or with the end of a glass slide.
- Wait for 5 minutes and look for agglutination.

Observations If any agglutination occurs it is visible with naked eyes as dark reddish clumps of different

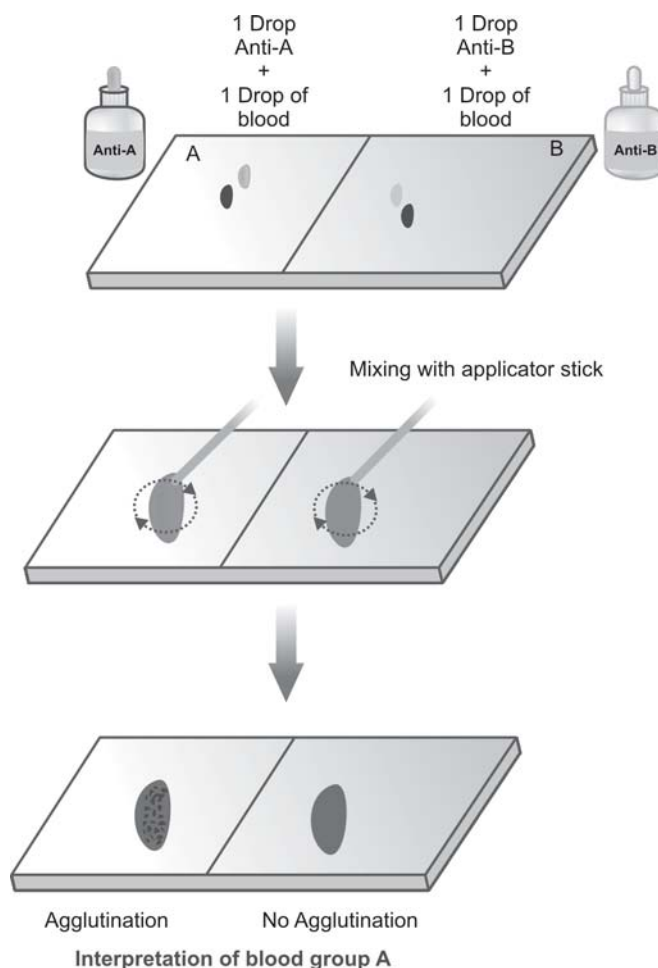


FIGURE 52.1: Slide method for ABO blood grouping.

sizes. If agglutination is minimal it can be confirmed by examining it under a microscope.

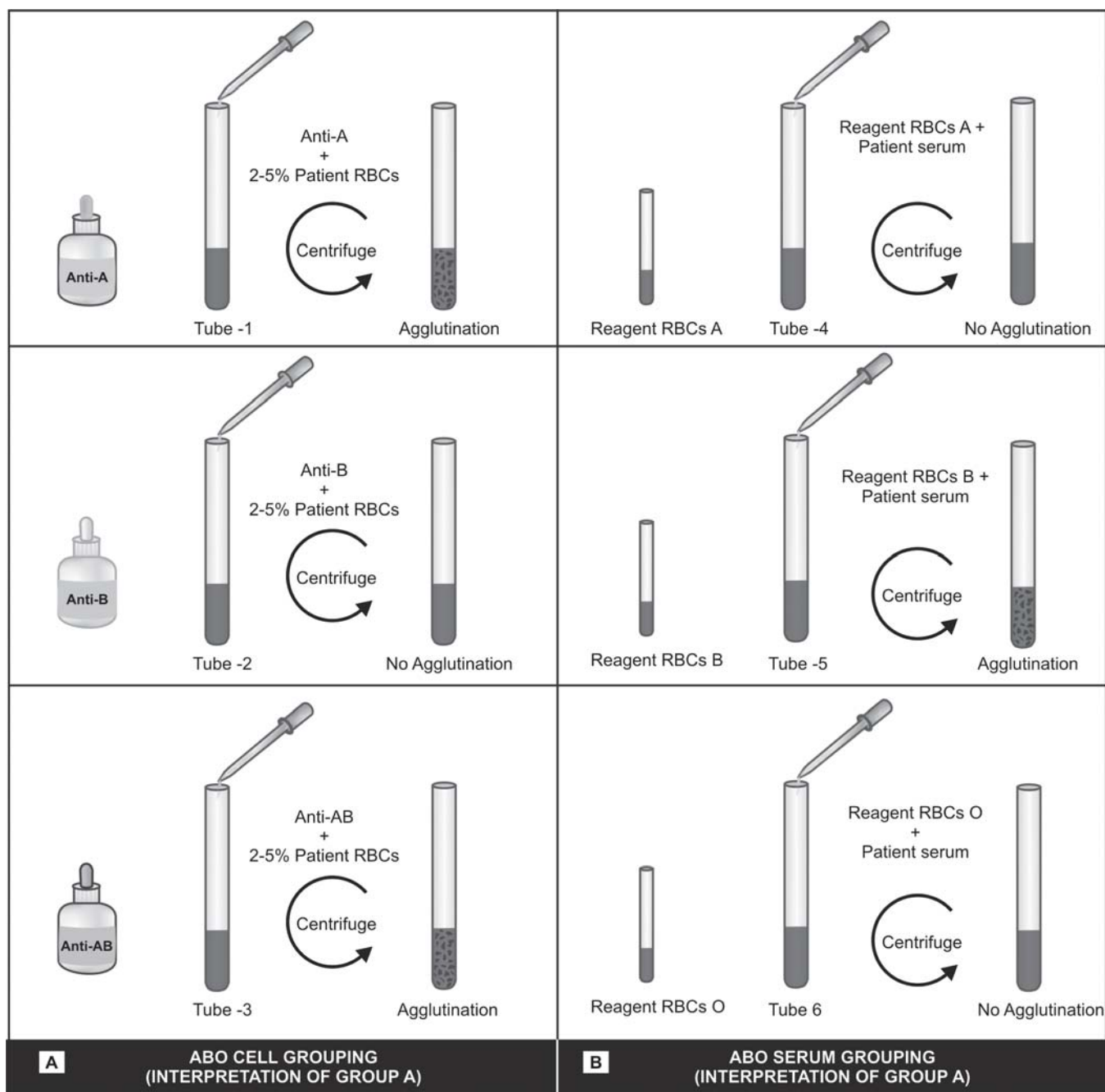


FIGURE 52.2: ABO blood grouping by tube method.

2. Tube Method

In this method cells and serum of the unknown blood sample to be tested are separated. Cells as well as serum grouping is done (Fig. 52.1).

Cell Grouping (Fig. 52.2,A)

- Prepare a 2-5% cell suspension in saline from the unknown blood sample.
- Take 3 test tubes 1,2,3 and put a drop of anti-A, anti-B and anti-AB serum (pink) to them.

- Add one drop of red cell suspension in each test tube.
- Centrifuge at 1500 rpm for one minute.
- Look for agglutination either with naked eye or under the microscope.

Serum Grouping (Fig. 52.2,B)

- Let the unknown blood sample stand for some time and separate the serum.
- Add 2 drops of unknown serum in test tubes 4,5,6.
- Add 1 drop of 2-5% cell suspension of known blood of A, B and O group into these test tubes.
- Centrifuge at 1500 rpm for one minute.
- Look for agglutination either with naked eye or under the microscope.

Advantages of Tube Method Over Slide Method

Tube method is more accurate because both red cell and serum grouping is done.

The interpretation of ABO grouping by any of the above methods is given in Table 52.1.

Note

- Red blood cells from person with *blood group O* contain no antigen; so no agglutination occur and thus such a person is a *universal donor*.
- Red blood cells from persons with *blood group AB* contain both A and B antigens, so agglutination occurs in both as no antibodies are present in their serum and thus such individual is a *universal recipient*.

RHESUS (Rh) SYSTEM

Rh blood group system was first reported by Landsteiner and Weiner in 1940. In contrast to ABO system, Rh antigens are present on red blood cells only and Rh antibodies develop only in response to a known stimulus (e.g. blood transfusion, or after first pregnancy). Rh factor is present in 85-95% of human beings.

Table 52.1: Results of ABO grouping		
Blood group of person	Agglutination with anti-A serum	Agglutination with anti-B serum
A	+	-
B	-	+
AB	+	+
O	-	-

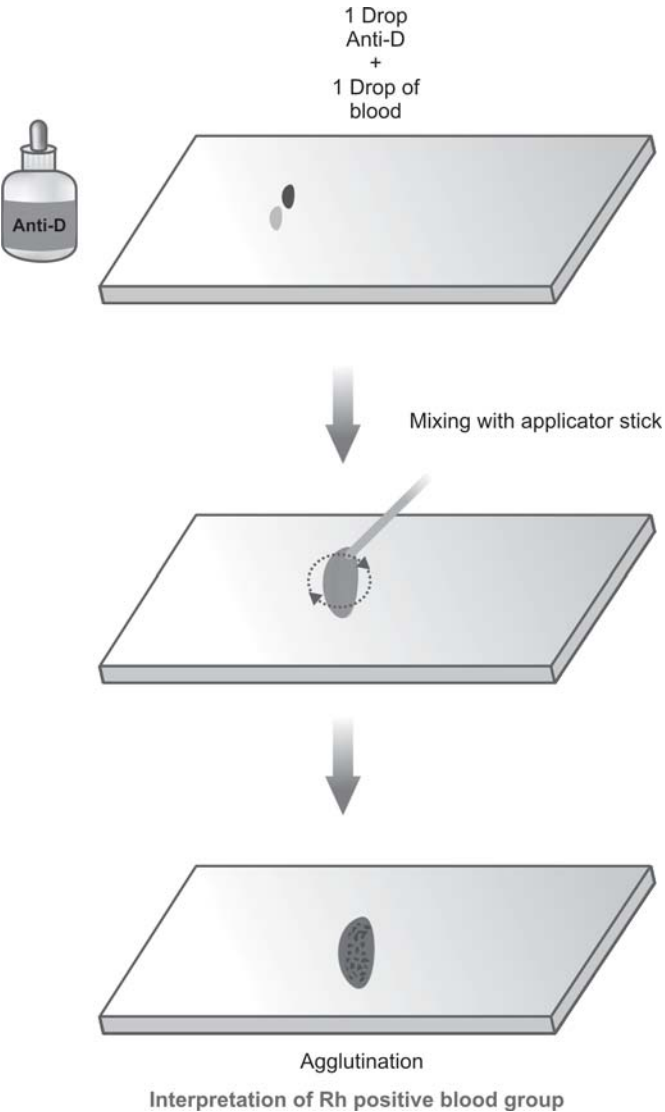


FIGURE 52.3: Rh grouping by slide method.

Methods for Rh Grouping

1. Slide method
2. Tube method

1. Slide Method

- Take a clean glass slide.
- Place a drop of anti-D serum on the slide.
- Place a drop of blood near anti-D serum and mix them as for ABO grouping.
- Wait for 5 minutes and see for agglutination (Fig. 52.3).

Table 52.2: Results of Rh grouping

Agglutination	Rh Group
Present	+
Absent	—

2. Tube Method

- Prepare a 2-5% cell suspension in saline from the blood to be tested.
- Take a test tube and put a drop of anti-D serum.
- Add one drop of red cell suspension in the tube.
- Centrifuge at 1500 rpm for 1 minute.
- Look for agglutination either with naked eye or under the microscope (Fig. 52.4).

The interpretation of Rh grouping is given in Table 52.2 below.

Note

- Rh positive subjects have Rh antigen on their red blood cells but no Rh antibody in their serum.
- Rh negative subjects have neither Rh antigen on their red blood cells nor Rh antibody in their serum. The most common Rh antigen is D.

Incidence of ABO Blood Groups in India

Incidence of ABO blood groups varies in different parts of the world and in different ethnic groups. Generally reported data in India are as under:

- A = 22-27%
- B = 31-33%
- O = 34-40%
- AB = 5-8%

Importance of Blood Grouping

1. *Blood transfusion:* Blood grouping and cross matching are always done prior to blood transfusion to any person.

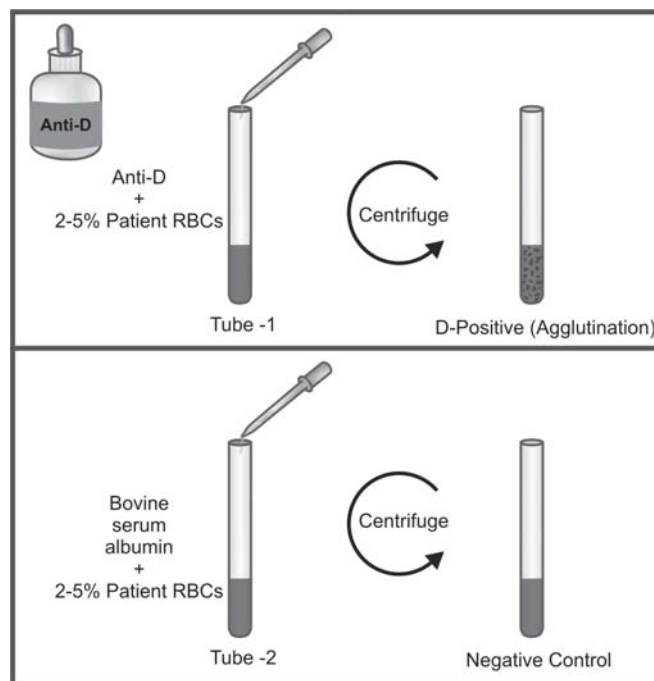


FIGURE 52.4: Rh grouping by tube method.

2. *Haemolytic disease of newborn:* There is a role of Rh and ABO blood grouping in HDN.
3. *Paternity disputes:* ABO and Rh blood grouping are used as a routine test in such cases. It is possible to disprove parentage but impossible to prove parent-hood.
4. *Medicolegal use:* In criminal cases, whether a stain is blood or not, and its blood group can be detected.
5. *Usefulness of blood groups:* In immunology, genetics and anthropology.
6. *Susceptibility to various diseases:* Persons with blood group O are more susceptible to peptic ulcer while persons with blood group A are more susceptible to gastric cancer.



Peripheral Blood Film Examination in Anaemias

- ◆ Plan for Investigation for Anaemia
- ◆ PBF in Microcytic Hypochromic Anaemia: Iron Deficiency
- ◆ PBF in Macrocytic Anaemia: Megaloblastic Anaemia
- ◆ PBF in Haemolytic Anaemia: Thalassemia

Exercise

53

Anaemia is defined as a haemoglobin concentration in blood below the lower limit of the normal range for the age and sex of the individual:

- *In adults*, the lower extreme of the normal haemoglobin is taken as 13 g/dl for males and 11.5 g/dl for females.
- *In pregnancy* due to haemodilution the lower limit of Hb is taken as 10.5 g/dl.
- *For newborn infants*, 15 g/dl is taken as the lower limit at birth and 9.5 g/dl at 3 months.

However, according to WHO criteria, haemoglobin less than 12 gm/dl in males and less than 10.5 gm/dl in females is taken as anaemia.

After obtaining the full medical history pertaining to different general and specific signs and symptoms, the patient is examined for evidence of anaemia. Special emphasis is placed on colour of skin, conjunctivae, sclerae and nails. Changes in the retina, atrophy of the papillae of the tongue, rectal examination for evidence of bleeding, and presence of hepatomegaly, splenomegaly, lymphadenopathy and bony tenderness are looked for.

PLAN OF INVESTIGATION FOR ANAEMIA

In order to confirm or deny the presence of anaemia, its type and its cause, the following plan of investigations is generally followed:

- Screening tests:**
 - A. Haemoglobin estimation
 - B. Peripheral blood film examination
 - C. Red cell indices
 - D. Leucocyte and platelet count
 - E. Reticulocyte count
- Confirmatory tests:**
 - A. Bone marrow examination
 - B. Biochemical tests.

Haemoglobin Estimation

The first and foremost investigation in any suspected case of anaemia is to carry out the haemoglobin estimation. Several methods are available but most reliable and accurate is the cyanmethaemoglobin (HiCN) method employing Drabkin's solution and a spectrophotometer (Exercise 45). If the haemoglobin value is below the lower limit of the normal range for particular age and sex, the patient is said to be anaemic.

Peripheral Blood Film (PBF) Examination

The haemoglobin estimation is invariably followed by examination of a peripheral blood film for morphologic features after staining it with the Romanowsky dyes (e.g. Leishman's stain, May-Grunwald-Giemsa's stain, Jenner-Giemsa's stain etc). The blood smear is evaluated in an area where there is neither rouleaux formation nor so thin as to cause red cell distortion. Such an area can usually be found towards the tail of the film, but not actually at the tail (Exercise 48). The following abnormalities in erythroid series of cells are particularly looked for in a blood smear:

Variation in Size (Anisocytosis)

Normally, there is slight variation in diameter of the red cells from 6.7-7.7 μm (mean value 7.2 μm). Increased variation in size of the red cell is termed anisocytosis. Anisocytosis may be due to the presence of cells larger than normal (macrocytosis) or cells smaller than normal (microcytosis). Sometimes both microcytosis and macrocytosis are present (dimorphic).

- **Macrocytes** are classically found in megaloblastic anaemia; other causes are aplastic anaemia, other dyserythropoietic anaemias, chronic liver disease and in conditions with increased erythropoiesis.

- *Microcytes* are present in iron-deficiency anaemia, thalassaemia and spherocytosis. They may also result from fragmentation of erythrocytes such as in haemolytic anaemia.

Variation in Shape (Poikilocytosis)

Increased variation in shape of the red cells is termed poikilocytosis. The nature of the abnormal shape determines the cause of anaemia. Poikilocytes are produced in various types of abnormal erythropoiesis e.g. in megaloblastic anaemia, iron-deficiency anaemia, thalassaemia, myelofibrosis and microangiopathic haemolytic anaemia.

Inadequate Haemoglobin Formation (Hypochromasia)

Normally, the intensity of pink-staining of haemoglobin in a Romanowsky-stained blood smear gradually decreases from the periphery to the centre of the cell. Increased central pallor is referred to as *hypochromasia*. It may develop either from lowered haemoglobin content (e.g. in iron-deficiency anaemia, chronic infections), or due to thinness of the red cells (e.g. in thalassaemia, sideroblastic anaemia). Unusually deep pink staining of the red cells due to increased haemoglobin concentration is termed hyperchromasia and may be found in megaloblastic anaemia, spherocytosis and in neonatal blood.

Compensatory Erythropoiesis

A number of changes are associated with compensatory increase in erythropoietic activity. These are as under:

- i. *Polychromasia* is defined as the red cells having more than one type of colour. Polychromatic red cells are slightly larger, generally stain bluish-grey and represent reticulocytes and, thus, correlate well with reticulocyte count.
- ii. *Normoblastaemia* is presence of nucleated red cells in the peripheral blood film. A small number of normoblasts (or erythroblasts) may be normally found in cord blood at birth. They are found in large numbers in haemolytic disease of the newborn, other haemolytic disorders and in extramedullary erythropoiesis. They may also appear in the blood in various types of severe anaemias except in aplastic anaemia. Normoblastaemia may also occur after splenectomy.
- iii. *Punctate basophilia* or *basophilic stippling* is diffuse and uniform basophilic granularity in the cell which does not stain positively with Perl's reaction (in contrast to Pappenheimer bodies which stain positively). Classical punctate basophilia is seen in aplastic anaemia, thalassaemia, myelodysplasia, infections and lead poisoning.
- iv. *Howell-Jolly bodies* are purple nuclear remnants, usually found singly, and are larger than basophilic stippling. They are present in megaloblastic anaemia and after splenectomy.

Miscellaneous Changes

In addition to the morphologic abnormalities of red cells described above, several other abnormal red cells may be found in different haematological disorders. Some of these are as follows (Fig. 53.1):

- i. *Spherocytosis* is characterised by presence of spheroidal rather than biconcave disc-shaped red cells. Spherocytes are seen in hereditary spherocytosis, autoimmune haemolytic anaemia and in ABO haemolytic disease of the newborn.
- ii. *Schistocytosis* is identified by fragmentation of erythrocytes. Schistocytes are found in thalassaemia, hereditary elliptocytosis, megaloblastic anaemia, iron-deficiency anaemia, microangiopathic haemolytic anaemia and in severe burns.
- iii. *Irregularly contracted red cells* are found in drug- and chemical-induced haemolytic anaemia and in unstable haemoglobinopathies.
- iv. *Leptocytosis* is the presence of unusually thin red cells. Leptocytes are seen in severe iron deficiency and thalassaemia. *Target cell* is a form of leptocyte in which there is central round stained area and a peripheral rim of haemoglobin. Target cells are found in iron deficiency, thalassaemia, chronic liver disease, and after splenectomy.
- v. *Sickle cells* or *drepanocytes* are sickle-shaped red cells found in sickle cell disease.
- vi. *Crenated red cells* are the erythrocytes which develop numerous projections from the surface. They are present in blood films due to alkaline pH, presence of traces of fatty substances on the slides and in cases where the film is made from blood that has been allowed to stand overnight.
- vii. *Acanthocytosis* is the presence of coarsely crenated red cells. Acanthocytes are found in large number in blood film made from splenectomised subjects, in chronic liver disease and in abetalipoproteinaemia.
- viii. *Burr cells* are cell fragments having one or more spines. They are particularly found in uraemia and in pyruvate kinase deficiency.
- ix. *Stomatocytosis* is the presence of stomatocytes which have central area having slit-like or mouth-

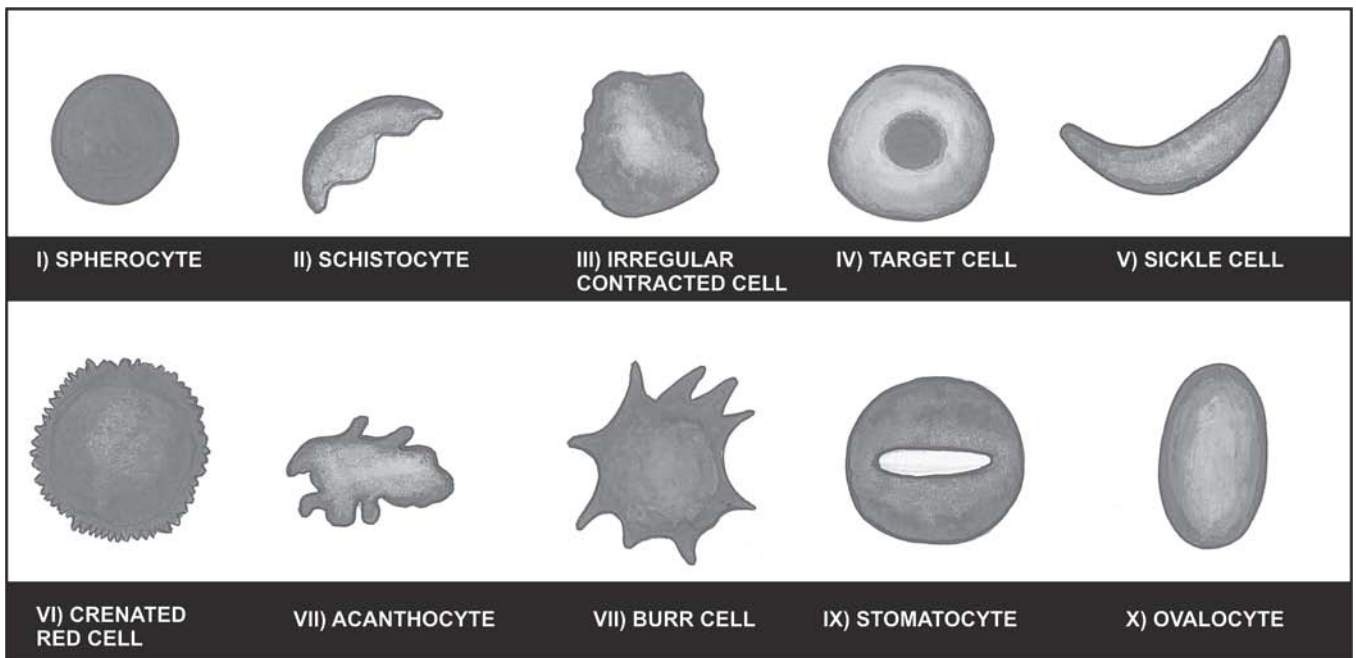


FIGURE 53.1: Some of the common morphologic abnormalities of red cells (The serial numbers in the illustrations correspond to the order in which they are described in the text).

like appearance. They are found in alcoholism or as an artefact.

- x. *Ovalocytosis* or *elliptocytosis* is the oval or elliptical shape of red cells. Their highest proportion (79%) is seen in hereditary ovalocytosis and elliptocytosis; other conditions showing such abnormal shapes of red cells are megaloblastic anaemia and hypochromic anaemia.

Red Cell Indices

An alternative method to diagnose and detect the severity of anaemia is by measuring the red cell indices (Exercise 50):

1. In iron deficiency and thalassaemia, MCV, MCH and MCHC are reduced.
2. In anaemia due to acute blood loss and haemolytic anaemias, MCV, MCH and MCHC are all within normal limits.
3. In megaloblastic anaemias, MCV is raised above the normal range.

Leucocyte and Platelet Count

Measurement of leucocyte and platelet count helps to distinguish pure anaemia from pancytopenia in which red cells, granulocytes and platelets are all reduced. In

anaemias due to haemolysis or haemorrhage, the neutrophil count and platelet counts are often elevated. In infections and leukaemias, the leucocyte counts are high and immature leucocytes appear in the blood (Exercise 49).

Reticulocyte Count

Reticulocyte count (normal 0.5-2.5%) is done in each case of anaemia to assess the marrow erythropoietic activity (Exercise 47). In acute haemorrhage and in haemolysis, the reticulocyte response is indicative of impaired marrow function.

Bone Marrow Examination

Bone marrow aspiration is done in cases where the cause for anaemia is not obvious. The procedures involved for marrow aspiration and trephine biopsy and their relative advantages and disadvantages have been discussed in Exercise 56.

Biochemical Tests

In addition to these general tests, certain specific tests are done in different types of anaemias which include biochemical tests, radio-assay and others.

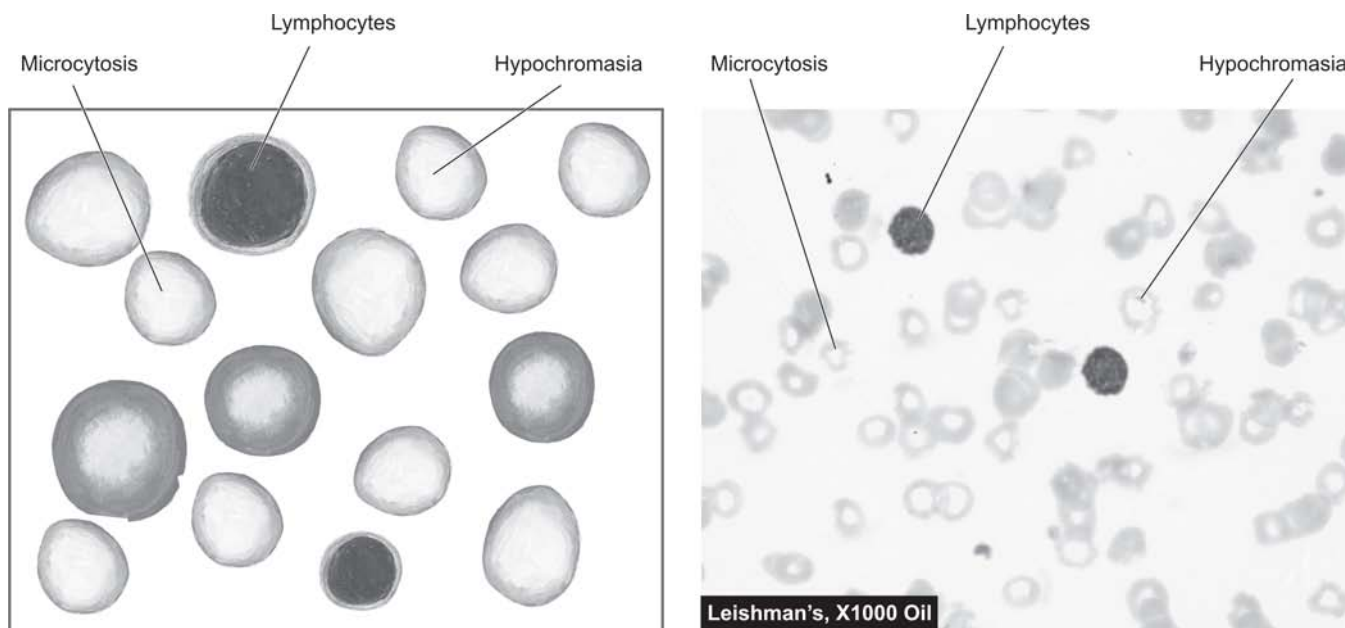


FIGURE 53.2: PBF in microcytic hypochromic anaemia. There is marked microcytosis and hypochromasia and a few target cells.

PERIPHERAL BLOOD FILM IN IRON DEFICIENCY ANAEMIA (IRON DEFICIENCY ANAEMIA)

The degree of anaemia in iron deficiency varies. It is usually mild to moderate but occasionally it may be marked (haemoglobin less than 6 g/dl) due to persistent and severe blood loss. The salient haematological findings in these cases are as under:

- i. **Haemoglobin.** The essential feature is a fall in haemoglobin concentration up to a variable degree.
- ii. **Red cells.** The red cells in the blood film are hypochromic and microcytic, and there is anisocytosis and poikilocytosis. Hypochromia generally precedes microcytosis. Hypochromia is due to poor filling of the red cells with haemoglobin so that there is increased central pallor. In severe cases, red cells reveal only a thin rim of haemoglobin at the periphery (ring or pessary cell). Target cells, elliptical forms and polychromatic cells are often present. Normoblasts are uncommon. RBC count is below normal but is generally not proportionate to the fall in haemoglobin value. When iron deficiency is associated with severe folate or vitamin B₁₂ deficiency, a dimorphic blood picture occurs with dual population of red cells, macrocytic as well as microcytic-hypochromic (Fig. 53.2).
- iii. **Reticulocyte count.** The reticulocyte count is normal or reduced but may be slightly raised (2-5%) in cases after haemorrhage.

- iv. **Absolute values.** The red cell indices reveal a diminished MCV (below 50 fl), diminished MCH (below 15 pg) and diminished MCHC (below 20 g/dl).
- v. **Leucocytes.** The total and differential white cell counts are usually normal. However, in cases in which iron deficiency is due to parasitic infestations such as hookworm infestation, there may be associated eosinophilia.
- vi. **Platelets.** Platelet count is usually normal but may be slightly to moderately raised in patients who have had recent bleeding.

PERIPHERAL BLOOD FILM IN MACROCYTIC ANAEMIA (MEGALOBlastic ANAEMIA)

The investigations of a suspected case of megaloblastic anaemia are aimed at 2 aspects:

- A. General laboratory investigations of anaemia** which include blood picture, red cell indices, bone marrow findings, and biochemical tests.
- B. Special tests** to establish the cause of megaloblastic anaemia as to know whether it is due to deficiency of vitamin B₁₂ or folate.

The estimation of haemoglobin, examination of a blood film and evaluation of absolute values are essential preliminary investigations.

- i. **Haemoglobin.** The haemoglobin estimation reveals values below the normal range. The fall in haemoglobin concentration may be of a variable degree.

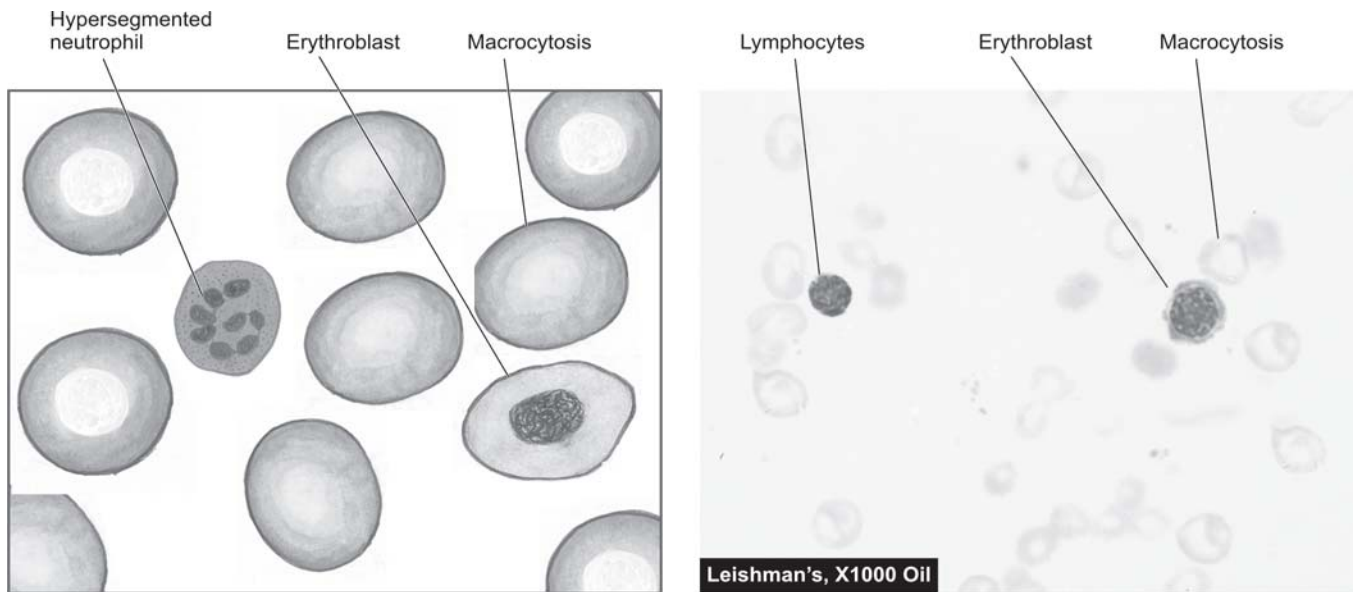


FIGURE 53.3: PBF in megaloblastic anaemia. There is prominent macrocytosis, oval red cells, hypersegmented neutrophil and a megaloblast.

- ii. **Red cells.** The red blood cell morphology in a blood film shows the characteristic macrocytosis. However, macrocytosis can also be seen in several other disorders such as: haemolysis, liver disease, alcoholism, hypothyroidism, aplastic anaemia, myeloproliferative disorders and reticulocytosis. In addition, the blood smear demonstrates marked anisocytosis, poikilocytosis and presence of macro-ovalocytes. Basophilic stippling, Howell-Jolly bodies, Cabot ring and occasional normoblast may also be seen (Fig. 53.3).
- iii. **Reticulocyte count.** The reticulocyte count is generally low to normal in untreated cases.
- iv. **Absolute values.** The red cell indices reveal an elevated MCV (above 120 fl) proportionate to the severity of macrocytosis, elevated MCH (above 50 pg) and normal or reduced MCHC.
- v. **Leucocytes.** The total white blood cell count may be reduced. Presence of characteristic hypersegmented neutrophils (5% neutrophils having more than 5 nuclear lobes) in the blood film should raise the suspicion of megaloblastic anaemia. An occasional myelocyte may also be seen.
- vi. **Platelets.** Platelet count may be moderately reduced in severely anaemic patients. Bizarre forms of platelets may be seen.

PERIPHERAL BLOOD FILM IN HAEMOLYTIC ANAEMIA (THALASSAEMIA)

The thalassaemias are a diverse group of hereditary disorders in which there is reduced rate of synthesis of

one or more of the globin polypeptide chains. Thus, thalassaemias, unlike haemoglobinopathies which are qualitative disorders of haemoglobin, are *quantitative abnormalities of polypeptide globin chain synthesis*.*

A classification of various types of thalassaemias alongwith the clinical syndromes produced and salient laboratory findings is given in Table 53.1.

The β -thalassaemia major, also termed Mediterranean or Cooley's anaemia is the most common form of congenital haemolytic anaemia. More commonly, β -thalassaemia major is a homozygous state with either complete absence of β -chain synthesis (β^0 thalassaemia major) or only small amounts of β -chains are formed (β^+ thalassaemia major). These result in excessive formation of alternate haemoglobins, HbF ($\alpha_2\gamma_2$) and HbA₂ ($\alpha_2\delta_2$).

Clinical manifestations appear insidiously and are as under:

1. **Anaemia** starts appearing within the first 4-6 months of life when the switch from γ -chain to β -chain production occurs.
2. **Marked hepatosplenomegaly** occurs due to excessive red cell destruction, extramedullary haematopoiesis and iron overload.
3. **Expansion of bones** occurs due to marked erythroid hyperplasia leading to thalassaemic facies and malocclusion of the jaw.

*In a normal adult, distribution of haemoglobin is as under: HbA ($\alpha_2\beta_2$) = 95-98%, HbA₂ ($\alpha_2\delta_2$) (a minor variant of HbA) = 1.5-3.5%, HbF ($\alpha_2\gamma_2$) = less than 1%. But the level of HbF in children under 6 months is slightly higher.

Table 53.1: Classification of thalassaemias.

Type	Hb	Hb-electrophoresis	Genotype	Clinical syndrome
α-Thalassaemias				
1. <i>Hydrops foetalis</i>	3-10 g/dl	Hb Barts (γ_4) (100%)	Deletion of four α -genes	Fatal <i>in utero</i> or in early infancy
2. <i>Hb-H disease</i>	2-12 g/dl	HbF (10%)	Deletion of three α -genes	Haemolytic anaemia
3. <i>α-Thalassaemia trait</i>	10-14 g/dl	Normal	Deletion of two α -genes	Microcytic hypochromic blood picture but no anaemia
β-Thalassaemias				
1. <i>β-Thalassaemia major</i>	< 5 g/dl	HbA (0-50%), HbF (50-98%)	$\beta^{\text{thal}}/\beta^{\text{thal}}$	Severe congenital haemolytic anaemia, requires blood transfusions
2. <i>β-Thalassaemia intermedia</i>	5-10 g/dl	Variable	Multiple mechanisms	Severe anaemia, but regular blood transfusions not required
3. <i>β-Thalassaemia minor</i>	10-12 g/dl	HbA ₂ (4-9%) HbF (1-5%)	$\beta^A/\beta^{\text{thal}}$	Usually asymptomatic

4. *Iron overload* due to repeated blood transfusions causes damage to the endocrine organs resulting in slow rate of growth and development, delayed puberty, diabetes mellitus and damage to the liver and heart.

The haematological investigations reveal the following findings (Fig. 53.4):

- Anaemia*, usually severe.
- Blood film* shows severe microcytic hypochromic red cell morphology, marked anisopoikilocytosis,

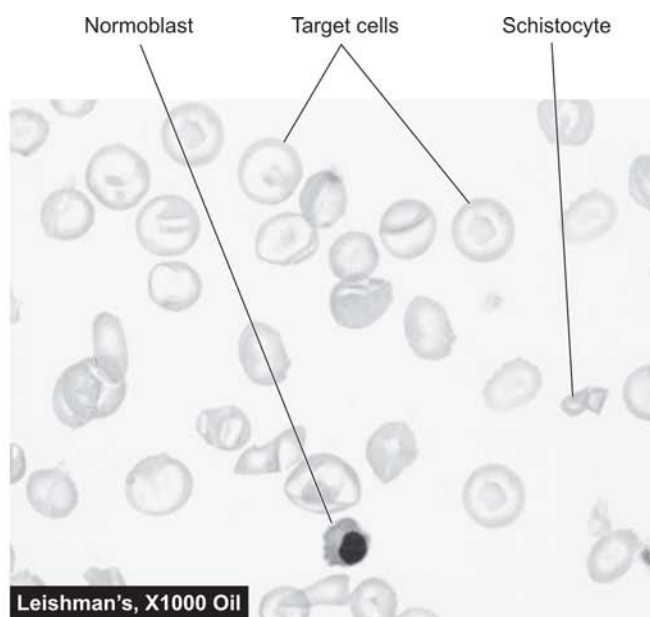
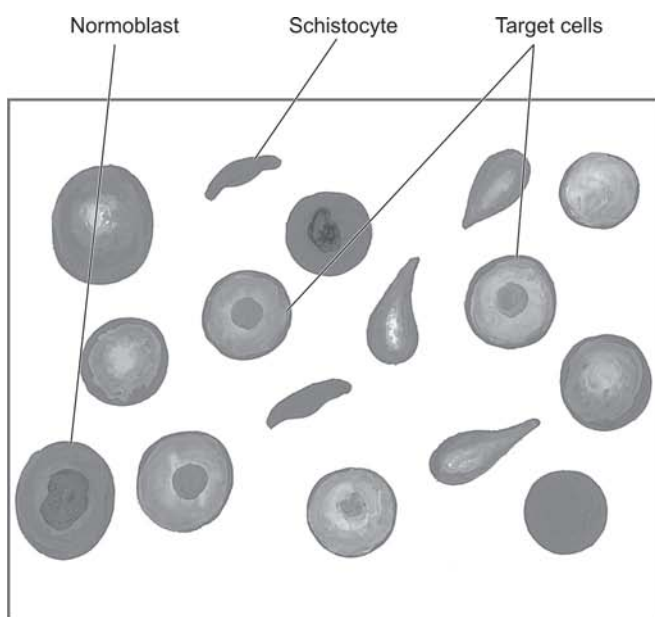


FIGURE 53.4: PBF in haemolytic anaemia (Thalassaemia). There is marked anisopoikilocytosis and presence of nucleated red cells.

- basophilic stippling, presence of many target cells, tear drop cells and normoblasts.
- iii. *Serum bilirubin* (unconjugated) is generally raised.
 - iv. *Reticulocytosis* is generally present.
 - v. *MCV, MCH and MCHC* are significantly reduced.
 - vi. *WBC count* is often raised with some shift to left of the neutrophil series, with presence of some myelocytes and metamyelocytes.
 - vii. *Platelet count* is usually normal but may be reduced in patients with massive splenomegaly.
 - viii. *Osmotic fragility* done by NESTROF (Naked Eye Single Tube Rapid Osmotic Fragility) test characteristically reveals increased resistance to saline haemolysis i.e. decreased osmotic fragility.
 - ix. *Alkali denaturation test* for HbF reveals increased HbF levels in thalassaemia major.
 - x. *HbA₂ denaturation* may be carried out by Hb electrophoresis, column chromatography or HPLC.
 - xi. *Haemoglobin electrophoresis* shows presence of increased amounts of HbF, increased amount of HbA₂ and almost complete absence or presence of variable amounts of HbA. The increased level of HbA₂ has not been found in any other haemoglobin abnormality except β -thalassaemia. The increased synthesis of HbA₂ is probably due to increased activity at both δ -chain loci.
 - xii. *Bone marrow aspirate examination* shows normoblastic erythroid hyperplasia with predominance of intermediate and late normoblasts which are generally smaller in size than normal. Iron staining demonstrates siderotic granules in the cytoplasm of normoblasts, increased reticuloendothelial iron but ring sideroblasts are only occasionally seen.



Blood Smear Examination in Leukaemias

- ◆ PBF in Acute Leukaemias: AML
- ◆ PBF in CML
- ◆ PBF in CLL

Exercise

54

The leukaemias are a group of disorders characterised by malignant transformation of blood-forming cells. The proliferation of leukaemic cells takes place primarily in the bone marrow, and in certain forms, in the lymphoid tissues. Ultimately, the abnormal cells appear in the peripheral blood raising the total white cell count to high level. In addition, features of bone marrow failure (e.g. anaemia, thrombocytopenia, neutropenia) and involvement of other organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin etc) occur.

In general, leukaemias are classified on the basis of cell types predominantly involved, into *myeloid* and *lymphoid*, and on the basis of natural history of the disease, into *acute* and *chronic*. Thus, the main types are:

- acute myeloblastic and acute lymphoblastic (AML and ALL);
- chronic myeloid and chronic lymphocytic leukaemias (CML and CLL); and
- hairy cell leukaemia (HCL) is an unusual variant of lymphoid neoplasia.

Leukaemias account for 4% of all cancer deaths. Generally, acute leukaemias have a rapidly downhill course, whereas chronic leukaemias tend to have more indolent behaviour. The incidence of both acute and chronic leukaemias is higher in men than in women. ALL is primarily a disease of children and young adults, whereas AML occurs at all ages. CLL tends to occur in the elderly, while CML is found in middle age.

PBF IN ACUTE LEUKAEMIAS: AML

Acute leukaemias are characterised by predominance of undifferentiated leucocyte precursors or leukaemic blasts. Acute leukaemias may be derived from the myeloid stem cells called acute myeloblastic leukaemia (AML), or from the lymphoid stem cells termed acute lymphoblastic leukaemia (ALL).

Now, more definite criteria for diagnosis and classification of acute leukaemias have been laid down by a group of French, American and British haematologists commonly called the FAB classification. According to this system, a leukaemia is acute if the bone marrow consists of more than 30% blasts. FAB classification divides AML into 8 subtypes (M0 to M7) and ALL into 3 subtypes (L1 to L3). Another classification called revised European-American classification of lymphoid neoplasms (REAL classification) has been formulated which includes acute and chronic lymphoid leukaemias as well as non-Hodgkin's lymphomas since the latter represent the neoplastic counterparts in the bone marrow and lymphoid tissue. Currently, WHO classification has evolved which not only includes morphology (as in FAB classification) but also takes into consideration clinical, immunophenotypic and cytogenetic features (Table 54.1).

The diagnosis of acute leukaemia is made by a combination of routine blood picture and bone marrow examination, coupled with cytochemical stains and certain biochemical investigations.

Findings of routine haematologic investigations are as under:

Anaemia

Anaemia is almost always present in acute leukaemias. It is generally severe, progressive and normochromic in type. A moderate reticulocytosis upto 5% and a few nucleated red cells may be present.

Thrombocytopenia

The platelet count is usually moderately to severely decreased (below 50,000/ μ l) but occasionally it may be normal. Bleeding tendencies in acute leukaemia are

Table 54.1: Revised FAB classification of acute leukaemias.

FAB class	Percent cases	Morphology	Cytochemistry
ACUTE MYELOBLASTIC LEUKAEMIA (AML)			
M0: Minimally differentiated AML	2	Blasts lack definite cytologic and cytochemical features but have myeloid lineage antigens	Myeloperoxidase –
M1: AML without maturation	20	Myeloblasts predominate; few if any granules or Auer rods	Myeloperoxidase +
M2: AML with maturation	30	Myeloblasts with promyelocytes predominate; Auer rods may be present	Myeloperoxidase +++
M3: Acute promyelocytic leukaemia	5	Hypergranular promyelocytes; often with multiple Auer rods per cell	Myeloperoxidase +++
M4: Acute myelomonocytic leukaemia (Naegeli type)	30	Mature cells of both myeloid and monocytic series in peripheral blood; myeloid cells resemble M2	Myeloperoxidase ++ Non-specific esterase +
M5: Acute monocytic leukaemia (Schilling type)	10	Two subtypes: M5a shows poorly-differentiated monoblasts, M5b shows differentiated promonocytes and monocytes	Non-specific esterase ++
M6: Acute erythroleukaemia (Di Guglielmo's syndrome)	<5	Erythroblasts predominate (>50%); myeloblasts and promyelocytes also increased	Erythroblasts: PAS + Myeloblasts: myeloperoxidase +
M7: Acute megakaryocytic leukaemia	<5	Pleomorphic undifferentiated blasts predominate; react with antiplatelet antibodies	Platelet peroxidase +
ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)			
L1: Childhood-ALL (B-ALL, and T-ALL)	More common in children	Homogeneous small lymphoblasts; scanty cytoplasm, regular round nuclei, inconspicuous nucleoli	PAS ± Acid phosphatase ±
L2: Adult-ALL (mostly T-ALL)	More frequent in adults	Heterogeneous lymphoblasts; variable amount of cytoplasm, irregular or cleft nuclei, large nucleoli	PAS ± Acid phosphatase ±
L3: Burkitt type-ALL (B-ALL)	Uncommon	Large homogeneous lymphoblasts; round nuclei, prominent nucleoli, cytoplasmic vacuolation	PAS – Acid phosphatase –

usually correlated with the level of thrombocytopenia but most serious spontaneous haemorrhagic episodes develop in patients with fewer than 20,000/ μ l platelets. Acute promyelocytic leukaemia (M3) may be associated with a serious coagulation abnormality called disseminated intravascular coagulation (DIC).

White Blood Cells

The total WBC count ranges from subnormal-to-markedly elevated values. In 25% of patients, the total WBC count at presentation is reduced to 1,000-4,000/ μ l. More often, however, there is progressive rise in white cell count which may exceed 100,000/ μ l in more advanced disease. Majority of leucocytes in the peripheral blood are blasts and there is often neutropenia

due to marrow infiltration by leukaemic cells. Some patients present with pancytopenia and have a few blasts (subleukaemic leukaemia) or no blasts (aleukaemic leukaemia) in the blood. Both these conditions are now-a-days included under 'myelodysplastic syndrome'. The basic morphologic features of myeloblasts and lymphoblasts are given in Table 54.3. Typical characteristics of different forms of AML (M0 to M7) and ALL (L1 to L3) are given in Tables 54.1 and 54.2. In some instances, the identification of blast cells is greatly aided by the company they keep i.e. by more mature and easily identifiable leucocytes in the company of blastic cells of myeloid or lymphoid series. It is usual to find some 'smear cells' in the peripheral blood which represent degenerated leucocytes (Fig. 54.1).

Table 54.2: WHO-REAL classification of ALL on the basis of immunologic and cytogenetic features.

Subtype	Incidence	Markers	FAB subtype	Cytogenetic abnormalities
Pre-B ALL	75%	CD10+ (90%), TdT+	L1, L2	t(9;22) i.e. Philadelphia+ALL
B cell ALL	5%	CD10+ (50%), TdT-	L3	t(8;14) (Burkitt's leukaemia)
T cell ALL	20%	CD10+ (30%), TdT+	L1, L2	14q11

(TdT= terminal deoxynucleotidyl transferase)

Table 54.3: Morphologic characteristics of the blast cells in Romanowsky stains.

Feature	Myeloblast	Lymphoblast
1. Size	10-18 µm	10-18 µm
2. Nucleus	Round or oval	Round or oval
3. Nuclear chromatin	Fine meshwork	Slightly clumped
4. Nuclear membrane	Very fine	Fairly dense
5. Nucleoli	2-5	1-2
6. Cytoplasm	Scanty, blue, agranular, Auer's rods may be present	Scanty, clear blue, agranular

PBF IN CHRONIC MYELOID LEUKAEMIA

Chronic leukaemias are those haematologic malignancies in which the predominant leukaemic cells are initially well-differentiated and easily recognisable as regards

their cell type. Chronic leukaemias are divided into 2 main types: chronic myeloid (granulocytic) leukaemia (CML or CGL), and chronic lymphocytic leukaemia (CLL). Less common variants include: chronic eosinophilic, chronic basophilic, chronic monocytic, chronic neutrophilic and chronic lymphosarcoma cell leukaemias. An unusual chronic lymphoproliferative variant is hairy cell leukaemia (HCL). In general, chronic leukaemias have a better prognosis than the acute leukaemias. Currently CML has come to be classified along with other myeloproliferative syndromes due to common histogenesis from haematopoietic stem cells.

Chronic myeloid (myelogenous, granulocytic) leukaemia comprises about 20% of all leukaemias and its peak incidence is seen in 3rd and 4th decades of life. A distinctive variant of CML seen in children under 3 years of age is called juvenile CML. Both the sexes are affected equally.

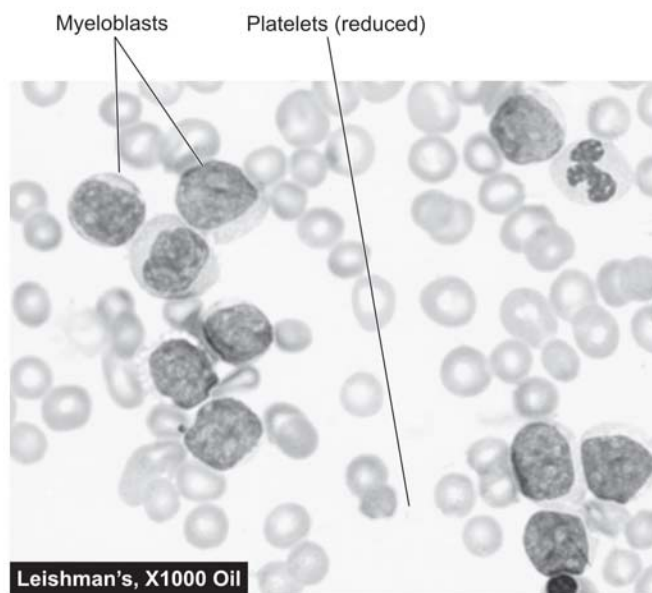
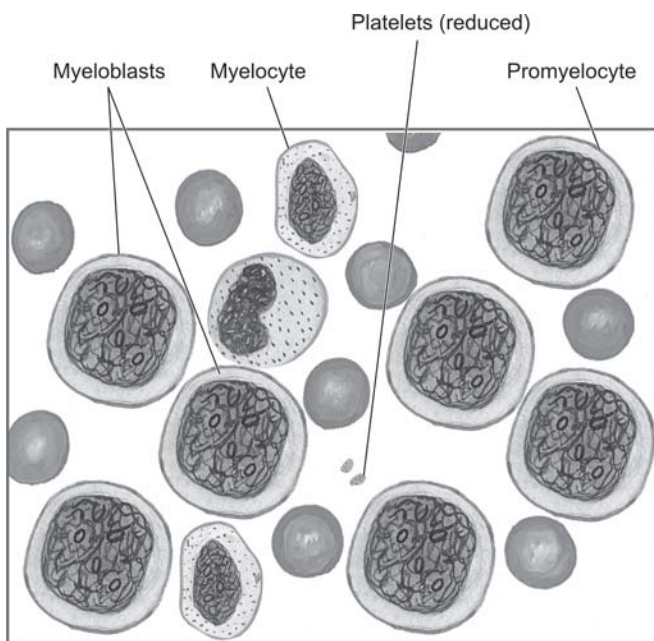


FIGURE 54.1: PBF in acute myeloblastic leukaemia. Numerous myeloblasts and a few promyelocytes.

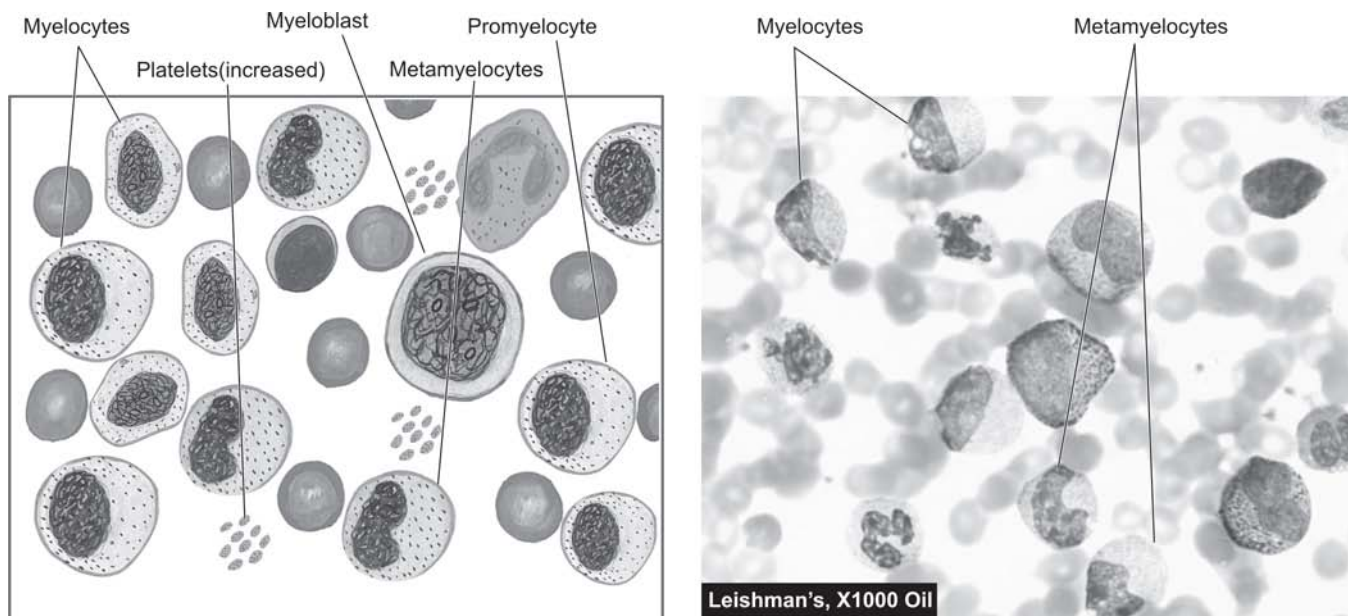


FIGURE 54.2: PBF in chronic myeloid leukaemia. There is rise in leucocyte count and late immature myeloid cells including basophils.

The diagnosis of CML is generally possible on blood picture alone. However, bone marrow, cytochemical stains and other investigations are of help. The characteristic Ph chromosome or BCR-ABL translocation between chromosome 9 and 22 can be detected using bone marrow or peripheral blood sample. Another important test is leucocyte alkaline phosphatase (LAP) score is low in cases of chronic phase of CML while it rises during accelerated phase and blast crisis.

The typical blood picture in a case of CML at the time of presentation shows the following features (Fig. 54.2):

Anaemia

Anaemia is usually of moderate degree and is normocytic normochromic in type. Occasional normoblasts may be present.

White Blood Cells

Characteristically, there is marked leucocytosis (approximately 200,000/ μ l or more at the time of presentation). The natural history of CML consists of 2 phases—chronic and blastic.

- The *chronic phase of CML* begins as a myeloproliferative disorder and consists of excessive proliferation of myeloid cells of intermediate grade (i.e. myelocytes and metamyelocytes) and mature segmented neutrophils. Myeloblasts usually do not

exceed 10% of cells in the peripheral blood and bone marrow. An accelerated phase of CML is also described in which there is progressively rising leucocytosis associated with thrombocytosis or thrombocytopenia and splenomegaly.

- The *blastic phase or blast crisis in CML* may be myeloid or lymphoid in origin. An increase in the proportion of basophils up to 10% is a characteristic feature of CML. There may be associated eosinophilia. A rising basophilia (more than 20%) in peripheral blood is indicative of impending blastic transformation. Myeloid blast crisis in CML is more common (two-third cases) and resembles AML. However, unlike AML, Auer rods are rarely seen in myeloblasts of CML in blast crisis. Lymphoid blast crisis in CML having the characteristics of lymphoblasts such as presence of TdT is seen in one-third cases of blastic phase in CML.

Platelets

The platelet count may be normal but is raised in about half the cases.

PBF IN CHRONIC LYMPHOCYTIC LEUKAEMIA

Chronic lymphocytic leukaemia (CLL) constitutes about 25% of all leukemias and is predominantly a disease of the elderly (over 60 years of age in 80% of patients) with

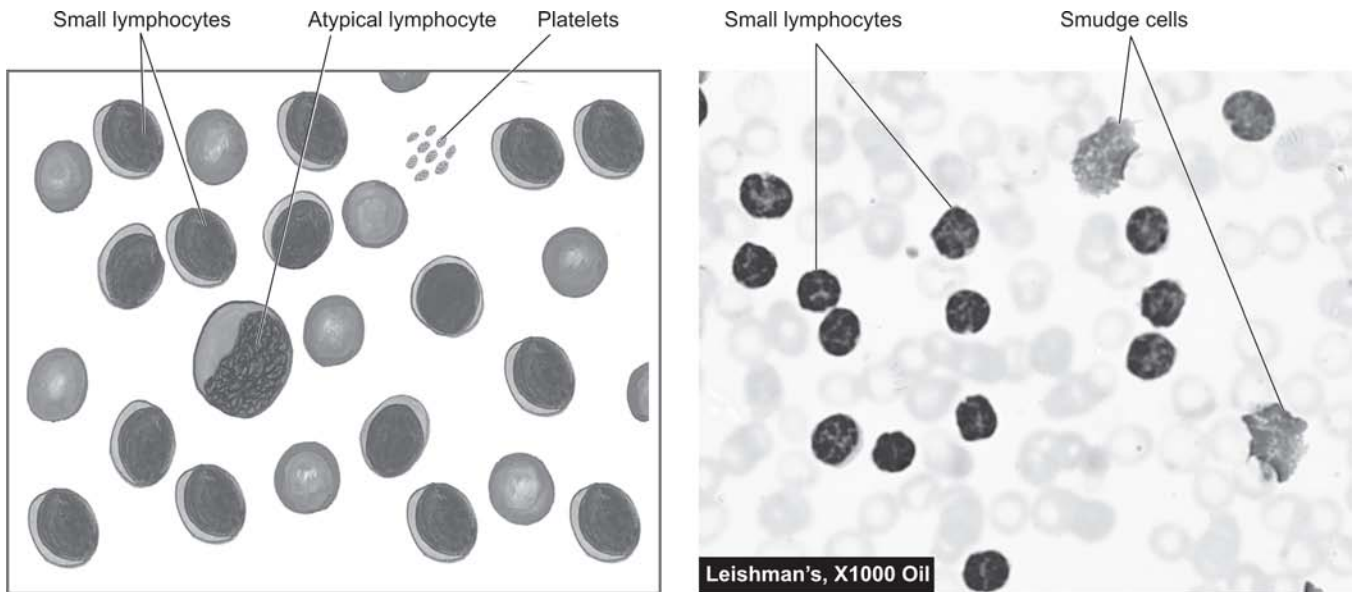


FIGURE 54.3: PBF in chronic lymphocytic leukaemia. The white cell count is increased with predominance of small lymphocytes and a few degenerated forms.

a male preponderance (male-female ratio 2:1). The diagnosis of CLL can usually be made on the basis of physical findings and blood smear examination. Bone marrow examination/ biopsy is generally not required for diagnosis of CLL but is useful to assess normal marrow reserve and the pattern of involvement by CLL which has prognostic significance.

The findings of routine blood picture are as under (Fig. 54.3):

Anaemia

Anaemia is usually mild to moderate and normocytic normochromic in type. Anaemia in CLL is related to marrow replacement but in about 20% cases develop a Coomb's-positive autoimmune haemolytic anaemia. Mild reticulocytosis may be present. About 20% cases develop a Coomb's-positive autoimmune haemolytic anaemia.

White Blood Cells

Typically, there is marked leucocytosis but less than that seen in CML (50,000-200,000/ μ l). Usually, more than 90% of leucocytes are mature-appearing lymphocytes. Smear cells (smudge cells or basket cells) which are degenerated forms are frequently present. The absolute neutrophil count is, however, generally within normal range. Granulocytopenia occurs in fairly advanced disease only.

Platelets

The platelet count is normal or moderately reduced. When thrombocytopenia is present in CLL, it is indicative of higher grade and worse prognosis.



Haemoparasites in Blood

- ◆ PBF in Malarial Parasite
- ◆ PBF in Filariasis
- ◆ Bone Marrow in Leishmaniasis

Exercise

55

Infection with blood parasites particularly malaria and filaria exacts an enormous toll of human suffering in the Indian subcontinent. Both can be easily diagnosed by careful examination of well prepared and stained peripheral blood smear, while Leishmania can be identified in bone marrow smears.

PBF IN MALARIAL PARASITE

Four species of malarial parasite (MP) are encountered in human disease: *Plasmodium vivax* and *P. ovale*, both

of which cause benign tertian malaria (febrile episodes typically occurring at 48 hourly intervals); *P. falciparum*, which is responsible for most fatalities; and *P. malariae*, which causes quartan malaria (febrile episodes typically occurring at 72 hourly intervals). The differences in appearances of various stages of *P. falciparum* and *P. vivax* are contrasted in Table 55.1.

M/E

1. Blood smears should be made a few hours after peak of febrile paroxysms.

Table 55.1: Contrasting features of common forms of malarial parasite.

Features	<i>P. falciparum</i>	<i>P. vivax</i>
1. Schizogony period	48 hours	48 hours or less
2. Forms in peripheral blood	Trophozoites, schizonts and gametocytes	Rings and crescents only; growing trophozoites and schizonts rarely seen
3. Trophozoite and ring form	Size 2.5 μ ; cytoplasm opposite the nucleus is thicker	Size 1.25 to 1.5 μ ; cytoplasm fine and regular in outline, often with 2 nuclei, may have accolé and multiple infection.
4. Growing forms	Irregular with a vacuole; actively amoeboid	Assume a compact form; pigments collect into a single mass early
5. Schizont	Size 9 to 10 μ ; regular, almost completely fills an enlarged red blood cell	Size 4.5 to 5 μ ; fills two-thirds of a red cell mass which is not enlarged
6. Merozoites	12 to 24, arranged in an irregular grape-like cluster	18 to 24 or more, arranged in a grape-like cluster
7. Haemozoin	Yellowish-brown, fine granules	Dark brown or blackish, one solid block
8. Changes in infected host RBCs	Enlarged, pale, Schuffner's dots present	Usually unaltered, crenation, reddish violet colour and Maurer's dots
9. Gametocyte	Spherical or globular; much larger than a red blood cell; host cell enlarged with Schuffner's dots	Crescentic; larger than a red blood cell; host cell hardly recognizable

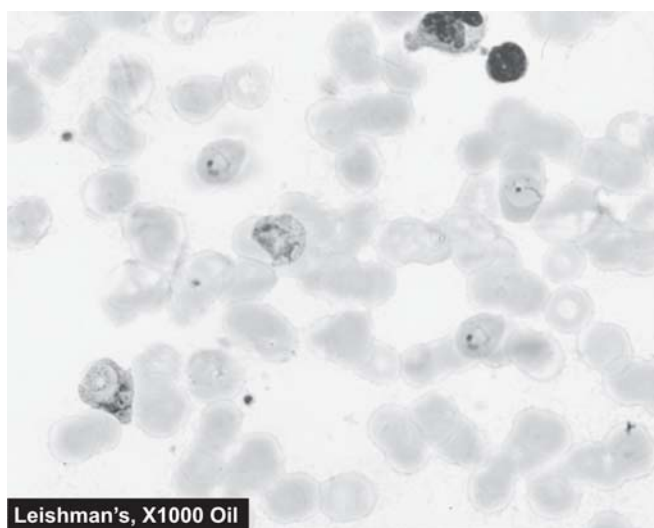
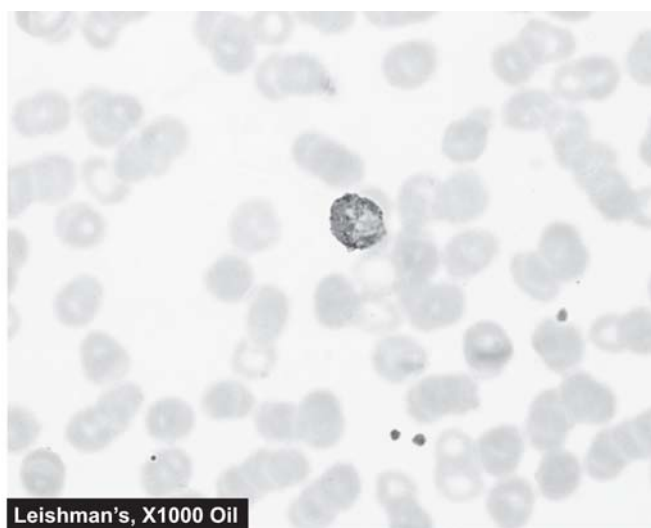
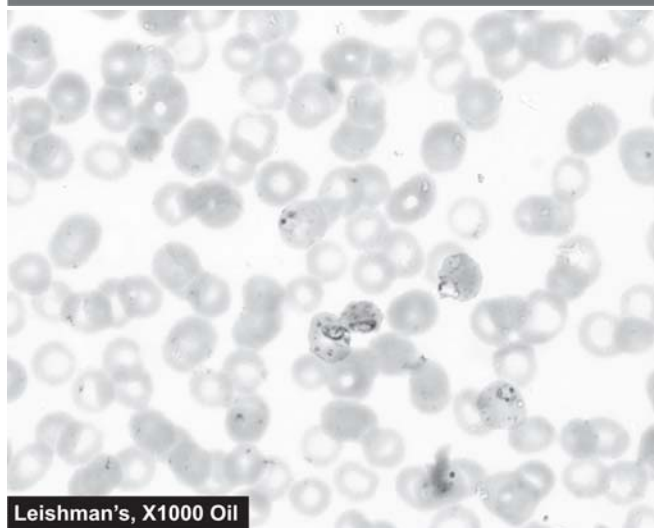
A, Ring stage of *P. vivax*A, Amoeboid form of *P. vivax*C, Ring stage of *P. falciparum*D, Gametocytes of *P. falciparum*

FIGURE 55.1: Malarial parasite—various stages of two main species of *Plasmodium* (*P. vivax* and *P. falciparum*).

- Thin and thick blood smears can be made to demonstrate the malarial parasite.
 - Thick smear* is prepared by spreading a drop of blood on the slide in an area of about 2 cm diameter and letting it dry. The smear is then given a few dips in tap water until red coloured solution comes out (dehaemoglobinization of the smear). The smear is then fixed in the methanol and stained with Leishman's or Giemsa or Field's stain. Thick smear is of use in quick detection of malarial parasite while thin smear is useful for studying morphology.
 - The detailed features in morphology of two main species of malarial parasite (*P. vivax* and *P. falciparum*) are given in Table 55.1 and shown in Figure 55.1.
 - Besides, PBF also shows monocytosis with moderate leucopenia. Two-thirds of patients infected with *P. falciparum* infection also show anaemia with increased reticulocyte count (due to haemolysis) and thrombocytopenia.
- In addition to examination of PBF, malaria can also be detected by other methods as follows:

- i. *Fluorescent microscopy*: Nucleic acids of the parasite are stained with fluorescent dyes and visualized by fluorescent microscopy.
- ii. *pLDH based immunochromatographic test*: Presence of *Plasmodium* LDH enzyme is detected using monoclonal antibodies against specific epitopes of pLDH.
- iii. *PCR based test*: These are based on the detection of nucleic acid sequences specific to *Plasmodium* species.

PBF IN FILARIASIS

Wechereria bancrofti is largely confined to the tropics and subtropics. In India, it is distributed chiefly along the sea-coast and along the banks of big rivers. The adult worms are long hair like transparent nematodes which remain localized to lymphatic vessels and lymph nodes causing massive lymphoedema and elephantiasis. Embryonic forms (microfilariae) reach into the circulating blood and can be demonstrated in the peripheral blood.

M/E

1. The blood sample should be collected at night between 10 pm and 2 am as microfilariae exhibit *nocturnal periodicity*.
2. *Unstained wet preparation* can be examined. In this, 2-3 drops of blood are taken of the midnight blood sample on a clean glass slide and a coverslip is put on it. The coverslip is sealed with vaseline to prevent drying of blood drop. The slide is examined next morning under low power objective and microfilariae, if present, may be seen wriggling about in the blood.
3. *Thin and thick blood smears* as described for malaria above can be made.
4. *Morphologically*, microfilariae measures about 290 μ in length and 6 μ in breadth. A hyaline sheath engulfs the larval body. The somatic cells appear as granules and extend from head to tail except five percent terminal tail sheath (Fig. 55.2).
5. In addition, PBF shows *eosinophilia* as compared to monocytosis seen in case of malaria.
6. *Concentration methods* can be employed for higher yield of the organism. Five to ten ml of blood is taken in EDTA vial and centrifuged at 2000 rpm for 2 to 5 minutes. The supernatant fluid is decanted and the sediment is examined for microfilariae.

BONE MARROW IN LEISHMANIASIS

The parasite *Leishmania donovani* (LD) causes visceral leishmaniasis or kala azar. In India, it is endemic in the states of Bihar, Orissa, Madras and eastern parts of Uttar Pradesh.



FIGURE 55.2: Microfilariae in thick peripheral blood smear.

M/E

- i. Amastigote forms or LD bodies are observed in reticuloendothelial cells of the bone marrow and spleen.
- ii. LD body measures 2 to 4 μ in size having a nucleus and kinetoplast (Fig. 55.3).

Examination of the bone marrow provides an invaluable diagnostic help in some cases, while in others it is of value in confirming a diagnosis suspected on clinical examination or on the blood film. A peripheral blood examination, however, must always precede bone marrow examination.



FIGURE 55.3: *Leishmania donovani* (LD) bodies in the reticulum cells of the bone marrow aspirate smear.



Bone Marrow Examination

- ◆ Bone Marrow Aspiration
- ◆ Trephine Biopsy

Exercise

56

Examination of the bone marrow provides an invaluable diagnostic help in some cases, while in others it is of value in confirming a diagnosis suspected on clinical examination or on the blood film. A peripheral blood examination, however, must always precede bone marrow examination.

Bone marrow examination may be performed by two methods: aspiration and trephine biopsy. A comparison of the two methods is summarised in Table 56.1 below.

BONE MARROW ASPIRATION

The method involves suction of marrow via a strong, wide bore, short-bevelled needle fitted with a stylet and an adjustable guard in order to prevent excessive penetration; for instance Salah bone marrow aspiration needle (Fig. 56.1,A) or Klima bone marrow needle. Smears are prepared immediately from the bone marrow

aspirate and are fixed in 95% methanol after air-drying. The usual Romanowsky technique is employed for staining and a stain for iron is performed routinely so as to assess the reticuloendothelial stores of iron.

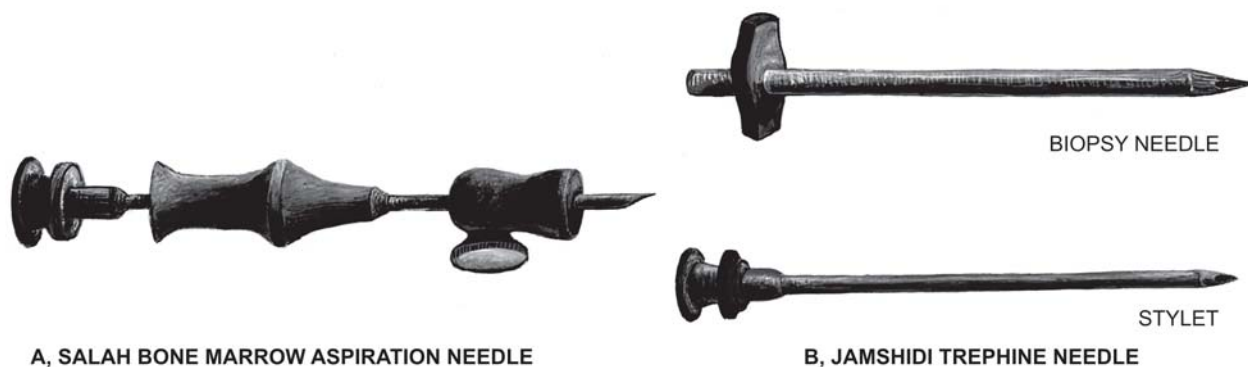
The marrow film provides assessment of the following features (Fig. 56.2):

- Cellularity
- Details of developing blood cells (i.e. normoblastic or megaloblastic, myeloid, lymphoid, macrophages and megakaryocytic)
- Ratio between erythroid and myeloid cells in storage diseases
- For the presence of cells foreign to the marrow such as secondary carcinoma, granulomatous conditions, fungi (e.g. histoplasmosis) and parasites (e.g. malaria, leishmaniasis, trypanosomiasis).

Estimation of the proportion of cellular components in the marrow, however, can be provided by doing a

Table 56.1: Comparison of bone marrow aspiration and trephine biopsy.

Feature	Aspiration	Trephine
1. Site	Sternum, posterior iliac crest; tibial head in infants	Posterior iliac crest
2. Instrument	Salah BM aspiration needle	Jamshidi trephine needle
3. Stains	Romanowsky, Perls' reaction for iron on smears	Haematoxylin and eosin, reticulin on tissue sections
4. Time	Within 1-2 hours	Within 1-7 days
5. Morphology	Better cellular morphology of aspiration smears but marrow architecture is indistinct	Better marrow architectural pattern but cell morphology is not as distinct since tissue sections are examined and not smears
6. Indications	Anaemias, suspected leukaemias, neutropenia thrombocytopenia, polycythaemia, myeloma, lymphomas, carcinomatosis, lipid storage diseases, granulomatous conditions, parasites, fungi, and unexplained enlargements of liver, spleen or lymph nodes.	Additional indications are: myelosclerosis, aplastic anaemia and in cases with 'dry tap' on aspiration.

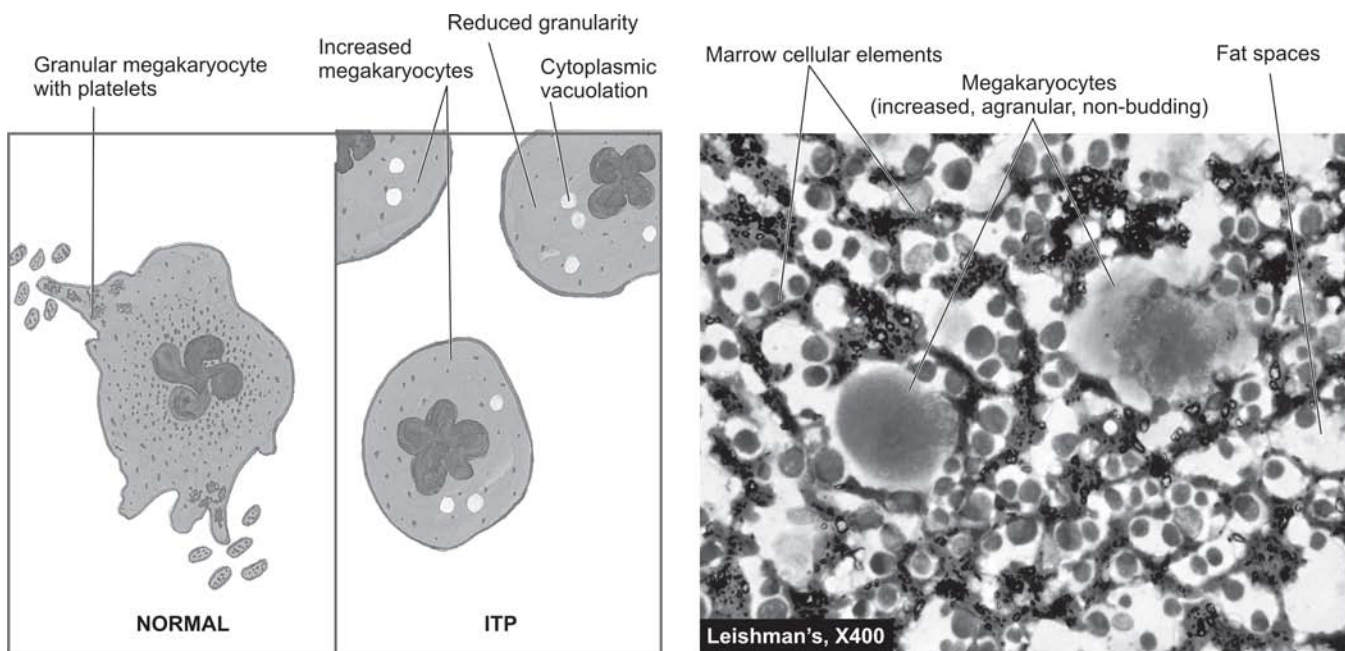


FIGURES 56.1: The Salah bone marrow aspiration needle (A) Jamshidi trephine needle (B).

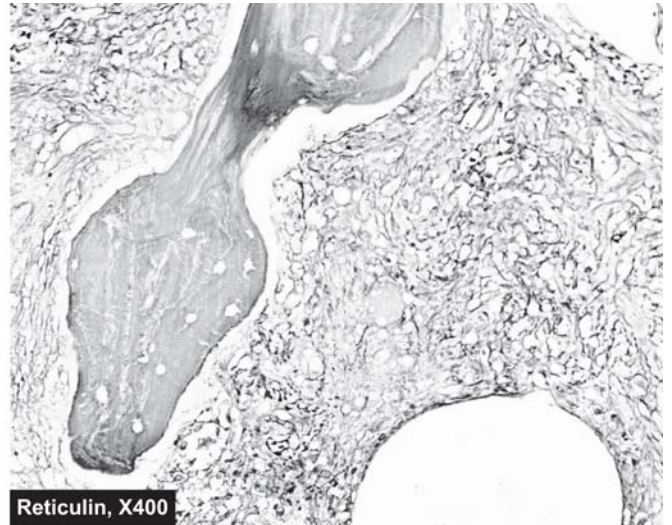
differential count of at least 500 cells called myelogram. Parameters of normal myelogram are given in Table 56.2. In some conditions, the marrow cells can be used for more detailed special tests such as cytogenetics, microbiological culture, biochemical analysis, and immunological and cytological markers.

TREPHINE BIOPSY

Trephine biopsy is performed by a simple Jamshidi trephine needle by which a core of tissue from periosteum to bone marrow cavity is obtained (Fig. 56.1,B). As the core of the bone marrow is obtained, it is rolled between two glass slides to obtain imprint smears. These smears



FIGURES 56.2: Bone marrow aspirate in ITP. Megakaryocytes are increased in number but do not show any budding of platelets.



FIGURES 56.3: Trephine biopsy in myelofibrosis. The soft tissue enclosed in the marrow spaces is composed of rich fibrous tissue.

can be used to study the morphology and to perform special stains. The tissue is then fixed, decalcified and processed for histological sections and stained with haematoxylin and eosin and for reticulin (Fig. 56.3).

Trephine biopsy is useful over aspiration since it provides an excellent view of the overall marrow architecture, cellularity, and presence or absence of infiltrates, but is less valuable than aspiration as far as individual cell morphology is concerned, as summed up in Table 56.2.

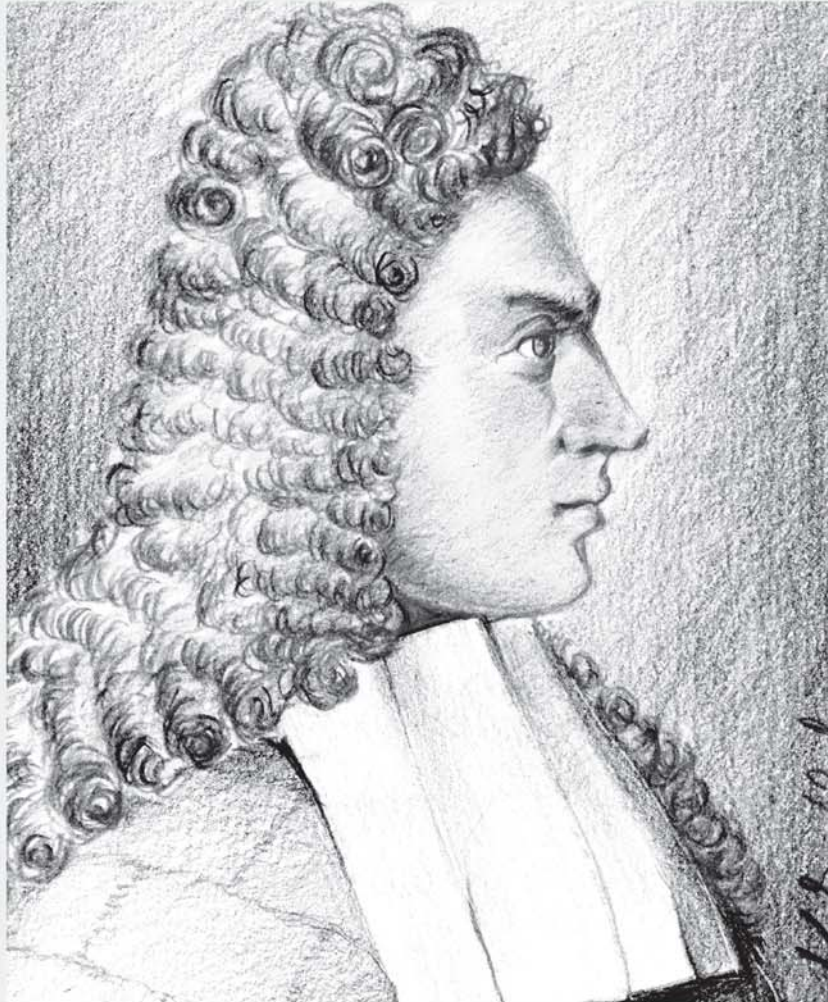
Table 56.2: Normal adult bone marrow counts (Myelogram)

Fat/cell ratio : 50 : 50
Myeloid/erythroid (M/E) ratio : 2-4:1 (mean 3:1)
Myeloid series: 30-45% (37.5%)
Myeloblasts : 0.1-3.5%
Promyelocytes: 0.5-5%
Erythroid series: 10-15% (mean 12.5%)
Megakaryocytes: 0.5%
Lymphocytes: 5-20%
Plasma cells: < 3%
Reticulum cells: 0.1-2%



SECTION SEVEN

AUTOPSY PATHOLOGY



GIOVANNI B MORGAGNI (1682-1771)
'FOUNDER OF CPC'

Italian anatomist and pathologist, who, for the first time made correlation between pathology found at post-mortem and clinical findings. Virchow believed that Morgagni introduced modern pathology.

SECTION VII: AUTOPSY PATHOLOGY

Objectives

After studying this section, the student should be able to gain knowledge and learn the following skills:

- ✧ To have the basic knowledge of autopsy techniques along with preparation of Autopsy Request Form and writing up of Autopsy Report, i.e. findings at prosection and gross and microscopic pathological findings.
- ✧ To gain knowledge about basic concepts of CPC and practice on ten common corollaries involving multiple organ systems.

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Introduction to Autopsy Protocol

- ◆ Introduction
- ◆ Autopsy Protocol

Exercise

57

INTRODUCTION

Professor William Boyd in his inimitable style wrote 'Pathology had its beginning on the autopsy table.' The significance of study of autopsy in pathology is summed up in Latin inscription in an autopsy room reproduced in English as 'The place where death delights to serve the living.'

There is still no substitute for a careful postmortem examination which enlightens the clinician about the pathogenesis of disease, reveals hazardous effects of therapy administered, and settles the discrepancies finally between antemortem and postmortem diagnosis.

The study of autopsy throws new light on the knowledge and skills of both physician as well as pathologist. The main *purposes* of autopsy are as under:

1. *Quality assurance of patient-care* by:
 - i. Confirming the cause of death;
 - ii. establishing the final diagnosis; and
 - iii. study of therapeutic response to treatment.
2. *Education of the entire team* involved in patient-care by:
 - i. Making autopsy diagnosis of conditions which are often missed clinically e.g. pneumonia, pulmonary embolism, acute pancreatitis, carcinoma prostate.
 - ii. Discovery of newer diseases made at autopsy e.g. Reye's syndrome, Legionnaire's disease.
 - iii. Study of demography and epidemiology of diseases.
 - iv. Affords education to students and staff of pathology.

Declining autopsy rate throughout the world in recent times are owing to the following reasons:

1. Higher diagnostic confidence made possible by advances in imaging techniques e.g. CT, MRI, etc.

2. Physician's fear of legal liability on being wrong.

Continued support for advocating autopsy by caring physicians as well as by discernible pathologists in tertiary-care hospitals is essential for improved patient-care and progress in medical science.

AUTOPSY PROTOCOL

Traditionally, there are two methods of carrying out autopsy, either of which may be followed:

1. Block extraction of abdominal and thoracic organs; and
2. *In situ* organ-by-organ dissection.

In conditions where multiple organs are expected to be involved, complete autopsy should be performed. But if a particular organ-specific disease is suspected, a mini-autopsy or limited autopsy may be sufficient.

For performing pathologic autopsy, also called clinical autopsy, there are two absolute essential requirements:

- i. To obtain permission for conducting autopsy including restrictions, if any.
- ii. To obtain as much history and laboratory data as possible about the deceased.

These two requirements can be incorporated in a standard *Autopsy Request Form* devised for the purpose so that the entire information has to be compulsorily filled by the clinical resident; a specimen proforma is shown in Fig. 57.1.

A. Prosection

Prosection means carefully programmed dissection for demonstration of anatomic structures. The prosector on his part conducts the autopsy keeping the following sequenced checklist in mind:

1. Assessment of the body as regards age, sex, race, general physique and nutrition.

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SECTOR-32, CHANDIGARH

DEPARTMENT OF PATHOLOGY

Referred by

Date of admission

Date of death

Name

Age/Sex

CR No.

Ward/OPD

Date

Income

HIV status

Autopsy No.....

Permission for autopsy:

Yes/No

Restrictions on permission:
(tick mark ✓)Abdominal with brain or without brain
Thoracoabdominal with brain or without brain
Brain only
Spinal Cord/eyes/testes/others

Laboratory data and radiological findings:

Operative procedures:

Provisional diagnosis:

Previous cytology/histopathology number, if any:

Date and time

Requested by
Doctor's name & signature

AUTOPSY REQUEST

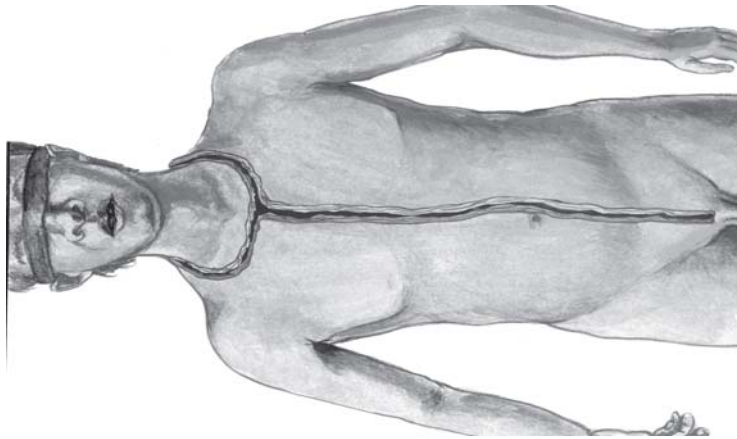


FIGURE 57.2: Thoracoabdominal midline incision from neck to the pubis but avoiding the umbilicus.

2. External examination including injuries (recent/old), if any.
 3. Internal examination by thoraco-abdominal midline incision from neck to pubis, but avoiding the umbilicus which has extensive fibrous tissue that may cause difficulty in sewing back (Fig. 57.2).
 4. Examination of the peritoneal cavity for presence of fluid, blood or pus followed by examination of the bowel and organs in the pelvis.
 5. Cutting through the structures in the neck up to the floor of mouth and base of the tongue.
 6. Divide the sterno-clavicular joints and cut with a saw from the costal margin upwards to the disarticulated sterno-clavicular joints and lift the sternum.
 7. Examine the pleural cavities for presence of fluid, blood or pus and visually assess the lungs.
 8. Divide the diaphragm close to the costal margin all around.
 9. Move the kidneys medially and cut the tissue behind them to free the kidneys.
 10. Now all the thoraco-abdominal organs are removed *en mass* and put on the dissecting table.
 11. Incise the scalp behind the ears on to the posterior parts (bitemporal incision), peeling it forwards as well as backwards. Then with a handsaw or electric saw, the skull is cut circumferentially removing the skull cap by a V-shaped incision. Brain is then removed alongwith meninges, part of the brainstem and pituitary gland.
- unexamined. Briefly, systematic examination of the organs can be done as under:
1. Examination of the tongue, thyroid, oesophagus, larynx and other neck structures.
 2. Examination of lungs by putting the hilum down on the cutting board and slicing them horizontally full length from apex to base.
 3. Heart is examined next. It is weighed after removal of blood clots, from the chambers. Enzyme studies on gross specimen can be done at this stage before fixing.
 4. Stomach is incised along the greater curvature down through the pylorus into the duodenum. At this stage gallbladder and ampulla of Vater are examined.
 5. Adrenals are examined next.
 6. Spleen is removed from the *en mass* and cuts given into the substance.
 7. Kidneys are then removed by cutting from the hilum and the capsule and the capsule is stripped off. Transverse cut is made from outer border towards the hilum.
 8. Urinary bladder and the other pelvic organs can be examined at this stage.
 9. Liver is examined next. Parallel incisions are given to the liver after weighing the organ.
 10. Brain is best examined after fixation in suspension than to cut the soft and wet brain.

C. Autopsy Report

Lastly, the most important part in the autopsy is formulation of carefully worded Autopsy Report based on foregoing examination (external, internal and gross examination) (Fig. 57.3,A) followed by ancillary investigations that include histopathologic examination and other ancillary investigations (Fig. 57.3,B).

B. Gross Examination of Organs

Examination of the structures and organs so removed can be done by various methods but whatever plan is followed it should be adhered to so that no part remains

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SECTOR-32, CHANDIGARH
DEPARTMENT OF PATHOLOGY**

Referred by
Clinical diagnosis

HIV status

Date & time of death

Date & time of autopsy

Name

Age/Sex

CR No.

Ward/OPD

Date

Income

Autopsy No.....

A. EXTERNAL EXAMINATION

(Body surface, nutritional status, height, weight, injuries if any, scars)

B. INTERNAL EXAMINATION

1. INCISION

2. EFFUSIONS

(Pericardial, pleural L/R, peritoneal)

3. CENTRAL NERVOUS SYSTEM

(Meninges, cerebral vessels, brain, spinal cord)

4. RESPIRATORY SYSTEM

(Larynx, trachea, bronchi, pleura, lungs)

5. CARDIOVASCULAR SYSTEM

(Heart, valves, myocardium, pericardium, coronaries, great vessels)

6. ALIMENTARY TRACT

(Mouth, tongue, oesophagus, stomach and its contents, duodenum, intestines, liver, gallbladder, pancreas, peritoneum)

7. GENITOURINARY SYSTEM

(Kidneys, ureters, bladder, gonads)

8. RETICULOENDOTHELIAL SYSTEM

(Spleen, lymph nodes, thymus)

9. ENDOCRINE SYSTEM

(Adrenals, thyroid, pituitary)

10. MUSCULOSKELETAL SYSTEM

(Skull, spine, rest of skeleton, muscles)

C. SAMPLE FOR ANCILLARY INVESTIGATIONS

(Toxicology/microbiology/virology/serology/EM)

Date & time

PROSECTOR
(Name & Signature)

AUTOPSY REQUEST (A)

**GOVERNMENT MEDICAL COLLEGE HOSPITAL
SECTOR-32, CHANDIGARH
DEPARTMENT OF PATHOLOGY**

Referred by
Clinical diagnosis

HIV status

Name	
Age/Sex	
CR No.	
Ward/OPD	
Date	Income

Autopsy No.....

MICROSCOPIC DESCRIPTION (ORGAN-WISE):

FINAL AUTOPSY DIAGNOSIS:

CAUSE OF DEATH:

Date of receiving.....

Date of reporting.....

PATHOLOGIST

AUTOPSY REQUEST (B)

FIGURE 57.3 (contd): Specimen proforma for autopsy report.

The autopsy report can be given in two formats:

- i. A *printed proforma* type of report listing various body systems with space for writing the description against each. This pattern has the advantage that no organ is left out for describing and the standardized information can be used for coding purposes. However, this type of pattern has the disadvantage that the space provided for each organ and system is prefixed and cannot be accommodated according to the requirement of the specific case for which additional sheets may be attached.
- ii. The *open type* of report is in the form of an essay, which can adjust any variation in the description of a particular organ or part of the body. But this pattern suffers from the disadvantage that description of some organs may be left out.

In general, the format of the Autopsy Report should consist of the following:

1. **Permission** for autopsy.
2. **Data pertaining to the deceased:** Name, age, sex, address, serial number of autopsy, date of admission and date and time of death, clinical history and laboratory data including radiological findings, operative procedure and clinical diagnosis.

3. **Data pertaining to the pathologist:** Autopsy number, date and time of autopsy, name of prosector, initial gross autopsy findings including weights and measurements of organs, and gross autopsy diagnosis.
4. **Postmortem findings:** These should be listed in the following sequence:
 - A. *External examination* as outlined above.
 - B. *Internal examination* as explained above.
 - C. *Pathologic examination* of formalin-fixed organs by description of gross and microscopic examination, with organs of interest in greater detail.
5. **Final autopsy diagnosis:** This is the conclusion of pathological findings of autopsy.
6. **Cause of death:** The final comment on the cause of death is of paramount importance. Summary and interpretation of causative sequence of various lesions observed by the pathologist about the case are given here.

Figure 57.3 provides a specimen proforma used for preparing an Autopsy Report by the pathologist that includes initial autopsy record and gross diagnosis (A) and subsequent pathologic examination of formalin-fixed tissues (B).



Clinicopathological Conference (CPC) and About CD on CPCs

- ◆ Clinicopathological Conference (CPC)
- ◆ About CD on CPCs

Exercise

58

CLINICOPATHOLOGICAL CONFERENCE (CPC)

G.B. Morgagni in Italy (1682-1771) and T.H.A. Laennec (1781-1826) in France started collecting the case records of hospital cases and began correlation of clinical features with the lesions observed at autopsy and thus marked the beginning of clinicopathologic correlation.

In the modern times, "Clinicopathological Conference" commonly known as "CPC" has about 100 years of long and distinguished history since its origin from Massachusetts General Hospital and Harvard Medical School, Boston. It started in 1890s as an informal discussion on case-based method of teaching medicine between two Harvard Internists, Dr. Richard C. Cabot and Walter B. Cannon, who got the idea from another roommate in college who attended Harvard Law School where case method of teaching law was practiced. Richard C. Cabot first published these case-based medical teaching exercises as the Cabot Case Records in the *Boston Medical and Surgical Journal* in 1924. In 1928, that journal became the *New England Journal of Medicine*, and has continued publication of the CPC since then regularly as *Case Records of the Massachusetts General Hospital*.

The purpose of the CPC is to teach anatomic-pathological correlation with the clinical sequence as it happened during life in disease, and the principles of differential diagnosis and pathophysiology. The process of the CPC has been described as an exercise by reasoning, i.e. a logical and instructive analysis of the conditions involved.

Classically, CPC has a *Moderator* to direct the discussion and to maintain the time discipline. The CPC involves presentation of clinical and laboratory data by a *Clinical Discussant* who is generally not a member of the treating unit of the selected case during life. The clinical discussant systematically analyses the data by suggesting various possible differential diagnosis and excluding one by one

by giving diagnostic reasoning and pathophysiological sequence, and then he concludes by offering the most likely clinical diagnosis. This is followed by a discussion in the open house on the proposed diagnosis or other clinical possibilities. Then, the *Pathology Discussant* presents the autopsy findings on gross and microscopy as well as results of some other ancillary studies which may have been carried out on autopsied organs. The pathologist also reconstructs the sequence as may have happened during life with autopsy findings to offer the pathophysiological aspect of the disease and assigns possible cause of death with reasoning. He concludes by providing autopsy diagnosis considered as "the final diagnosis". Moderator then invites the discussion for correlation of pathological findings with the preceding clinical and laboratory data and ends with the lessons learnt in the process.

In general, CPC is not designed as a "Clinicopathological Competition" or a "Clinicopathological Contest" between the Clinical Discussant and the Pathology Discussant but sometimes it actually turns so.

ABOUT CD ON CPCs

CPC continues to be the most important form of clinical teaching activity in medical institutions worldwide. The curriculum of undergraduate students in medicine lays down this learning activity as part of pathology teaching towards the end of their second professional i.e. in the 5th Semester of MBBS. Not many institutions have access to CPC as teaching material due to dwindling rates of pathologic autopsies as outlined already in previous exercise. Thus, it was considered prudent to include ten structured CPCs based on common diseases pertaining to different organ systems for the students as learning exercises which have been included in the enclosed CD.

The pattern of presentation in the CD is in the conventional sequence as follows: ID of the deceased, brief clinical signs and symptoms, results of relevant laboratory data, possible clinical diagnosis and autopsy findings (i.e. external examination, internal examination that includes findings on gross and microscopic examination with greater emphasis on the organ or system of interest, final autopsy diagnosis and cause of death).

While the details of ten CPCs as regards their clinical and laboratory data, clinical diagnosis, and gross and microscopic autopsy findings along with the representative photomicrographs, are given in the enclosed CD, the final autopsy diagnosis of these ten CPCs are given below which may serve as their index for ready reference:

CPC 1: Case of secondary systemic amyloidosis

- Secondary (Reactive) systemic amyloidosis involving the spleen, kidneys and the liver
- Bronchiectasis both lungs

CPC 2: Case of septic shock with DIC

- Septic shock due to infected retained products of intrauterine gestation, myometrial and cervical abscesses, left pyosalpinx
- DIC with ATN

CPC 3: Case of miliary tuberculosis with TBM

- Disseminated miliary tuberculosis affecting both lungs, hilar lymph nodes, heart, liver, spleen, both kidneys, small intestines and tubercular meningitis
- Senile emphysema both lungs

CPC 4: Case of RHD with infective endocarditis

- RHD
- Bacterial endocarditis
- Infarcts and abscesses in spleen, kidneys and brain

CPC 5: Case of cirrhosis with portal hypertension

- Post-necrotic macronodular cirrhosis and hepatocellular carcinoma
- Portal hypertension (ascites, gastroesophageal varices with ulcerations, congestive splenomegaly)

CPC 6: Case of bronchogenic carcinoma

- Bronchogenic carcinoma, left main bronchus with left lung collapse
- Metastatic deposits in the hilar lymph nodes
- Coronary atherosclerosis

CPC 7: Case of long-standing inflammatory bowel disease

- Ulcerative colitis with features of toxic megacolon
- CMV colitis
- Fatty change liver

CPC 8: Case of RPGN

- Crescentic glomerulonephritis
- Thromboemboli multiple organs
- Pulmonary haemorrhages

CPC 9: Case of NIDDM

- Diabetic nephropathy
- Coronary atherosclerosis with healed transmural myocardial infarcts
- Multiple lung abscesses

CPC 10: Case of choriocarcinoma

- Disseminated gestational choriocarcinoma of the uterus
- Metastatic deposits both lungs, liver, kidneys, heart and the brain.



Normal Values

- ◆ Weights and Measurements of Normal Organs
- ◆ Laboratory Values of Clinical Significance

Appendix

WEIGHTS AND MEASUREMENTS OF NORMAL ORGANS

In order to understand the significance of alterations in weight and measurement of an organ in disease, it is important to be familiar with the normal values. A comprehensive list of generally accepted normal weights

and measurements of most of the normal organs in fully-developed, medium-sized individual and a normal healthy newborn are compiled in Table A-1.

Single value and value within brackets are indicative of the average figure for that organ. Measurements have been given as width × breadth (thickness) × length. An alphabetic order has been followed.

Table A-1: Weights and Measurement of Normal Organs.

Organs	In adults	At birth (wherever applicable)
Adrenal gland:		
Weight	4–5 gm	8-11 gm
Brain:		
Weight (in males)	1400 gm	320-420 gm
Weight (in females)	1250 gm	—
Measurements (sagittal × vertical)	16.5 × 12.5 cm	—
Volume of cerebrospinal fluid	120–150 ml	—
Heart:		
Weight (in males)	300–350 gm	17-30 gm
Weight (in females)	250–300 gm	—
Thickness of right ventricular wall	0.3–0.5 cm	—
Thickness of left ventricular wall	1.3–1.5 cm	—
Circumference of mitral valve	10 cm	—
Circumference of aortic valve	7.5 cm	—
Circumference of pulmonary valve	8.5 cm	—

Organs	In adults	At birth (wherever applicable)
Circumference of tricuspid valve	12 cm	—
Volume of pericardial fluid	10–30 ml	—
Intestines:		
Length of duodenum	30 cm	—
Total length of small intestine	550–650 cm	—
Length of large intestine	150–170 cm	—
Kidneys:		
Weight each (in males)	150 gm	20-30 gm
Weight each (in females)	135 gm	—
Measurements	3.5 × 5.5 × 11.5 cm	—
Liver:		
Weight (in males)	1400–1600 (1500) gm	100-160 gm
Weight (in females)	1200–1400 (1300) gm	—
Measurements	27 × 8 × 20 cm	—
Lungs:		
Weight (right lung)	375–500 (450) gm	35-55 gm
Weight (left lung)	325–450 (400) gm	—
Volume of pleural fluid	< 15 ml	—

Table A-1: Weights and Measurement of Normal Organs. (contd...)

Organ	In adults	At birth (wherever applicable)
Oesophagus:		
Length (cricoid cartilage to cardia)	25 cm	—
Distance from incisors to gastro-oesophageal junction	40 cm	—
Ovaries:		
Weight (each)	4–8 (6) gm	—
Measurements	1 × 2.5 × 4.5 cm	—
Pancreas:		
Total weight	60–100 (80) gm	3–6 gm
Weight of endocrine pancreas	1–1.5 gm	—
Measurements	3.8 × 4.5 × 18 cm	—
Parotid glands:		
Weight (each)	30 gm	—
Pituitary gland (hypophysis):		
Weight	500 mg	—
Placenta:		
Weight at term	400–600 gm	—
Prostate:		
Weight	20 gm	—
Stomach:		
Length	25–30 cm	—
Spleen:		
Weight	125–175 (150) gm	6–14 gm
Measurements	3.5 × 8.5 × 13 cm	—
Testis and epididymis:		
Weight each (in adults)	20–27 gm	—
Thymus:		
Weight	5–10 gm	10–35 gm
Thyroid:		
Weight	15–40 gm	—
Uterus:		
Weight (in nonpregnant woman)	35–40 gm	—
Weight (in parous woman)	75–125 gm	—

LABORATORY VALUES OF CLINICAL SIGNIFICANCE

Currently, the concept of ‘normal values’ and ‘normal ranges’ is replaced by ‘reference values’ and ‘reference limits’ in which the variables for establishing the values for the reference population in a particular test are well defined. Reference ranges are valuable guidelines for the clinician. However, the following cautions need to be exercised in their interpretation:

- *Firstly*, they should not be regarded as absolute indicators of health and ill-health since values for healthy individuals often overlap with values for persons afflicted with disease.
- *Secondly*, laboratory values may vary with the method and mode of standardisation used; reference ranges given below are based on the generally accepted values by the standard methods in laboratory medicine.
- *Thirdly*, although in most laboratories in the West and in all medical and scientific journals, International Units (IU) conforming to the SI system are followed, but conventional units continue to be used in many laboratories in the developing countries.

The WHO as well as International Committee for Standardisation in Haematology (ICSH) have recommended adoption of SI system by the scientific community throughout world. In this section, laboratory values are given in both conventional and international units. Conversion from one system to the other can be done as follows:

$$\text{mg/dl} = \frac{\text{mmol/L} \times \text{atomic weight}}{10}$$

$$\text{mmol/L} = \frac{\text{mg/dl} \times 10}{\text{atomic weight}}$$

According to the SI system, the prefixes and conversion factors for metric units of length, weight and volume are as given in Table A-2.

Table A-2: Prefixes and Conversion Factors in SI System.

Prefix	Prefix symbol	Factor	Units of length	Units of weight	Units of volume
kilo-	k	10 ³	kilometre (km)	kilogram (kg)	kilolitre (kl)
		1	metre (m)	gram (g)	litre (l)
deci-	d	10 ⁻¹	decimetre (dm)	decigram (dg)	decilitre (dl)
centi-	c	10 ⁻²	centimetre (cm)	centigram (cg)	centilitre (cl)
milli-	m	10 ⁻³	millimetre (mm)	milligram (mg)	millilitre (ml)
micro-	μ	10 ⁻⁶	micrometre (μm)	microgram (μg)	microlitre (μl)
nano-	n	10 ⁻⁹	nanometre (nm)	nanogram (ng)	nanolitre (nl)
pico-	p	10 ⁻¹²	picometre (pm)	picogram (pg)	picolitre (pl)
femto-	f	10 ⁻¹⁵	femtometre (fm)	femtogram (fg)	femtolitre (fl)
atto-	a	10 ⁻¹⁸	attometre (am)	altogram (ag)	altolitre (al)

The laboratory values given below are divided into three sections: clinical chemistry of blood (Table A-3),

other body fluids (Table A-4), and haematologic values (Table A-5). In general, an alphabetic order has been followed.

Table A-3: Clinical Chemistry of Blood.

Components	Fluid	Reference value	
		Conventional	SI units
Alcohol, ethyl	Serum/whole blood	Negative	Negative
mild to moderate intoxication		80-200 mg/dl	
marked intoxication		250-400 mg/dl	
severe intoxication		>400 mg/dl	
Aminotransferases (transaminases)			
aspartate (AST, SGOT)	Serum	0-35 U/L	0-0.58 μkat*/L
alanine (ALT, SGPT)	Serum	0-35 U/L	0-0.58 μkat/L
Ammonia	Plasma	10-80 μg/dl	6-47 μmol/L
Amylase	Serum	60-180 U/L	0.8-3.2 μkat/L
Bicarbonate (HCO ₃ ⁻)	Whole blood	21-30 mEq/L	21-28 mmol/L
Bilirubin			
total	Serum	0.3-1.0 mg/dl	5.1-17 μmol/L
direct (conjugated)	Serum	0.1-0.3 mg/dl	1.7-5.1 μmol/L
indirect (unconjugated)	Serum	0.2-0.7 mg/dl	3.4-12 μmol/L
Calcium, ionised	Whole blood	4.5-5.6 mg/dl	1.1-1.4 mmol/L
Calcium, total	Plasma	9.0-10.5 mg/dl	2.2-2.6 mmol/L
CO ₂ content	Plasma	21-30 mEq/L (arterial)	21-30 mmol/L (arterial)
Chloride (Cl ⁻)	Serum	98-106 mEq/L	98-106 mmol/L
Cholesterol	Serum		
total desirable for adults		<200 mg/dl	<5.2 mmol/L
borderline high		200-239 mg/dl	5.20-6.18 mmol/L
high undesirable	>240 mg/dl	>6.21 mmol/L	
LDL-cholesterol, desirable range		<130 mg/dl	<3.36 mmol/L
HDL-cholesterol, protective range		>60 mg/dl	>1.15 mmol/L

*μkat (kat stands for katal, meaning catalytic activity) is a modern unit of enzymatic activity.

Table A-3: Clinical Chemistry of Blood (Contd...)

Components	Fluid	Reference value	
		Conventional	SI units
Copper	Serum	70-140 µg/dl	13-24 µmol/L
Creatine kinase (CK)	Serum		
males		60-400 U/L	1.00--6.67 µkat/L
females		40-150 U/L	0.67-2.50 µkat/L
Creatine kinase-MB (CK-MB)	Serum	0-7 ng/ml	0-7 µg/L
Creatinine	Serum	0.5-1.5 mg/dl	53-133 µmol/L
Electrophoresis, protein	Serum	See under proteins	
Fatty acids, free non-esterified	Plasma	<180 mg/dl	<18mg/L
Gamma-glutamyl trans-peptidase (transferase) (γ-GT)	Serum	1-94 U/L	1-94U/L
Gases, arterial			
HCO ₃ ⁻	Whole blood	21-30 mEq/L	21-28 mmol/L
pH	Whole blood	7.38-7.44	7.38-7.44
pCO ₂	Whole blood	35-45 mmHg	4.7-5.9 kPa
pO ₂	Whole blood	80-100 mmHg	11.0-13.0 kPa
Glucose (fasting)	Plasma		
normal		70-110 mg/dl	< 6.1 mmol/L
impaired fasting glucose (IFG)		110-126 mg/dl	6.1-7.0 mmol/L
diabetes mellitus		>126 mg/dl	>7.0 mmol/L
Glucose (2-hr post-prandial)	Plasma		
normal		<140 mg/dl	<7.8 mmol/L
impaired glucose tolerance (IGT)		140-200 mg/dl	7.8-11.1 mmol/L
diabetes mellitus		>200 mg/dl	>11.1 mmol/L
Immunoglobulins	Serum		
IgA		60-309 mg/dl	
IgD		0-14 mg/dl	
IgE		<0.025 mg/dl	
IgG		800-1500 mg/dl	
IgM		53-334 mg/dl	
Lactate dehydrogenase (LDH)	Serum	100-190 U/L	1.7-3.2 µkat/L
Lactate/pyruvate ratio		10/1	
Lipids	Serum	See under cholesterol	
Non-protein nitrogen (NPN)	Serum	<35 mg/dl	
Oxygen (% saturation)			
arterial blood	Whole blood	94-100%	
venous blood	Whole blood	60-85%	
pH		Blood	7.38-7.44
Phosphatases			
acid phosphatase	Serum	0-5.5 U/L	0.90 nkat/L
alkaline phosphatase	Serum	30-120 U/L	0.5-2.0 nkat/L
Phosphorus, inorganic	Serum	3-4.5 mg/dl	1.0-1.4 mmol/L
Potassium	Serum	3.5-5.0 mEq/L	3.5-5.0 mmol/L

Table A-3: Clinical Chemistry of Blood (Contd...)

Components	Fluid	Reference value	
		Conventional	SI units
Proteins	Serum		
total		5.5-8 g/dl	
albumin		3.5-5.5 g/dl (50-60%)	
globulin		2.0-3.5 g/dl (40-50%)	
α 1 globulin		0.2-0.4 g/dl	
α 2 globulin		0.5-0.9 g/dl	
β globulin		0.6-1.1 g/dl	
γ globulin		0.7-1.7 g/dl	
A/G ratio		1.5-3 : 1	
Renal blood flow		1200 ml/min	
Sodium	Serum	136-145 mEq/L	136-145 mmol/L
Triglycerides	Serum	<160 mg/dl	11-22 μ mol/L
Thyroid function tests			
radioactive iodine uptake (RAIU) 24-hr		5-30%	
thyroxine (T4)	Serum	5-12 μ g/dl	64-154 nmol/L
triiodothyronine (T3)	Serum	70-190 ng/dl	1.1-2.9 nmol/L
thyroid stimulating hormone (TSH)	Serum	0.4-5.0 μ U/ml	0.4-5.0 mU/L
Troponins, cardiac (cTn)			
troponin I (cTnI) Serum	0-0.4 ng/ml	0-0.4 μ g/L	
troponin T (cTnT)	Serum	0-0.1 ng/ml	0-0.1 μ g/L
Urea	Blood	20-40 mg/dl	3.3-6.6 mmol/L
Urea nitrogen (BUN)	Blood	10-20 mg/dl	
Uric acid	Serum		
males		2.5-8.0 mg/dl	150-480 μ mol/L
females		1.5-6.0 mg/dl	90-360 μ mol/L

TABLE A-4: Other Body Fluids.

Components	Fluid	Reference value	
		Conventional	SI units
Catecholamines	24-hr urinary excretion		
epinephrine		< 10 ng/day	
free catecholamines		<100 μ g/day	
metanephrine		<1.3 mg/day	
vanillyl mandelic acid (VMA)		<8 mg/day	
Cerebrospinal fluid (CSF)	CSF		
CSF volume		120-150 ml	
CSF pressure		60-150 mm water	
leucocytes		0-5 lymphocytes/ μ l	
pH		7.31-7.34	
glucose		40-70 mg/dl	
proteins		20-50 mg/dl	
FIGLU	24-hr urine	<3 mg/day	<17.2 μ mol/day

Table A-4: Other Body Fluids (Contd...)

Components	Fluid	Reference value	
		Conventional	SI units
Gastric analysis	Gastric juice		
24-hr volume		2-3 L	
pH		1.6-1.8	1.6-1.8
basal acid output (BAO)		1-5 mEq/hr	1-5 mmol/hr
maximal acid output (MAO)		5-40 mEq/hr	5-40 mmol/hr
after injection of stimulant			
BAO/MAO ratio	<0.6		
Glomerular filtration rate (GFR)	Urine	180 L/day (about 125 ml/min)	
5-HIAA	24-hr urinary excretion	2-8 mg/day	
17-Ketosteroids	24-hr urinary excretion		
males		7-25 mg/day	
females		4-15 mg/day	
Seminal fluid	Semen		
liquefaction		Within 20 min	
sperm morphology		>70% normal, mature spermatozoa	
sperm motility		>60%	
pH		>7.0 (average 7.7)	
sperm count		60-150 million/ml	60-150 × 10 ⁶ /ml
volume		1.5-5.0 ml	
Schilling's test	24-hr urinary excretion	>10% of ingested dose of 'hot'	
(intrinsic factor test)		vitamin B ₁₂ (Details on page 383)	
Urine examination	24-hr volume	600-1800 ml (variable)	
specific gravity	urine (random)	1.002-1.028 (average 1.018)	
protein excretion	24-hr urine	<150 mg/day	
protein, qualitative	urine (random)	Negative	
glucose excretion	24-hr urine	50-300 mg/day	
glucose, qualitative	urine (random)	Negative	
porphobilinogen	urine (random)	Negative	
urobilinogen	24-hr urine	1.0-3.5 mg/day	
Urobilinogen	Urine (random)	Present in 1: 20 dilution	
D-Xylose excretion	Stool	5-8 g within 5 hr after oral dose of 25 g	

TABLE A-5: Normal Haematologic Values.

Components	Fluid	Reference value	
		Conventional	SI units
Myelogram		See Table 56.2 on page 223	
Erythrocytes and Haemoglobin			
Erythrocyte count	Blood		
males		4.5-6.5 × 10 ¹² /L (mean 5.5 × 10 ¹² /L)	
females		3.8-5.8 × 10 ¹² /L (mean 4.8 × 10 ¹² /L)	
Erythrocyte diameter		6.7-7.7 µm (mean 7.2 µm)	
Erythrocyte thickness			
peripheral		2.4 µm	
central		1.0 µm	

Table A-5: Normal Haematologic Values(Contd...)

Components	Fluid	Reference value	
		Conventional	SI units
Erythrocyte indices (Absolute values)	Blood		
mean corpuscular haemoglobin (MCH)		27-32 pg	
mean corpuscular volume (MCV)		77-93 fl	
mean corpuscular haemoglobin concentration (MCHC)		30-35 g/dl	
Erythrocyte life-span	Blood	120 days	
Erythrocyte sedimentation rate (ESR)	Blood		
Westergren 1st hr, males		0-15 mm	
females		0-20 mm	
Wintrobe, 1st hr, males		0-9 mm	
females		0-20 mm	
Ferritin	Serum		
males		15-200 ng/ml	15-200 µg/L
females		12-150 ng/ml	15-150 µg/L
Folate			
body stores		2-3 mg	
daily requirement		100-200 µg	
red cell level	Red cells	150-450 ng/ml	
Serum level	Serum	6-12 ng/ml	11-57 nmol/L
Free erythrocyte protoporphyrin (FEP)	Red cells	20 µg/dl	
Haematocrit (PCV)	Blood		
males		40-54%	0.47 ± 0.07 L/L
females		37-47%	0.42 ± 0.05 L/L
Haptoglobin	Serum	60-270 mg/dl	0.6-2.7 g/L
Haemoglobin (Hb)			
adult haemoglobin (HbA)	Whole blood		
males		13.0-18.0 g/dl	130-180 g/L
females		11.5-16.5 g/dl	115-165 g/L
plasma Hb (quantitative)		0.5-5 mg/dl	5-50 mg/L
haemoglobin A ₂ (HbA ₂)		1.5-3.5%	
haemoglobin, foetal (HbF) in adults		<1%	
HbF, children under 6 months		<5%	
Iron, total	Serum	80-180 µg/dl	10.7-26.9 µmol/L
total iron binding capacity (TIBC)	Serum	250-460 µg/dl	44.8-71.6 µmol/L
iron saturation	Serum	20-45% (mean 33%)	
Iron intake		10-15 mg/day	
Iron loss			
males		0.5-1.0 mg/day	
females		1-2 mg/day	
Iron, total body content			
males		50 mg/kg body weight	
females		35 mg/kg body weight	
Iron, storage form (ferritin and haemosiderin)	30% of body iron		
Osmotic fragility	Blood		
slight haemolysis		at 0.45 to 0.39 g/dl NaCl	
complete haemolysis		at 0.33 to 0.36 g/dl NaCl	
mean corpuscular fragility		0.4-0.45 g/dl NaCl	

Table A-5: Normal Haematologic Values(Contd...)

Components	Fluid	Reference value	
		Conventional	SI units
Reticulocytes	Blood		
adults		0.5-2.5%	
infants		2-6%	
Transferrin	Serum	1.5-2.0 mg/dl	
Vitamin B ₁₂			
body stores		10-12 mg	
daily requirement		2-4 µg	
serum level	Serum	200-900 pg/ml	200-900 pmol/L
Leucocytes			
Total leucocyte count (TLC)	Blood		
adults		4,000-11,000/µl	
infants (full term, at birth)		10,000-25,000/µl	
infants (1 year)		6,000-16,000/µl	
Differential leucocyte count (DLC)	Blood film		
P (polymorphs or neutrophils)		40-75% (2,000-7,500/µl)	
L (lymphocytes)		20-50% (1,500-4,000/µl)	
M (monocytes)		2-10% (200-800/µl)	
E (eosinophils)		1-6% (40-400/µl)	
B (basophils)		< 1% (10-100/µl)	
Muramidase	Serum	5-20 µg/ml	
Platelets and Coagulation			
Bleeding time (BT)			
Ivy's method	Finger prick blood	2-7 min	
template method		2.5-9.5 min	
Clot retraction time	Clotted blood		
qualitative		Visible in 60 min (complete in <24-hr)	
quantitative		48-64% (55%)	
Clotting time (CT)	Whole blood		
Lee and White method		4-9 min at 37°C	
Partial thromboplastin time			
with kaolin (PTTK) or activated			
partial thromboplastin time			
(APTT)	Plasma	30-40 sec	
Prothrombin time (PT)	Plasma	10-14 sec	
(Quick's one-stage method)			
Thrombin time (TT)	Plasma	<20 sec (control ± 2 sec)	
Platelet count	Blood	150,000-400,000/µl	



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