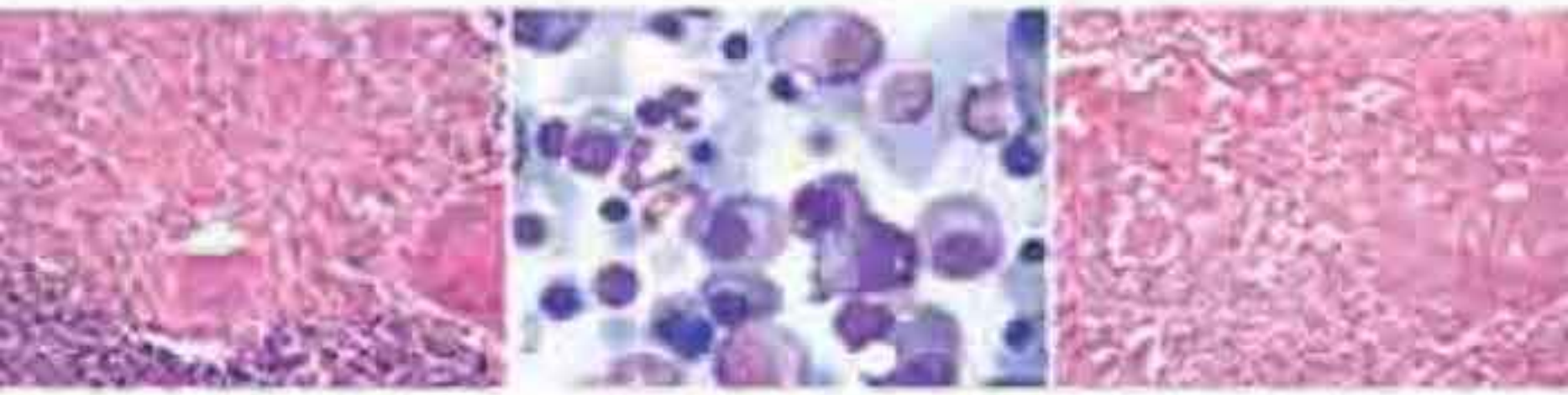
HARSH MOHAN

Textbook of PATHOLOGY



Foreword
Ivan Damjanov

SEVENTH EDITION

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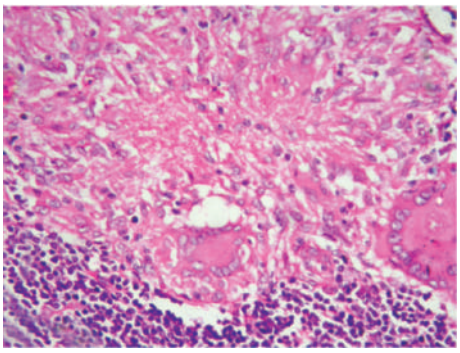

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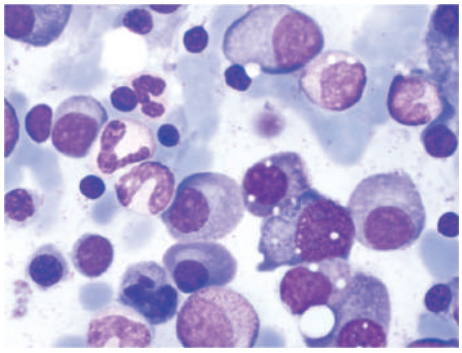

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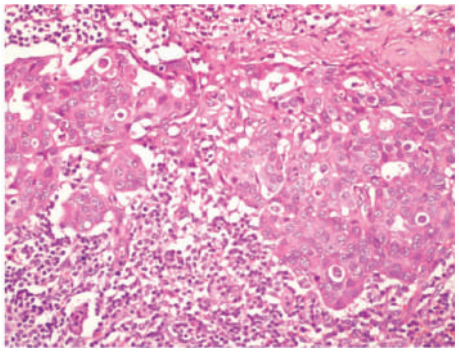

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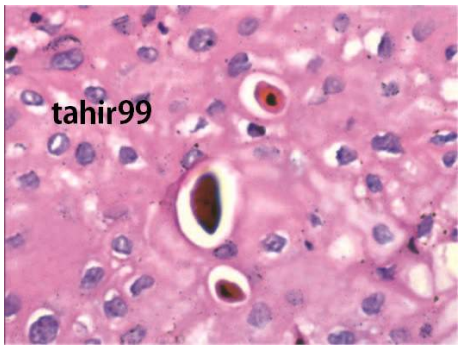
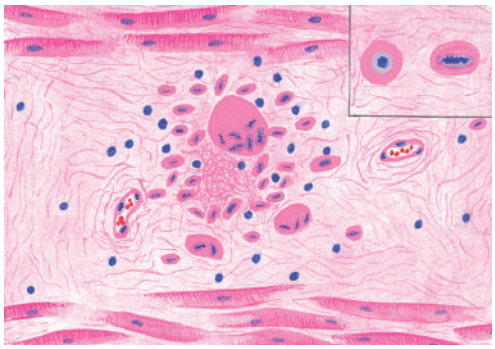


Apoptotic cells in squamous mucosa



Aschoff body in the myocardium

Textbook of
PATHOLOGY

Seventh Edition

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To deeds alone you have a right and
never at all to its fruits;
Let not the fruits of deeds be your motive;
Neither let there be in you
any detachment to performing your duty.

The Bhagvadgita (Chapter II, verse 47)

Dedicated to
My loving soulmate, Praveen, for being there for me and with me always,
Tanya-Vivek, for giving happiness of being together forever,
Sugandha, for being the daughter who is my best friend,
&
To all my students and colleagues, present and past:
For their enduring inspiration.

Foreword

This is the third time that I was asked to write a foreword to Dr Mohan's Textbook, and again I am at a loss for words to adequately express my enthusiasm for this book which has such a long history of excellence. Over all those years spanning the previous six editions, it has served as an introduction to clinical medicine to generations of medical students, and I am sure that it will continue in that function way into the twenty first century. A book of this caliber does not need introductions, forewords and endorsements for its continuous success. Its value has been proven over and over again by those for whom it was written and those who have used it in its previous editions—the medical students and their professors.

For the new edition, Dr Mohan has thoroughly revised his previous text, expanding it with novel data selected judiciously from both laboratory and clinical research papers. Yet the basic structure of the book remains unchanged, with an unconditional dedication to the systematic coverage of the basics, strong clinical underpinning, and a good sense for didactics. It reflects the author's lifelong experience in the classroom and his passion for teaching of pathology to medical students. The text is illustrated with informative artists' drawings and photographs. It also contains highlighted summary boxes and valuable tables. At the end of each chapter there are clinical cases, designed to stimulate further studies and discussions. The condensed Quick Review Book, appended to the textbook as a lagniappe, will remain attractive to medical students preparing for their examinations.

In a short foreword of this kind, it is not possible to mention all the strong points of this textbook. It should suffice to say that Dr Mohan's Textbook has retained all the features which have made previous editions so popular with medical students and their teachers alike. It was masterfully updated and with the new didactic elements it will be even more attractive to its readers. It will remain an essential must-have for all medical undergraduate students, serving them as a pathfinder and bridge during their transition from basic medical sciences to clinical medicine.

Ivan Damjanov, MD, PhD
Professor
Department of Pathology
The University of Kansas School of Medicine
Kansas City, Kansas, USA

Preface

As I sit down to put my thoughts into words for the 7th revised edition of my *Textbook of Pathology*, I look back with satisfaction how this book has grown phenomenally since its modest beginning in 1992. During these years, successive editions of the textbook have brought me closer to enumerable well-wishers, won me life-long friends, rewarded me with respect and faith of my colleagues, got me blessings of senior professionals, and earned me affection of innumerable students and fans. It has been really highly gratifying journey so far. At the same time, such an abiding trust by users of previous editions of my textbook certainly puts an onerous responsibility on me to come up to their expectations and make it better with every new edition. It is this motivation and zeal which I pursued while preparing the thoroughly transformed and updated 7th revised edition which I am pleased to present to users.

Diagnostic pathology has been growing exponentially with advances in molecular methods, cytogenetics and immunology, besides the ready availability of immunohistochemistry. In fact, in the current era, immunophenotyping and cytogenetics have been recommended as defining criteria for classification, diagnosis and prognostication of growing number of cancers. In such a scenario, it is quite natural that undergraduate students of pathology should be made aware of what is happening in the realm of diagnostic science while at the same time not forgetting to lose hold of the fundamental aspects of pathology of diseases. Thus, for beginners in pathology, for whom this textbook is primarily meant, a balanced approach for learning of pathology is recommended i.e. the students must learn basic morphologic pathology including recent knowledge of etiology and pathogenesis of diseases, and simultaneously they should know the contribution of modern diagnostic techniques mentioned above towards achieving the goal of an objective 'final diagnosis' that is prognostically relevant as well. This philosophy for teaching and learning of pathology has been followed in the present edition but without disturbing the basic format of the book.

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

Revised and Updated Text Most of the topics in chapters have undergone revision and updating of various aspects of diseases including their newer causes and recent mechanisms by insertion of latest information between the lines. Emphasis has also been placed on contemporary diagnostic modalities in a simple and lucid manner. In doing so, the basic accepted style of the book—simple, easy-to-understand and reproduce the subject matter, and emphasis on clarity and accuracy, has not been disturbed. Considering their utility, a dozen new tables have been added in different chapters in the revised edition while many others have been updated.

Reorganisation of the Book The redistribution of the textbook into three sections (General Pathology, Haematology and Lymphoreticular Tissues, and Systemic Pathology) done in the previous edition has been widely accepted and appreciated for its ease for locating material and has, therefore, been retained. In order to make space for addition of new information, topics of normal cell structure and function and laboratory techniques have been relocated, after editing them, to relevant chapters to which they belong.

Newer and Revised Images Morphologic pathology has always been regarded as a highly visual branch of medicine, and therefore, there is always need and scope of doing more and making this aspect better in the new edition. In the revised edition, many newer illustrations have been added while some old ones have been replaced with better quality images or improved after eliminating their shortcomings. Inboxes have been incorporated in some photomicrographs for a close-up microscopic view. In general, the effort has been to give soft and pleasing colours for soothing visual look to the new edition.

Gist Boxes In the revised edition, at the end of every topic a short summary of the subject covered has been given. These 'Gist Boxes' (226 in all) include salient must-know features of the covered topic in bulleted points for a rapid revision in ultrashort time. These Gist Boxes have been given a distinctive eye-catching colour throughout the book to be visible on a glance and for looking up quickly by turning the pages of the book for a last minute revision before facing an evaluation in examination.

Clinical Focus on Learning A novel modality of learning and self-assessment has been added in the revised edition by including 30 structured clinical cases. At the end of most of the chapters, one or more clinical cases with history and findings of examination have been given based on a common or an important disease pertaining to the system of that chapter. Questions framed at the end of these cases have been rationally answered and discussed in an analytical manner in Appendix II.

Revised Pathology Quick Review and MCQs The 7th edition of textbook is accompanied with the new revised baby-book popular with many students and interns. This small book has been found profoundly useful by the students just before practical examination to face viva voce when they need to revise huge course content in a short time, or by those preparing to take postgraduate entrance examinations. The revised edition has over 50 more new MCQs while some old ones have either been edited or replaced.

A Word on Foreword Professor Ivan Damjanov, MD, PhD, Kansas University, USA, has been very generous and gracious in writing Foreword for the last three successive editions of my textbook which has brought the textbook closer to users in other

parts of the world, which is appreciated and gratefully acknowledged. He is gifted with qualities of perfection, clarity of mind and meticulous approach, besides having an excellent knack for choosing measured words in his language. I wholeheartedly express my gratitude to this adorable teacher and an eminent author and editor.

In essence, the revised edition is a comprehensive text of pathology meant primarily for students of pathology; however, the practicing clinicians and students of other branches of medicine, dentistry, pharmacy, alternate system of medicine, and paramedical courses may also find it useful.

ACKNOWLEDGEMENTS

The magnitude of work pertaining to revision of the textbook after 5 years is massive and would not have been possible without active cooperation and support from friends and well-wishers in general, and my departmental colleagues in particular. The task of fresh photomicrography for the present edition was ably assisted by my colleagues, Dr Shilpa, Senior Resident, Department of Pathology; and Ms Agam Verma, Senior Lab Technician, which is greatly appreciated. Here, I wish to recall and put on record the sincere and selfless services rendered by my former students and colleagues in preparation of images in earlier editions of the book and thank them once again. As always, I remain indebted to those from whom I had the opportunity to learn basics of pathology—Professor K Joshi, MD, PhD, formerly at PGIMER, Chandigarh, Late Professor TS Jaswal, MD, and Professor Uma Singh, MD, formerly at PGIMS, Rohtak, Haryana, India.

Constant strategic encouragement and support extended by the Department of Medical Education and Research, Chandigarh Administration, during the completion of this academic work is gratefully acknowledged.

I have strived to be as accurate and perfect as possible, and in doing so, I may have been quite harsh and demanding with Production team at the M/s Jaypee Brothers Medical Publishers (P) Ltd. But I must appreciate their patience, cooperation and commitment in general, and Mr Manoj Pahuja, Senior Graphic Designer and Mrs Y Kapoor, Senior Desktop Operator, in particular, for acceding to all my requests for amendments smilingly and ungrudgingly till the very last minute, and to Mr Sunil Dogra, Production Executive, for overseeing the entire project vigilantly and efficiently.

Lastly, the passionate involvement of Shri Jitendar P Vij (Group Chairman) and enthusiasm of Mr Ankit Vij (Group President), M/s Jaypee Brothers Medical Publishers (P) Ltd, in the revised edition of the textbook has raised the bar for a high standard for which I am deeply thankful to both of them. While the content and product quality of the revised edition of the textbook are of an uncompromising quality, the book continues to be of reasonable volume and has been kept affordable.

Finally, I owe gratitude to the users of previous editions who have been generous in giving feedback and suggestions. Every suggestion helps me to introspect and attempt to make the textbook better. I request all the users of present edition to continue giving their valuable suggestions and point out errors, if any, to help me to improve it further.

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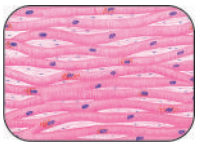

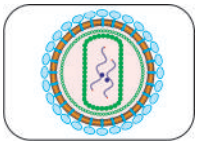

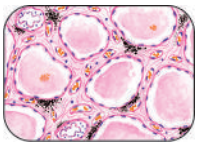

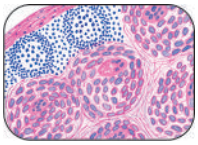

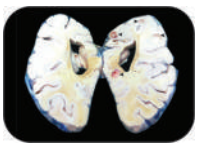

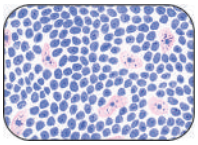

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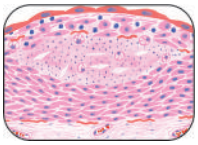

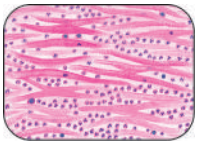

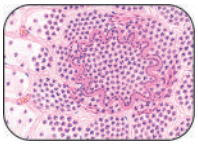

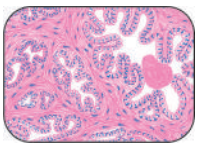

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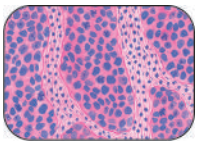

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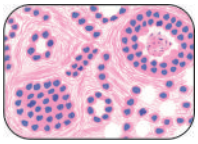

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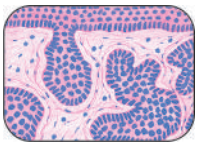

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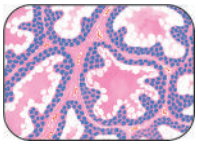

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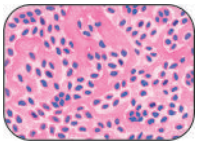

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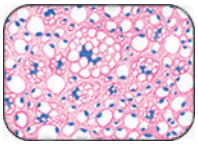

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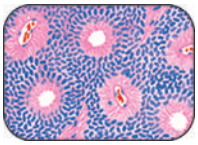
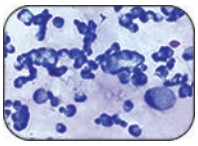


APPENDICES



Section I GENERAL PATHOLOGY

1 Introduction to Pathology

STUDY OF PATHOLOGY

The word *'Pathology'* is derived from two Greek words—*pathos* (meaning suffering) and *logos* (meaning study). Pathology is, thus, scientific study of changes in the structure and function of the body in disease. In other words, pathology consists of the abnormalities in normal anatomy (including histology) and normal physiology owing to disease. Another commonly used term with reference to study of diseases is '*pathophysiology'* (*patho*=suffering, *physiology*=study of normal function). Pathophysiology, thus, includes study of disordered function (i.e. physiological changes) and breakdown of homeostasis in diseases (i.e. biochemical changes). Pathologists contribute in patient management by providing final diagnosis of disease. Therefore, knowledge and understanding of pathology is essential for all would-be doctors, as well as general medical practitioners and specialists because unless they have knowledge and understanding of the language in the form of pathology laboratory reports, they would not be able to institute appropriate treatment or suggest preventive measures to the patient.

For the student of any system of medicine, the discipline of pathology forms a vital bridge between initial learning phase of preclinical sciences and the final phase of clinical subjects. The role and significance of learning of pathology in clinical medicine is quite well summed up by *Sir William Osler* (1849-1919), acclaimed physician and teacher in medicine considered as 'Father of Modern Medicine' by his famous quote "your practice of medicine will be as good as is your understanding of pathology" **(Fig. 1.1)**.

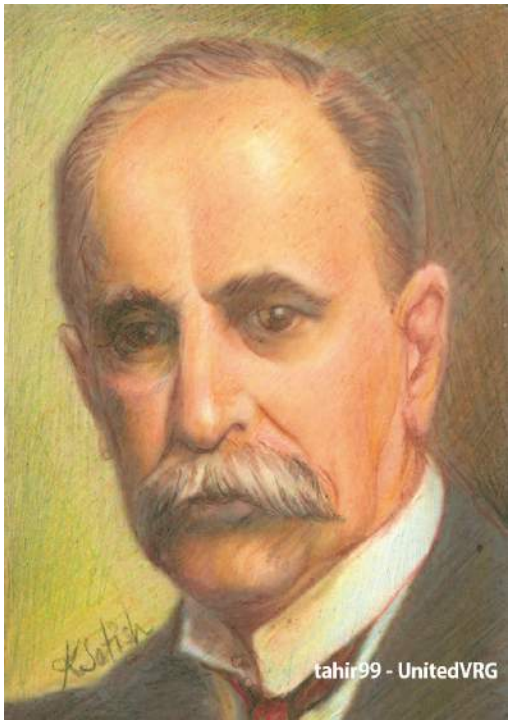


Figure 1.1 Sir William Osler (1849–1919). Canadian physician and one of the four founding Professors of Johns Hopkins Hospital, Baltimore, US, is regarded as '*Father of Modern Medicine*', Sir Osler had keen interest in pathology, was an acclaimed teacher and is also remembered for his famous quotations.

HEALTH AND DISEASE

Before there were humans on earth, there was disease, albeit in early animals. Since pathology is the study of disease, then what is *disease*? In simple language, disease is opposite of health i.e. what is not healthy is disease. *Health* may be defined as a condition when the individual is in complete accord with the surroundings, while *disease* is loss of ease (or comfort) to the body (i.e. dis+ease). However, it must be borne in mind that in health there is a wide range of 'normality' e.g. in height, weight, blood and tissue chemical composition etc. It also needs to be appreciated that at cellular level, the cells display wide range of activities within the broad area of health similar to what is seen in diseased cells. Thus, a disease or an illness means a condition marked by pronounced deviation from the normal healthy state. The term s*yndrome* (meaning running together) is used for a combination of several clinical features caused by altered physiologic processes.

COMMON TERMS IN PATHOLOGY

It is important for a beginner in pathology to be familiar with the language used in pathology **(Fig.1.2)**:

- *Patient* is the person affected by disease.
- *Lesions* are the characteristic changes in tissues and cells produced by disease in an individual or experimental animal.
- *Pathologic changes* or *morphology* consist of examination of diseased tissues. These can be recognised with the naked eye *(gross or macroscopic changes)* or studied by *microscopic examination* of tissues.

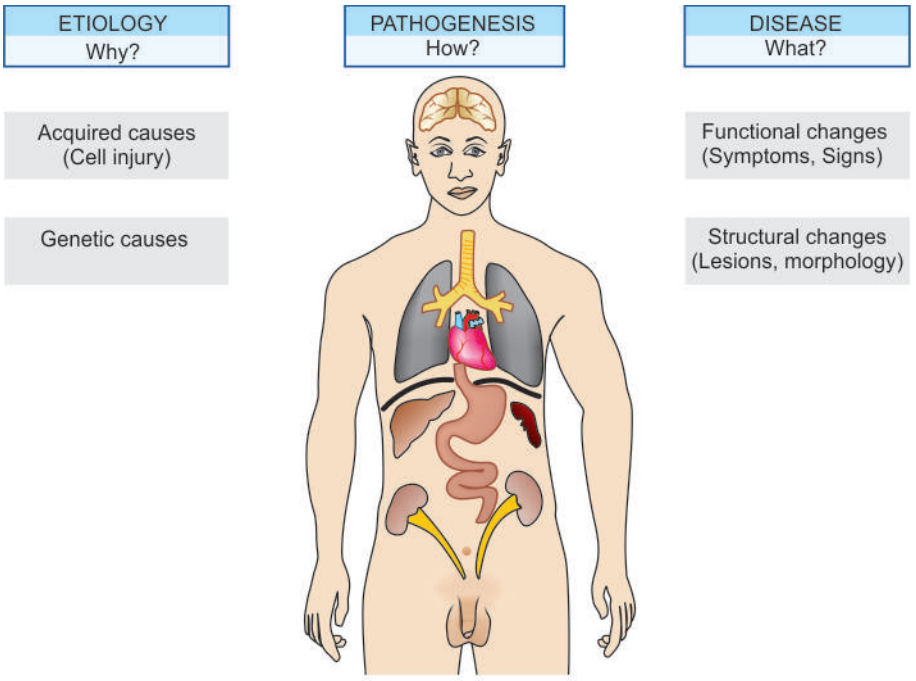


Figure 1.2 Diagrammatic depiction of disease and various terms used in pathology.

- Causal factors responsible for the lesions are included in *etiology* of disease (i.e. 'why' of disease).
- Mechanism by which the lesions are produced is termed *pathogenesis* of disease (i.e. 'how' of disease).
- Functional implications of the lesion felt by the patient are *symptoms* and those discovered by the clinician are the *physical signs.*
- Clinical significance of the morphologic and functional changes together with results of other investigations help to arrive at an answer to what is wrong *(diagnosis)*, what is going to happen *(prognosis),* what can be done about it *(treatment)*, and finally what should be done to avoid complications and spread *(prevention)* (i.e. 'what' of disease).

EVOLUTION OF PATHOLOGY

Pathology as the scientific study of disease processes has its deep roots in medical history. Since the beginning of mankind, there has been desire as well as need to know more about the causes, mechanisms and nature of diseases. The answers to these questions have evolved over the centuries—from supernatural beliefs to the present state of our knowledge of modern pathology. However, pathology is not separable from other multiple disciplines of medicine and owes its development to interaction and interdependence on advances in diverse neighbouring branches of science, in addition to the strides made in medical technology. As we shall see in the pages that follow, pathology has evolved over the years as a distinct discipline from anatomy, medicine and surgery, in that sequence.

The following brief review of fascinating history of pathology and its many magnificent personalities with their outstanding contribution in the form of a disease or a process known by their names, is meant to stimulate and generate interest in the inquisitive beginner in pathology as to how this colourful specialty has emerged.

PREHISTORIC TIMES TO MEDIEVAL PERIOD

Present-day knowledge of primitive culture which was prevalent in the world in prehistoric times reveals that religion, magic and medical treatment were quite linked to each other in those times. The earliest concept of disease understood by the patient and the healer was the religious belief that disease was the outcome of 'curse from God' or the belief in magic that the affliction had supernatural origin from 'evil eye of spirits.' To ward them off, priests through prayers and sacrifices, and magicians by magic power used to act as faith-healers and invoke supernatural powers and please the gods. Remnants of ancient superstitions still exist in some parts of the world. The link between medicine and religion became so firmly established throughout the world that different societies had their gods and goddesses of healing; for example: mythological Greeks had *Aesculapius* and *Apollo* as the principal gods of healing, *Dhanvantri* as the deity of medicine in India, and orthodox Indians' belief in *Mata Sheetala Devi* as the pox goddess.

The insignia of healing, the Caduceus, having snake and staff, is believed to represent the god Hermes or Mercury, which according to Greek mythology has power of healing since snake has regenerative powers expressed by its periodic sloughing of its skin. God of Greek medicine, *Aesculapius*, performed his functions with a staff having a single serpent wound around it. Later (around AD1800), however, the Caduceus got replaced with twin-serpents wound around a staff topped by a round knob and flanked by two wings and now represents the symbol of medicine instead of cross **(Fig. 1.3)**.

The period of ancient religious and magical beliefs was followed by the philosophical and rational approach to disease by the methods of observations. This happened at the time when great Greek philosophers—*Socrates, Plato* and *Aristotle,* introduced philosophical conc

But the real practice of medicine began with *Hippocrates* (460–370 BC), the great Greek clinical genius of all times and regarded as 'the father of medicine' **(Fig. 1.4)**. Hippocrates dissociated medicine from religion and magic. Instead, he firmly believed in study of patient's symptoms and described methods of diagnosis. He recorded his observations on cases in the form of collections of writings called Hippocratic Corpus which remained the mainstay of learning of medicine for nearly two thousand years. However, the prevailing concept at that time on mechanism of disease based on disequilibrium of four basic humors (water, air, fire, and earth) was propagated by Hippocates too but this concept was later abandoned.

Hippocrates followed rational and ethical attitudes in practice and teaching of medicine and is revered by the medical profession by taking *'Hippocratic oath'* at the time of entry into practice of medicine.

After Hippocrates, Greek medicine reached Rome (now Italy) which controlled Greek world after 146 BC and, therefore, it dominated the field of development of medicine in ancient Europe then. In fact, since old times, many tongue-twisting terminologies in medicine have their origin from Latin language which was the official language of countries included in ancient Roman empire (Spanish, Portuguese, Italian, French and Greek languages have their origin from Latin).

In Rome, Hippocratic teaching was propagated by Roman physicians, notably by *Cornelius Celsus* (53 BC-7 AD) and *Claudius Galen* (130–200 AD). Celsus first described four cardinal signs of inflammation—rubor (redness), tumor (swelling), calor (heat), and dolor (pain). Galen postulated humoral theory, later called Galenic theory. This theory suggested that the illness resulted from imbalance between *four humors* (or body fluids): blood, lymph, black bile (believed at that time to be from the spleen), and biliary secretion from the liver.

The hypothesis of disequilibrium of four elements constituting the body *(Dhatus)* similar to Hippocratic doctrine finds mention in ancient Indian medicine books compiled about 200 AD—*Charaka Samhita*, a finest document by *Charaka* on medicine listing 500 remedies, and *Sushruta Samhita,* similar book of surgical sciences by *Sushruta,* and includes about 700 plant-derived medicines.

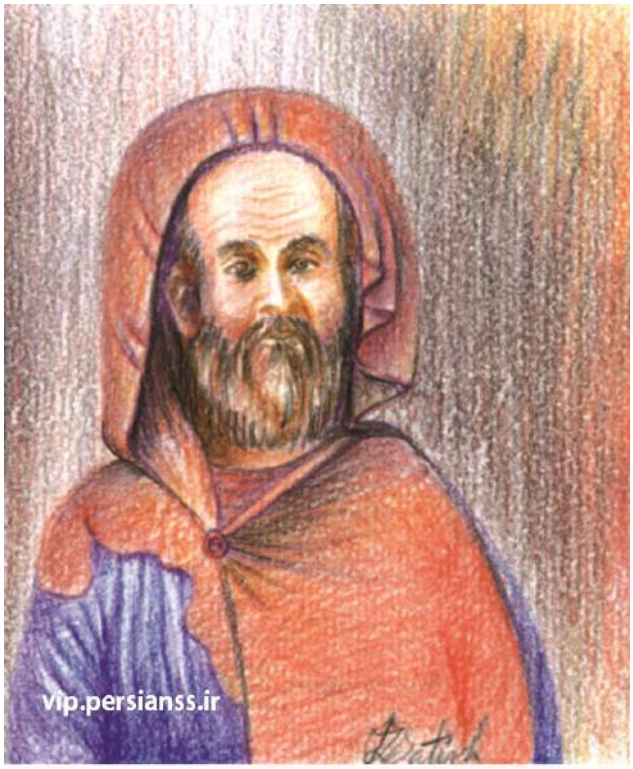


Figure 1.4 Hippocrates (460–370 BC). The great Greek clinical genius and regarded as *'the Father of Medicine'*. He introduced ethical aspects to medicine.

Figure 1.3 The Caduceus, representing symbol of medicine, is the traditional symbol of god Hermes or Mercury. It features twin serpents winding around a winged staff.

The end of Medieval period was marked by backward steps in medicine. There were widespread and devastating epidemics which reversed the process of rational thinking again to supernatural concepts and divine punishment for 'sins.' The dominant belief during this period was that life was due to influence of vital substance under the control of soul *(theory of vitalism)*. Thus, dissection of human body was strictly forbidden at that time as that would mean hurting the 'soul.'

HUMAN ANATOMY AND ERA OF GROSS PATHOLOGY

The backwardness of Medieval period was followed by the Renaissance period i.e. revival of learning. The Renaissance began from Italy in late 15th century and spread to whole of Europe. During this period, there was quest for advances in art and science. Since there was freedom of thought, there was emphasis on philosophical and rational attitudes again.

The beginning of the development of human anatomy took place during this period with the art works and drawings of human muscles and embryos by famous Italian painter *Leonardo da Vinci* (1452–1519). Dissection of human body was started by *Vesalius* (1514–1564) on freshly executed criminals. His pupils, *Gabriel Fallopius* (1523–1562) who described human oviducts (Fallopian tubes) and *Fabricius* who discovered lymphoid tissue around the intestine of birds (bursa of Fabricius) further popularised the practice of human anatomic dissection for which special postmortem amphitheatres came in to existence in various parts of ancient Europe.

Antony van Leeuwenhoek (1632–1723), a cloth merchant by profession in Holland, during his spare time invented the first ever microscope by grinding the lenses himself through which he recognised male spermatozoa as tiny preformed men (or "homunculi") and other single-celled organisms which he called animalcules. He also introduced histological staining in 1714 using saffron to examine muscle fibres.

Marcello Malpighi (1624–1694) used microscope extensively and observed the presence of capillaries and described the malpighian layer of the skin, and lymphoid tissue in the spleen (malpighian corpuscles). Malpighi is known as 'the father of histology.'

The credit for beginning of the study of morbid anatomy (pathologic anatomy), however, goes to Italian anatomist-pathologist, *Giovanni B. Morgagni* (1682–1771). Morgagni was an excellent teacher in anatomy, a prolific writer and a practicing clinician. By his work, Morgagni demolished the ancient humoral theory of disease and published his life-time experiences based on 700 postmortems and their corresponding clinical findings. He, thus, laid the foundations of clinicopathologic methodology in the study of disease and introduced the concept of clinicopathologic correlation (CPC), establishing a coherent sequence of cause, lesions, symptoms, and outcome of disease **(Fig. 1.5)**.

Sir Percivall Pott (1714–1788), famous surgeon in England, described arthritic tuberculosis of the spine (Pott's disease) and identified the first ever occupational cancer (cancer of scrotal skin) in the chimney sweeps in 1775 and discovered chimney soot as the first carcinogenic agent. The study of anatomy in England during the latter part of 18th Century was dominated by the two Hunter brothers. These were *John Hunter* (1728–1793), a student of Sir Percivall Pott, who rose to become the greatest surgeon-anatomist of all times **(Fig. 1.6)** and his elder brother *William Hunter* (1718–1788) who was a reputed anatomist-obstetrician. These brothers together started the first ever museum by collection of surgical specimens from their flourishing practice, arranged them into separate organ systems, made comparison of specimens from animals and plants with humans, and included many clinical pathology specimens as well, and thus developed the first museum of comparative anatomy and pathology in the world which became the Hunterian Museum, now housed in Royal College of Surgeons of London. Among many pupils of John Hunter was *Edward Jenner* (1749–1823) whose work on inoculation in smallpox is well known. Another prominent English pathologist was *Matthew Baillie* (1760–1823), nephew of Hunter brothers, who published first-ever systematic textbook of morbid anatomy in 1793. The era of gross pathology had three more illustrious and brilliant physician-pathologists in England who were colleagues at Guy's Hospital in London:

- *Richard Bright* (1789–1858) who described non-suppurative nephritis, later termed glomerulonephritis or Bright's disease;
- *Thomas Addison* (1793–1860) who gave an account of chronic adrenocortical insufficiency termed Addison's disease; and
- *Thomas Hodgkin* (1798–1866), who observed the complex of chronic enlargement of lymph nodes, often with enlargement of the liver and spleen, later called Hodgkin's disease.

Towards the end of 18th century, *Xavier Bichat* (1771–1802) in France described that organs were composed of tissue and divided the study of morbid anatomy into General Pathology and Systemic Pathology. *R.T.H. Laennec* (1781–1826), another French physician, dominated the early part of 19th century by his numerous discoveries. He described several lung diseases (tubercles, caseous lesions, miliary lesions, pleural effusion, and bronchiectasis), chronic sclerotic liver disease (later called Laennec's cirrhosis) and invented stethoscope.

Morbid anatomy attained its zenith with appearance of *Carl F. von Rokitansky* (1804–1878), self-taught German pathologist who performed nearly 30,000 autopsies himself. He described acute yellow atrophy of the liver, wrote an outstanding monograph on diseases of arteries and congenital heart defects. Unlike most other surgeons of that time, Rokitansky did not do clinical practice of surgery but instead introduced the concept that pathologists should confine themselves to making diagnosis which became the accepted role of pathologist later.

ERA OF TECHNOLOGY DEVELOPMENT AND CELLULAR PATHOLOGY

Up to middle of the 19th century, correlation of clinical manifestations of disease with gross pathological findings at autopsy became the major method of study of disease. Sophistication in surgery led to advancement in pathology. The anatomist-surgeons of earlier centuries got replaced largely with surgeon-pathologists in the 19th century.

Pathology started developing as a diagnostic discipline in later half of the 19th century with the evolution of cellular pathology which was closely linked to technology advancements in machinery manufacture for cutting thin sections of tissue, improvement in microscope, and development of chemical industry and dyes for staining.

The discovery of existence of disease-causing microorganisms was made by French chemist *Louis Pasteur* (1822–1895), thus demolishing the prevailing theory of spontaneous generation of disease and firmly established germ theory of disease. Subsequently, *G.H.A. Hansen* (1841–1912) in Germany identified Hansen's bacillus in 1873 as the first microbe causative for leprosy (Hansen's disease). While the study of infectious diseases was being made, the concept of immune tolerance and allergy emerged which formed the basis of immunisation initiated by Edward Jenner. *Metchnikoff* (1845-1916), a Russian zoologist, introduced the existence of phenomenon of phagocytosis by human defense cells against invading microbes.

Developments in chemical industry helped in switch over from earlier dyes of plant and animal origin to synthetic dyes; aniline violet being the first such synthetic dye prepared by *Perkin* in 1856. This led to emergence of a viable dye industry for histological and bacteriological purposes. The impetus for the flourishing and successful dye industry came from the works of numerous pioneers as under:

- *Paul Ehrlich* (1854–1915), German physician, conferred Nobel prize in 1908 for his work in immunology, described Ehrlich's test for urobilinogen using Ehrlich's aldehyde reagent, staining techniques of cells and bacteria, and laid the foundations of clinical pathology **(Fig. 1.7)**.
- *Christian Gram* (1853–1938), Danish physician, developed bacteriologic staining by crystal violet.
- *D.L. Romanowsky* (1861–1921), Russian physician, developed stain for peripheral blood film using eosin and methylene blue derivatives.
- *Robert Koch* (1843–1910), German bacteriologist, besides Koch's postulate and Koch's phenomena, developed techniques

FATHER OF CPCs

Figure 1.5 Giovanni B. Morgagni (1682–1771), an Italian physician-anatomist who introduced clinicopathologic methodology in the study of disease by correlation of clinical findings with findings at postmortem examination.

FATHER OF MUSEUM IN PATHOLOGY

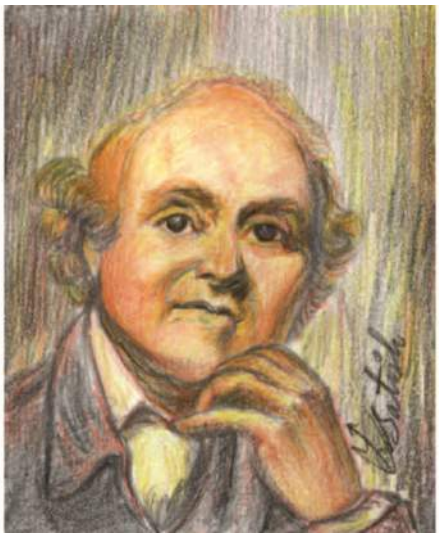


Figure 1.6 John Hunter (1728–1793). Scottish surgeon, regarded as the greatest surgeon-anatomist of all times who established first ever unique collection of pathological specimens that later resulted in the Hunterian Museum of the Royal College of Surgeons, London.

FATHER OF CLINICAL PATHOLOGY

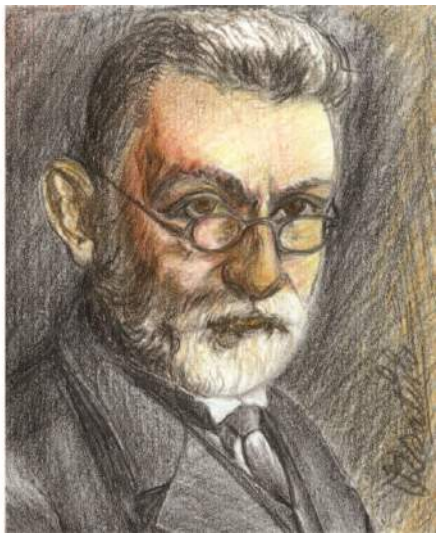


Figure 1.7 Paul Ehrlich (1854–1915). German physician, conferred Nobel prize for his work in immunology, described Ehrlich's test for urobilinogen, staining techniques of cells and bacteria, and laid the foundations of haematology and clinical pathology.

of fixation and staining for identification of bacteria, discovered tubercle bacilli in 1882 and cholera vibrio organism in 1883.

- *May-Grünwald* in 1902 and *Giemsa* in 1914 developed blood stains and applied them for classification of blood cells and bone marrow cells.
- *Sir William Leishman* (1865–1926) described Leishman's stain for blood films in 1914 and observed Leishman-Donovan bodies (LD bodies) in leishmaniasis.
- *Robert Feulgen* (1884–1955) described Feulgen reaction for DNA staining and laid the foundations of cytochemistry and histochemistry.

Simultaneous technological advances in machinery manufacture led to development and upgrading of microtomes for obtaining thin sections of organs and tissues for staining by dyes for enhancing detailed study of sections.

Though the presence of cells in thin sections of non-living object cork had been first demonstrated much earlier by *Robert Hooke* in 1667, it was revived as a unit of living matter in the 19th century by *F.T. Schwann* (1810–1882), the first neurohistologist, and *Claude Bernarde* (1813–1878), pioneer in pathophysiology.

Until the end of the 19th century, the study of morbid anatomy had remained largely autopsy-based and thus had remained a retrospective science. *Rudolf Virchow* (1821–1905) in Germany is credited with the beginning of microscopic examination of diseased tissue at cellular level and thus began histopathology as a method of investigation. Virchow hypothesised cellular theory having following two components:

- All cells come from other cells.
- Disease is an alteration of normal structure and function of these cells.

Virchow was revered as Pope in pathology in Europe and is aptly known as the 'father of cellular pathology' **(Fig. 1.8)**. Thus, sound foundation of diagnostic pathology based on microscopy had been laid which was followed and promoted by numerous brilliant successive workers. This gave birth to biopsy pathology and thus emerged the discipline of surgical pathology. Virchow also described etiology of embolism (Virchow's triad—slowing of blood-stream, changes in the vessel wall, changes in the blood itself), metastatic spread of tumours (Virchow's lymph node), and components and diseases of blood (fibrinogen, leukocytosis, leukaemia).

The concept of frozen section examination while the patient was still on the operation table was introduced by Virchow's student, *Julius Cohnheim* (1839–1884). In fact, during the initial period of development of surgical pathology around the turn of the 19th century, frozen section was considered more acceptable by the surgeons.

The concept of surgeon and physician doubling up in the role of pathologist which started in the 19th century continued as late as the middle of the 20th century in most clinical departments. Assigning biopsy pathology work to some faculty member in the clinical department was common practice; that is why some of the notable pathologists of the first half of 20th century had background of clinical training e.g. *James Ewing* (1866–1943), *A.P. Stout* (1885–1967) and *Lauren Ackerman* (1905–1993) in US, *Pierre Masson* (1880–1958) in France, and *R.A. Willis* in Australia.

A few other landmarks in further evolution of modern pathology in this era are as follows:

- *Karl Landsteiner* (1863–1943) described the existence of major human blood groups in 1900 and is considered "father of blood transfusion"; he was awarded Nobel prize in 1930 **(Fig. 1.9)**.
- *Ruska* and *Lorries* in 1933 developed electron microscope which aided the pathologist to view ultrastructure of cell and its organelles.

FATHER OF CELLULAR PATHOLOGY

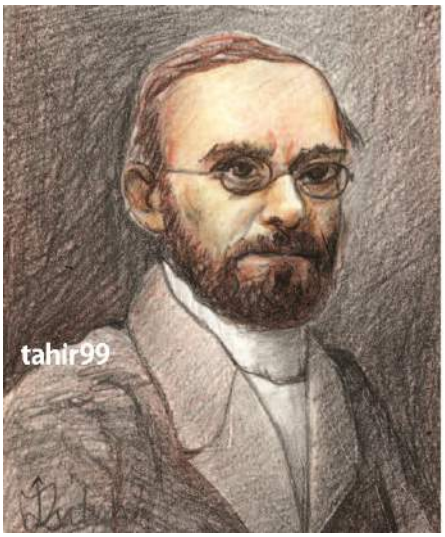


Figure 1.8 Rudolf Virchow (1821–1905). German pathologist who proposed cellular theory of disease and initiated biopsy pathology for diagnosis of diseases.

FATHER OF BLOOD TRANSFUSION

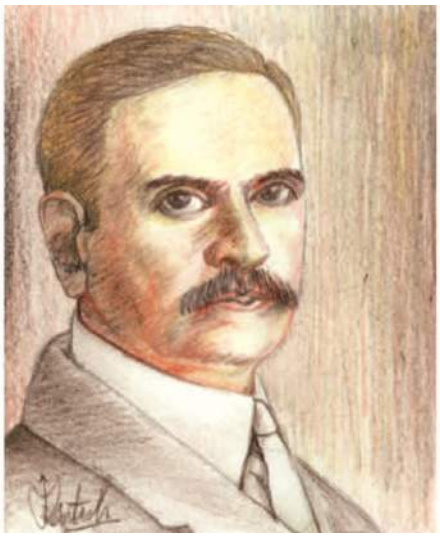


Figure 1.9 Karl Landsteiner (1863–1943). An Austrian pathologist who first discovered the existence of major human blood groups in 1900 and was recipient of Nobel prize in 1930.

FATHER OF EXFOLIATIVE CYTOLOGY

Figure 1.10 George N Papanicolaou (1883–1962). An American pathologist, who developed Pap test for diagnosis of cancer of uterine cervix.

◈ The development of exfoliative cytology for early detection of cervical cancer began with *George N. Papanicolaou* (1883–1962), a Greek-born, American pathologist, in 1930s and is known as 'father of exfoliative cytology' **(Fig. 1.10)**.

Another pioneering contribution in pathology in the 20th century was by an eminent teacher-author, *William Boyd* (1885–1979), psychiatrist-turned pathologist, whose textbooks—'Pathology for Surgeons' (first edition 1925) and 'Textbook of Pathology' (first edition 1932), dominated and inspired the students of pathology all over the world due to his flowery language and lucid style for a few generations. *M.M. Wintrobe* (1901–1986), a pupil of Boyd who discovered haematocrit technique, regarded him as a very stimulating teacher.

MODERN PATHOLOGY

The strides made in the latter half of 20th century until recent times in 21st century have made it possible to study diseases at genetic and molecular level, and provide an evidence-based and objective diagnosis that may enable the physician to institute targeted therapy. The major impact of advances in molecular biology are in the field of diagnosis and treatment of genetic disorders, immunology and in cancer. Some of the revolutionary discoveries during this time are as under **(Fig. 1.11)**:

◈ Description of *the structure of DNA* of the cell by Watson and Crick in 1953.

◈ Identification of *chromosomes and their correct number* in humans (46) by *Tijo* and *Levan* in 1956.

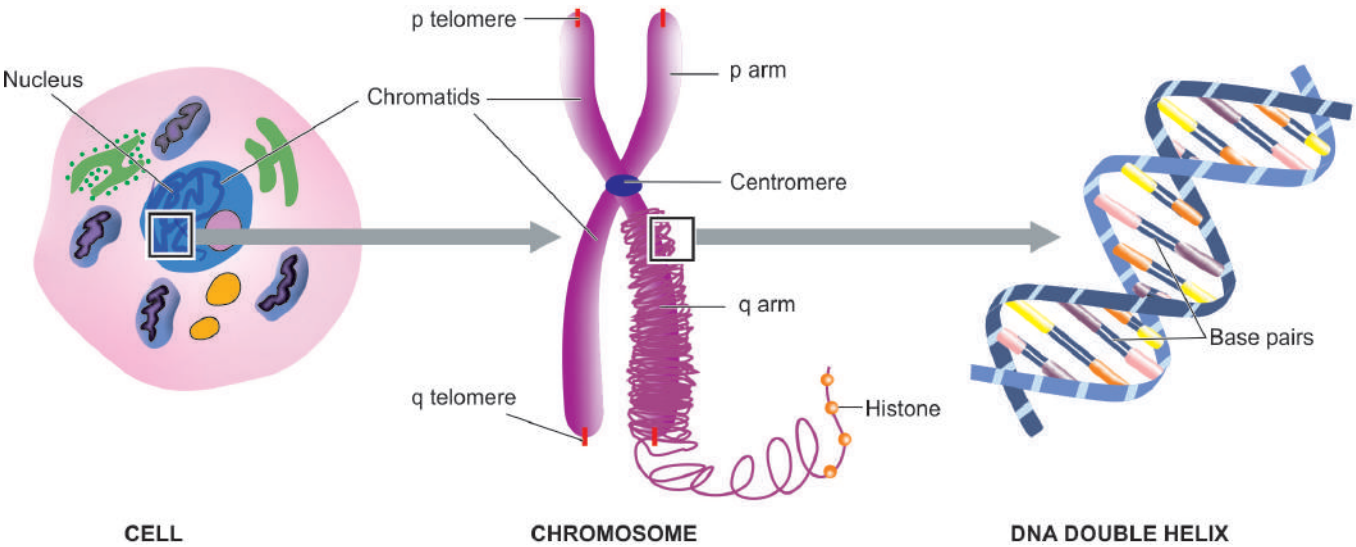


Figure 1.11 Molecular structure of human chromosome.

◈ Identification of *Philadelphia chromosome* t(9;22) in chronic myeloid leukaemia by Nowell and Hagerford in 1960 as the first chromosomal abnormality in any cancer.

◈ *In Situ hybridization* (ISH) introduced in 1969 in which a labelled probe is employed to detect and localise specific RNA or DNA sequences *'in situ'* (i.e. in the original place). Its later modification employs use of fluorescence microscopy (FISH) to detect specific localisation of the defect on chromosomes.

◈ *Recombinant DNA technique* developed in 1972 using restriction enzymes to cut and paste bits of DNA.

◈ Introduction of *polymerase chain reaction* (PCR) i.e. "xeroxing" of DNA fragments by Kary Mullis in 1983 has revolutionised the diagnostic molecular genetics. PCR analysis is more rapid than ISH, can be automated by thermal cyclers and requires much lower amount of starting DNA.

◈ Invention of *flexibility and dynamism of DNA* by Barbara McClintock for which she was awarded Nobel prize in 1983.

◈ *Mammalian cloning* started in 1997 by *Ian Wilmut* and his colleagues at Roslin Institute in Edinburgh, by successfully using a technique of somatic cell nuclear transfer to create the clone of a sheep named Dolly. Reproductive cloning for human beings, however, is very risky besides being absolutely unethical.

◈ The era of *stem cell research* started in 2000s by harvesting these primitive cells isolated from embryos and maintaining their growth in the laboratory. There are 2 types of sources of stem cells in humans: embryonic stem cells and adult stem cells, the former being more numerous. Stem cells are seen by many researchers as having virtually unlimited applications in the treatment of many human diseases such as Alzheimer's disease, diabetes, cancer, strokes, etc. At some point of time, stem cell therapy may be able to replace whole organ transplant and instead stem cells 'harvested' from the embryo may be used.

◈ *Human Genome Project (HGP)* consisting of a consortium of countries was completed in April 2003 coinciding with 50 years of description of DNA double helix by Watson and Crick in April 1953. The sequencing of human genome reveals that human genome contains approximately 3 billion base pairs of amino acids, which are located in the 23 pairs of chromosomes within the nucleus of each human cell. Each chromosome contains an estimated 30,000 genes in the human genome which carry the instructions for making proteins. The HGP has given us the ability to read nature's complete genetic blueprint used in making of each human being (i.e. gene mapping). Clinical trials by gene therapy on treatment of some single gene defects have resulted in some success, especially in haematological and immunological diseases. Future developments in genetic engineering may result in designing new and highly effective individualised treatment options for genetic diseases as well as suggest prevention against diseases.

TELEPATHOLOGY AND VIRTUAL MICROSCOPY

Telepathology is defined as the practice of diagnostic pathology by a remote pathologist utilising images of tissue specimens transmitted over a telecommunication network. The main *components* of a telepathology system are as under:

◈ Conventional light microscope.

◈ Method of image capture, commonly a camera mounted on light microscope.

◈ Telecommunications link between sending and receiving side.

◈ Workstation at receiving end with a high quality monitor.

Depending upon need and budget, telepathology system is of two *types*:

Static (store-and-forward, passive telepathology) In this, selected images are captured, stored and then transmitted over the internet via e-mail attachment, file transfer protocol, web page or CD-ROM. It is quite inexpensive and is more common but suffers from disadvantage of having sender's bias in selection of transmitted images.

Dynamic (Robotic interactive telepathology) Here, the images are transmitted in real-time from a remote microscope. Robotic movement of stage of microscope is controlled remotely and the desired images and fields are accessioned from a remote/local server. Thus, it almost duplicates to perfection the examination of actual slides under the microscope, hence is referred to as *Virtual Microscopy*. However, image quality and speed of internet can be major hurdles.

The era of "*digital pathology*" in 21st Century has reached its zenith with availability of technology for preparation of *virtual pathology slides (VPS)* by high speed scanners and then storing the scanned data in large memory output computers. VPS stored in the memory of the computer can then be examined and reported at any place on computer, without having to use microscope. However, the moot question remains whether current pathologists used to conventional microscopy will get the same perception on monitor. At present, this technology holds potential for pathology education, storage for records, clinical meetings and quality control.

SUBDIVISIONS OF PATHOLOGY

Human pathology is conventionally studied under two broad divisions: *General Pathology* dealing with general principles of disease, and *Systemic Pathology* that includes study of diseases pertaining to the specific organs and body systems. In general, the study of pathology includes morphological and non-morphological disciplines as follows:

MORPHOLOGICAL BRANCHES

These branches essentially involve application of microscope as an essential tool for the study and include histopathology, cytopathology and haematology.

A. HISTOPATHOLOGY Histopathology, used synonymously with anatomic pathology, pathologic anatomy, morbid anatomy, or tissue pathology, is the classic method of study and still the most useful one which has stood the test of time. The study includes structural changes observed by naked eye examination referred to as gross or macroscopic changes, and the changes detected by microscopy, which may be further supported by numerous special staining methods such as histochemistry and immunohistochemistry to arrive at the most accurate diagnosis. Modern time anatomic pathology includes sub-specialities such as cardiac pathology, pulmonary pathology, neuropathology, renal pathology, gynaecologic pathology, breast pathology, dermatopathology,

gastrointestinal pathology, oral pathology, and so on. Anatomic pathology includes the following subdivisions:

1. Surgical pathology It deals with the study of tissues removed from the living body by biopsy or surgical resection. Surgical pathology forms the bulk of tissue material for the pathologist and includes study of tissue by conventional *paraffin embedding* technique; intraoperative *frozen section* may be employed for rapid diagnosis.

2. Experimental pathology This is defined as production of disease in the experimental animal and study of morphological changes in organs after sacrificing the animal. However, all the findings of experimental work in animals may not be applicable to human beings due to species differences.

3. Forensic pathology and autopsy work This includes the study of organs and tissues removed at postmortem for medicolegal work and for determining the underlying sequence and cause of death. By this, the pathologist attempts to reconstruct the course of events how they may have happened in the patient during life which culminated in his death. Postmortem anatomical diagnosis is helpful to the clinician to enhance his knowledge about the disease and his judgement while forensic autopsy is helpful for medicolegal purposes. The significance of a careful postmortem examination is appropriately summed up in the old saying 'the dead teach the living'.

B. CYTOPATHOLOGY Though a branch of anatomic pathology, cytopathology has developed as a distinct subspeciality in recent times. It includes study of cells shed off from the lesions (exfoliative cytology) and fine-needle aspiration cytology (FNAC) of superficial and deep-seated lesions for diagnosis (Appendix I).

C. HAEMATOLOGY Haematology deals with the diseases of blood. It includes laboratory haematology and clinical haematology; the latter covers the management of patient as well.

NON-MORPHOLOGICAL BRANCHES

These diagnostic branches of pathology include clinical pathology, clinical biochemistry, microbiology, immunology, genetics and molecular pathology. In these diagnostic branches, qualitative, semi-quantitative or quantitative determinations are carried out in the laboratory. Microscope may also be required for at least some of these lab tests.

A. CLINICAL PATHOLOGY Analysis of various fluids including blood, urine, semen, CSF and other body fluids is included in this branch of pathology. Such analysis may be qualitative, semi-quantitative or quantitative.

B. CLINICAL BIOCHEMISTRY Quantitative determination of various biochemical constituents in serum and plasma, and in other body fluids is included in clinical biochemistry. Obviously, there is likely to be overlapping between clinical pathology and clinical biochemistry.

C. MICROBIOLOGY This is study of disease-causing microbes implicated in human diseases. Depending upon the type of microorganims studied, it has further developed into such as bacteriology, parasitology, mycology, virology etc.

D. IMMUNOLOGY Detection of abnormalities in the immune system of the body comprises immunology and immunopathology.

E. MEDICAL GENETICS This is the branch of human genetics that deals with the relationship between heredity and disease. There have been important developments in the field of medical genetics e.g. in blood groups, inborn errors of metabolism, chromosomal aberrations in congenital malformations and neoplasms etc.

F. MOLECULAR PATHOLOGY The detection and diagnosis of abnormalities at the level of DNA of the cell is included in molecular pathology such as in situ hybridisation, PCR etc. These methods are now not only used for research purposes but are also being used as a part of diagnostic pathology reports.

The above divisions of pathology into several subspecialities are quite artificial since overlapping of disciplines is likely, ultimate aim of pathologist being to establish the final diagnosis and learn the causes and mechanisms of disease. Towards this aim, the beginner as well as the teacher in pathology remain life-long students of pathology, eager to learn more in their quest to become better with every passing day.

GIST BOX 1.1 Introduction to Pathology

- Pathology is the study of structural and functional changes in disease.
- Pathologic changes present with clinical features (symptoms, signs) in the patient.
- In pathology, we study causes (etiology), mechanisms (pathogenesis) and arrive at final diagnosis by various laboratory methods; gross and microscopic examination of tissues is the major method.
- The Caduceus representing ancient Greek gods is symbol of medicine.
- 'Father of medicine' is Hippocrates; 'Father of modern medicine' is Sir William Osler.
- 'Father of pathology' is Rudolf Virchow; 'Father of CPCs' is Giovanni B. Morgagni; 'Father of museum' is John Hunter; 'Father of clinical pathology' is Paul Ehrlich; 'Father of blood transfusion' is Karl Landsteiner; 'Father of cytology' is George N. Papanicolaou.
- Morphologic branches of diagnostic pathology are histopathology, cytopathology and haematology.
- Important ancillary diagnostic techniques in pathology are immunohistochemistry, cytogenetics and molecular methods such as ISH and PCR.

2 Cell Injury, Cellular Adaptations and Cellular Ageing

Cells are the basic units of tissues, which form organs and systems in the human body. Traditionally, body cells are divided in to two main types: epithelial and mesenchymal cells. In health, the cells remain in accord with each other. In 1859, Virchow first published cellular theory of disease, bringing in the concept that diseases occur due to abnormalities at the level of cells. Since then, study of abnormalities in structure and function of cells in disease has remained the focus of attention in understanding of diseases. Thus, most forms of diseases begin with cell injury followed by consequent loss of cellular function.

CELL INJURY

Cell injury is defined as the effect of a variety of stresses due to etiologic agents a cell encounters resulting in changes in its internal and external environment. In general, cells of the body have inbuilt mechanism to deal with changes in environment to an extent. The cellular response to stress may vary and depends upon following two variables:

i) Host factors i.e. the type of cell and tissue involved.

ii) Factors pertaining to injurious agent i.e. extent and type of cell injury.

Various forms of cellular responses to cell injury may be as follows **(Fig. 2.1)**:

1. When there is increased functional demand, the cell may adapt to the changes which are expressed morphologically, which then revert back to normal after the stress is removed *(cellular adaptations, see* Fig. 2.36).
2. When the stress is mild to moderate, the injured cell may recover *(reversible cell injury)*, while persistent and severe form of cell injury may cause cell death *(irreversible cell injury)*.
3. The residual effects of reversible cell injury may persist in the cell as evidence of cell injury at subcellular level *(subcellular changes)*, or metabolites may accumulate within the cell *(intracellular accumulations)*.

In order to learn the fundamentals of disease processes at cellular level, it is essential to have an understanding of the causes (etiology) and mechanisms (pathogenesis) of cell injury and cellular adaptations which are discussed below.

ETIOLOGY OF CELL INJURY

The cells may be broadly injured by two major ways:

A. Genetic causes
B. Acquired causes

The genetic causes of various diseases are discussed in Chapter 9. The acquired causes of disease comprise vast majority of common diseases afflicting mankind. Based on underlying agent, the acquired causes of cell injury can be further categorised as under:

1. Hypoxia and ischaemia
2. Physical agents
3. Chemical agents and drugs
4. Microbial agents
5. Immunologic agents
6. Nutritional derangements
7. Ageing
8. Psychogenic diseases
9. Iatrogenic factors
10. Idiopathic diseases.

In a given situation, more than one of the above etiologic factors may be involved. These factors are briefly outlined below.

HYPOXIA AND ISCHAEMIA Cells of different tissues essentially require oxygen to generate energy and perform metabolic functions. Deficiency of oxygen or hypoxia results in failure to carry out these activities by the cells. Hypoxia is the

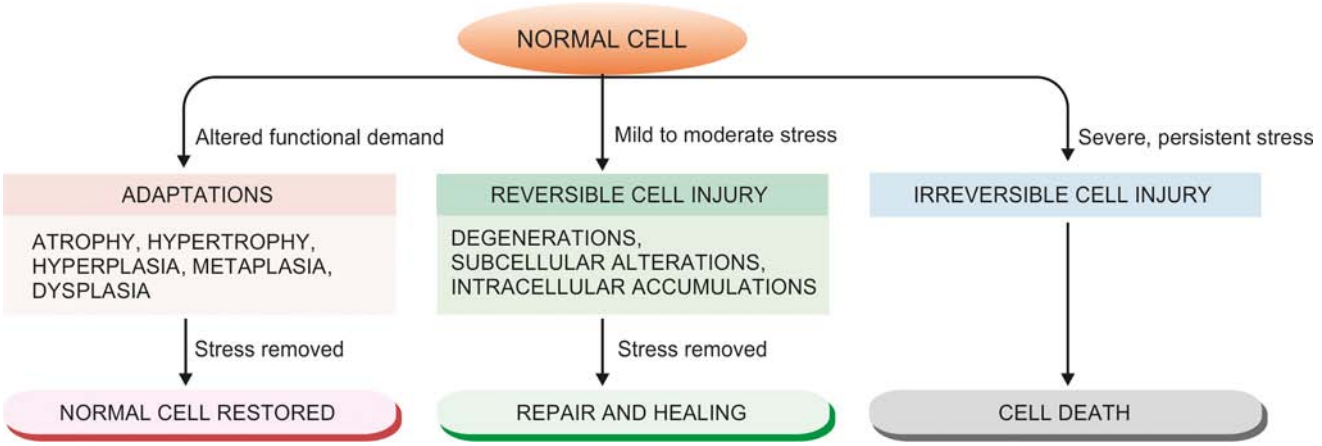


Figure 2.1 Cellular responses to cell injury.

most common cause of cell injury. Hypoxia may result from the following 2 ways:

◈ The most common mechanism of hypoxic cell injury is by reduced supply of blood to cells due to interruption i.e. ischaemia.

◈ Hypoxia may also result from impaired blood supply from causes other than interruption e.g. disorders of oxygen-carrying RBCs (e.g. anaemia, carbon monoxide poisoning), heart diseases, lung diseases and increased demand of tissues.

PHYSICAL AGENTS Physical agents in causation of disease are as under:

i) mechanical trauma (e.g. road accidents);
ii) thermal trauma (e.g. by heat and cold);
iii) electricity;
iv) radiation (e.g. ultraviolet and ionising); and
v) rapid changes in atmospheric pressure.

CHEMICALS AND DRUGS An ever-increasing list of chemical agents and drugs may cause cell injury. Important examples include the following:

i) chemical poisons such as cyanide, arsenic, mercury;
ii) strong acids and alkalis;
iii) environmental pollutants;
iv) insecticides and pesticides;
v) oxygen at high concentrations;
vi) hypertonic glucose and salt;
vii) social agents such as alcohol and narcotic drugs; and
viii) therapeutic administration of drugs.

MICROBIAL AGENTS Injuries by microbes include infections caused by bacteria, rickettsiae, viruses, fungi, protozoa, metazoa, and other parasites.

Diseases caused by biologic agents are discussed in Chapter 6.

IMMUNOLOGIC AGENTS Immunity is a 'double-edged sword'—it protects the host against various injurious agents but it may also turn lethal and cause cell injury e.g.

i) hypersensitivity reactions;
ii) anaphylactic reactions; and
iii) autoimmune diseases.

Immunologic tissue injury is discussed in Chapter 3.

NUTRITIONAL DERANGEMENTS A deficiency or an excess of nutrients may result in nutritional imbalances.

◈ Nutritional deficiency diseases may be due to overall deficiency of nutrients (e.g. starvation), of protein calorie (e.g. marasmus, kwashiorkor), of minerals (e.g. anaemia), or of trace elements.

◈ Nutritional excess is a problem of affluent societies resulting in obesity, atherosclerosis, heart disease and hypertension.

Nutritional diseases are discussed in Chapter 8.

AGEING Cellular ageing or senescence leads to impaired ability of the cells to undergo replication and repair, and ultimately lead to cell death culminating in death of the individual. This aspect is dealt at the end of this chapter.

PSYCHOGENIC DISEASES There are no specific biochemical or morphologic changes in common acquired mental diseases due to mental stress, strain, anxiety, overwork and frustration e.g. depression, schizophrenia. However, problems of drug addiction, alcoholism, and smoking result in various organic diseases such as liver damage, chronic bronchitis, lung cancer, peptic ulcer, hypertension, ischaemic heart disease etc.

IATROGENIC CAUSES Although as per Hippocratic oath, every physician is bound not to do or administer anything that causes harm to the patient, there are some diseases as well as deaths attributed to iatrogenic causes (owing to physician). Examples include occurrence of disease or death due to error in judgement by the physician and untoward effects of administered therapy (drugs, radiation).

IDIOPATHIC DISEASES Idiopathic means "of unknown cause". Finally, although so much is known about the etiology of diseases, there still remain many diseases for which exact cause is undetermined. For example, most common form of hypertension (90%) is idiopathic (or essential) hypertension. Similarly, exact etiology of many cancers is still incompletely known.

GIST BOX 2.1 Etiology of Cell Injury

- Cell injury is the effect of a variety of stresses due to etiologic agents a cell encounters resulting in changes in its internal and external environment.
- The cellular response to stress depends upon the type of cell and tissue involved, and the extent and type of cell injury.
- Initially, cells adapt to the changes due to injurious agent and may revert back to normal.
- Mild to moderate stress for shorter duration causes reversible cell injury; severe and persistent stress causes cell death.
- Among various etiologic factors, hypoxia-ischaemia is most important; others are chemical and physical agents, microbes, immunity, ageing etc.

PATHOGENESIS OF CELL INJURY

Injury to the normal cell by one or more of the above listed etiologic agents may result in a state of reversible or irreversible cell injury. The underlying alterations in biochemical systems of cells for reversible and irreversible cell injury by various agents are complex and varied. However, in general, irrespective of the type, following common scheme applies to most forms of cell injury by various agents:

1. Factors pertaining to etiologic agent and host As mentioned above, factors pertaining to host cells and etiologic agent determine the outcome of cell injury:

i) Type, duration and severity of injurious agent: The extent of cellular injury depends upon type, duration and severity of the stimulus e.g. small dose of chemical toxin or short duration of ischaemia cause reversible cell injury while large dose of the same chemical agent or persistent ischaemia cause cell death.

ii) Type, status and adaptability of target cell: The type of cell as regards its susceptibility to injury, its nutritional and metabolic status, and adaptation of the cell to hostile environment determine the extent of cell injury e.g. skeletal muscle can withstand hypoxic injury for long-time while cardiac muscle suffers irreversible cell injury after persistent ischaemia due to total coronary occlusion ≥20 minutes.

2. Common underlying mechanisms Irrespective of other factors, following essential intracellular biochemical phenomena underlie all forms of cell injury:

ii) Cell membrane damage disturbing the metabolic and trans-membrane exchanges.
iii) Release of toxic free radicals.

3. Usual morphologic changes Biochemical and molecular changes underlying cell injury from various agents become apparent first, and are associated with appearance of ultrastructural changes in the injured cell. However, eventually, gross and light microscopic changes in morphology of organ and cells appear. The morphologic changes of reversible cell injury (e.g. hydropic swelling) appear earlier while later morphologic alterations of cell death are seen (e.g. in myocardial infarction).

4. Functional implications and disease outcome Eventually, cell injury affects cellular function adversely which has bearing on the body. Consequently, clinical features in the form of symptoms and signs would appear. Further course or prognosis will depend upon the response to treatment versus the biologic behaviour of disease.

The interruption of blood supply (i.e. ischaemia) and impaired oxygen supply to the tissues (i.e. hypoxia) are most common form of cell injury in human beings. Pathogenesis of hypoxic and ischaemic cell injury is, therefore, described in detail below followed by brief discussion on pathogenesis of chemical and physical (principally ionising radiation) agents.

PATHOGENESIS OF ISCHAEMIC AND HYPOXIC INJURY

Ischaemia and hypoxia are the most common forms of cell injury. Although underlying intracellular mechanisms and ultrastructural changes seen in reversible and irreversible cell injury by hypoxia-ischaemia (depending upon extent of hypoxia and type of cells involved) are a continuation of the process, these mechanisms are discussed separately below and illustrated diagrammatically in **Figs. 2.2 and 2.3**:

REVERSIBLE CELL INJURY If the ischaemia or hypoxia is of short duration, the effects may be reversible on rapid restoration of circulation e.g. in coronary artery occlusion, myocardial contractility, metabolism and ultrastructure are reversed if the circulation is quickly restored. The sequential biochemical and ultrastructural changes in reversible cell injury are as under **(Fig. 2.3,A)**:

1. Decreased generation of cellular ATP: Damage by ischaemia from interruption *versus* hypoxia from other causes All living cells require continuous supply of oxygen to produce ATP which is essentially required for a variety of cellular functions (e.g. membrane transport, protein synthesis, lipid synthesis and phospholipid metabolism). ATP in human cell is derived from 2 sources:

- *Firstly,* by aerobic respiration or oxidative phosphorylation (which requires oxygen) in the mitochondria.
- *Secondly,* cells may subsequently switch over to anaerobic glycolytic oxidation to maintain constant supply of ATP (in which ATP is generated from glucose/glycogen in the absence of oxygen).

Ischaemia due to interruption in blood supply as well as hypoxia from other causes limit the supply of oxygen to the cells, thus causing decreased ATP generation from ADP:

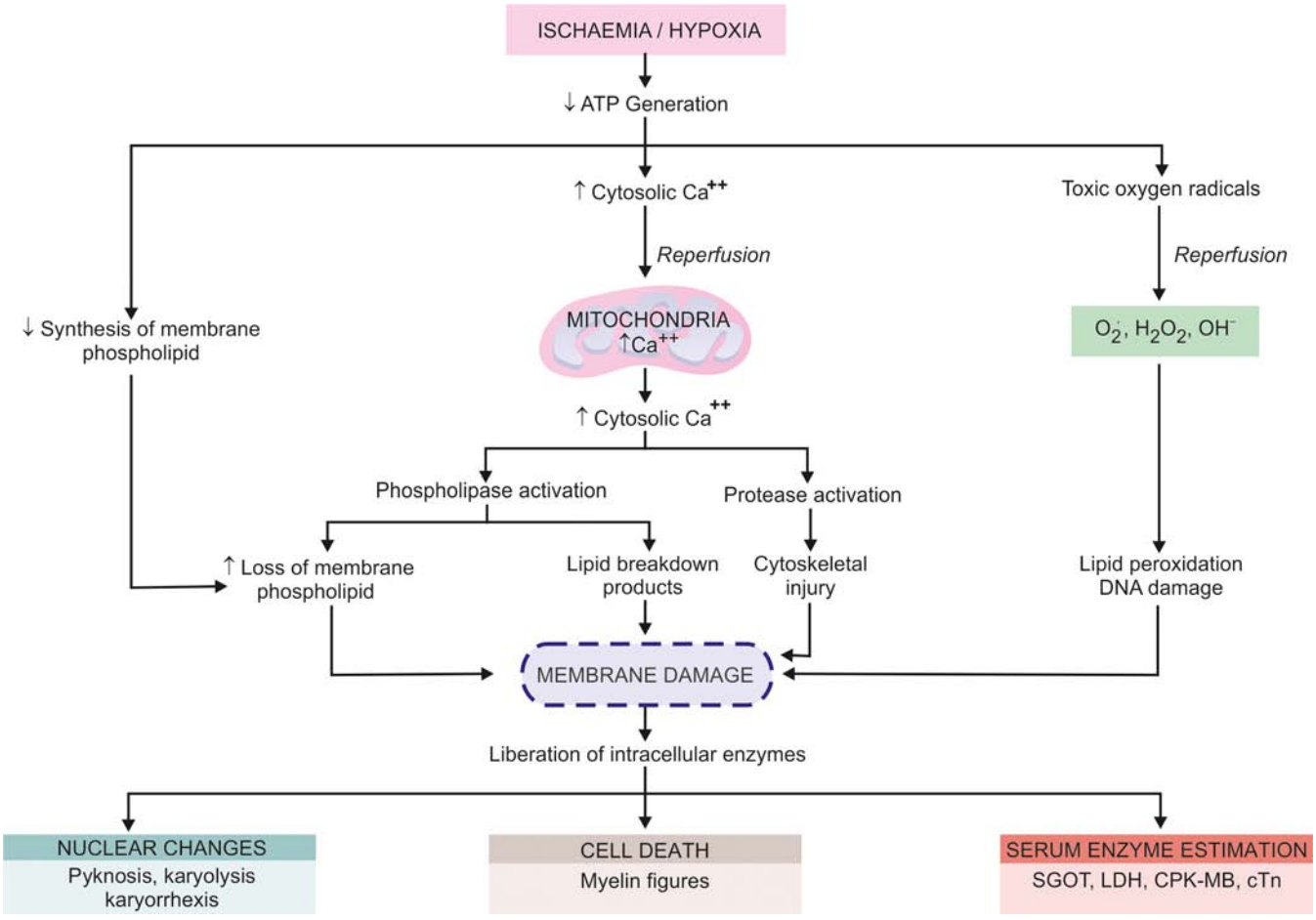


Figure 2.2 Sequence of events in the pathogenesis of reversible and irreversible cell injury caused by hypoxia/ischaemia.

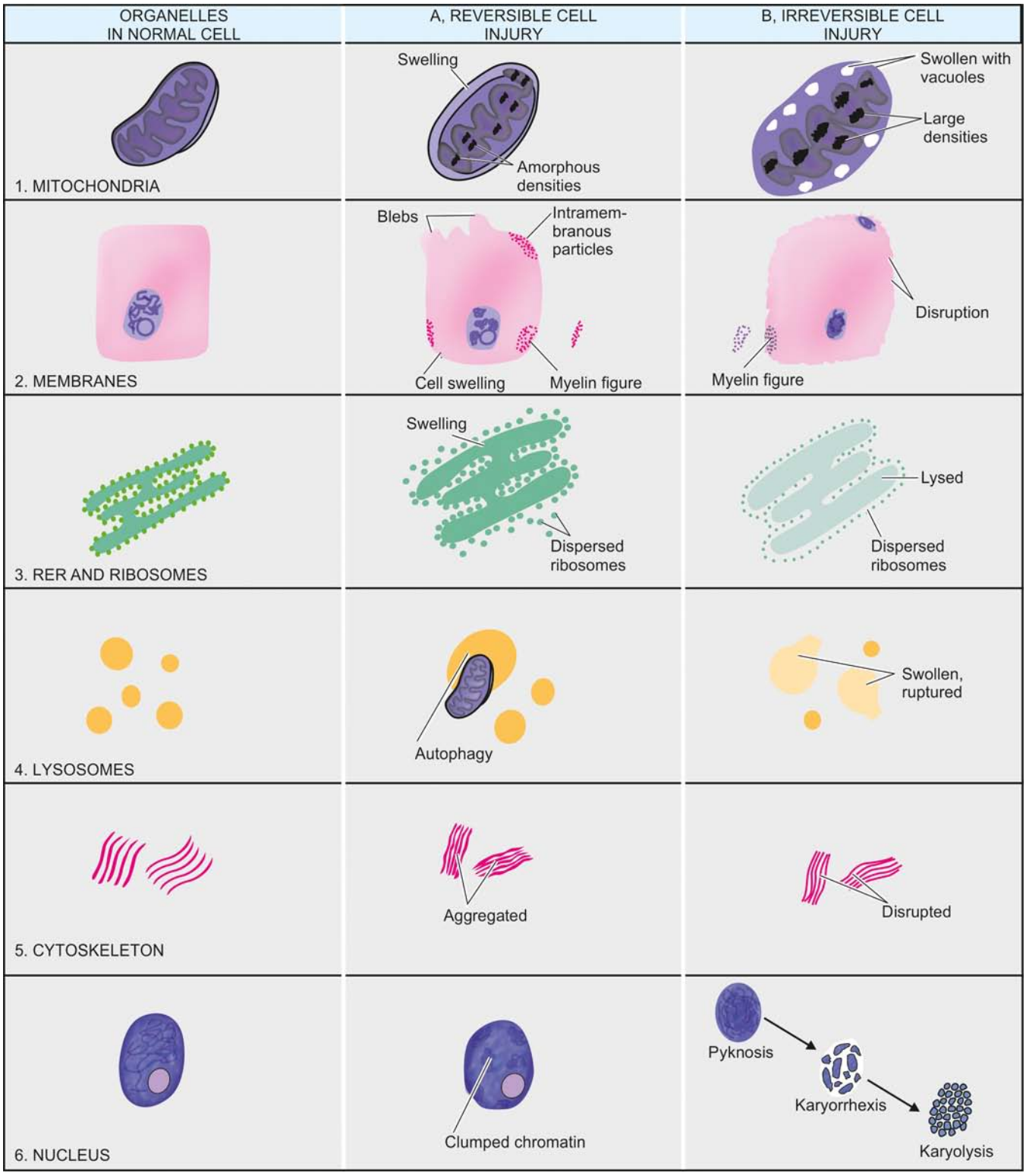


Figure 2.3 Ultrastructural changes during cell injury due to hypoxia-ischaemia.

◈ In *ischaemia* from interruption of blood supply, aerobic respiration as well as glucose availability are both compromised resulting in more severe and faster effects of cell injury. Ischaemic cell injury also causes accumulation of metabolic waste products in the cells.

◈ On the other hand, in *hypoxia from other causes* (RBC disorders, heart disease, lung disease), anaerobic glycolytic ATP generation continues, and thus cell injury is less severe.

However, highly specialised cells such as myocardium, proximal tubular cells of the kidney, and neurons of the CNS are dependent solely on aerobic respiration for ATP generation and thus these tissues suffer from ill-effects of ischaemia more severely and rapidly.

2. Intracellular lactic acidosis: Nuclear clumping Due to low oxygen supply to the cell, aerobic respiration by mitochondria fails first. This is followed by switch to anaerobic

glycolytic pathway for the requirement of energy (i.e. ATP). This results in rapid depletion of glycogen and accumulation of lactic acid lowering the intracellular pH. Early fall in intracellular pH (i.e. intracellular lactic acidosis) results in clumping of nuclear chromatin.

3. Damage to plasma membrane pumps: Hydropic swelling and other membrane changes Lack of ATP interferes in generation of phospholipids from the cellular fatty acids which are required for continuous repair of membranes. This results in damage to membrane pumps operating for regulation of sodium-potassium and calcium as under:

i) Failure of sodium-potassium pump Normally, the energy (ATP)-dependent sodium pump (also called Na^+-K^+ ATPase) operating at the plasma membrane allows active transport of sodium out of the cell and diffusion of potassium into the cell. Lowered ATP in the cell lowers the activity of sodium pump and consequently interferes with this membrane-regulated process. This results in intracellular accumulation of sodium and diffusion of potassium out of the cell. The accumulation of sodium in the cell leads to increase in intracellular water to maintain iso-osmotic conditions (i.e. hydropic swelling occurs, discussed later in the chapter).

ii) Failure of calcium pump Membrane damage causes disturbance in the calcium ion exchange across the cell membrane. Excess of calcium moves into the cell (i.e. calcium influx), particularly in the mitochondria, causing its swelling and deposition of phospholipid-rich amorphous densities.

4. Reduced protein synthesis: Dispersed ribosomes As a result of continued hypoxia, membranes of endoplasmic reticulum and Golgi apparatus swell up. Ribosomes are detached from granular (rough) endoplasmic reticulum and polysomes are degraded to monosomes, thus dispersing ribosomes in the cytoplasm and inactivating their function. Similar reduced protein synthesis occurs in Golgi apparatus.

Ultrastructural evidence of reversible cell membrane damage is seen in the form of loss of microvilli, intramembranous particles and focal projections of the cytoplasm (blebs). *Myelin figures* may be seen lying in the cytoplasm or present outside the cell; these are derived from membranes (plasma or organellar) enclosing water and dissociated lipoproteins between the lamellae of injured membranes.

Up to this point, withdrawal of acute stress that resulted in reversible cell injury can restore the cell to normal state.

IRREVERSIBLE CELL INJURY Persistence of ischaemia or hypoxia results in irreversible damage to the structure and function of the cell (cell death). The stage at which this *point of no return or irreversibility* is reached from reversible cell injury is unclear but the sequence of events is a continuation of reversibly injured cell. Two essential phenomena always distinguish irreversible from reversible cell injury **(Fig. 2.2)**:

- Inability of the cell to reverse *mitochondrial dysfunction* on reperfusion or reoxygenation.
- *Disturbance in cell membrane function* in general, and in plasma membrane in particular.

In addition, there is further reduction in ATP, continued depletion of proteins, reduced intracellular pH, and leakage of lysosomal enzymes into the plasma. These biochemical changes have effects on the ultrastructural components of the cell **(Fig. 2.3, B)**:

1. Calcium influx: Mitochondrial damage As a result of continued hypoxia, a large cytosolic influx of calcium ions occurs, especially after reperfusion of irreversibly injured cell. Excess intracellular calcium collects in the mitochondria disabling its function. Morphological changes are in the form of vacuoles in the mitochondria and deposits of amorphous calcium salts in the mitochondrial matrix.

2. Activated phospholipases: Membrane damage Damage to membrane function in general, and plasma membrane in particular, is the most important event in irreversible cell injury. Increased cytosolic influx of calcium in the cell activates endogenous *phospholipases*. These, in turn, degrade membrane phospholipids progressively which are the main constituent of the lipid bilayer membrane. Besides, there is also decreased replacement-synthesis of membrane phospholipids due to reduced ATP. Other lytic enzyme which is activated is *ATPase* which causes further depletion of ATP.

3. Intracellular proteases: Cytoskeletal damage The normal cytoskeleton of the cell (microfilaments, microtubules and intermediate filaments) which anchors the cell membrane is damaged due to degradation by activated intracellular proteases or by physical effect of cell swelling producing irreversible cell membrane injury.

4. Activated endonucleases: Nuclear damage DNA or nucleoproteins are damaged by the activated lysosomal enzymes such as proteases and endonucleases. Irreversible damage to the nucleus can be in three forms:

i) *Pyknosis:* Condensation and clumping of nucleus which becomes dark basophilic.
ii) *Karyorrhexis:* Nuclear fragmentation in to small bits dispersed in the cytoplasm.
iii) *Karyolysis:* Dissolution of the nucleus.

Damaged DNA activates proapoptotic proteins leading the cell to death.

5. Lysosomal hydrolytic enzymes: Lysosomal damage, cell death and phagocytosis The lysosomal membranes are damaged and result in escape of lysosomal hydrolytic enzymes. These enzymes are activated due to lack of oxygen in the cell and acidic pH. These hydrolytic enzymes: (e.g. hydrolase, RNAase, DNAase, protease, glycosidase, phosphatase, lipase, amylase, cathepsin etc) on activation bring about enzymatic digestion of cellular components and hence cell death. The dead cell is eventually replaced by masses of phospholipids called *myelin figures* which are either phagocytosed by macrophages or there may be formation of calcium soaps.

Liberated enzymes just mentioned leak across the abnormally permeable cell membrane into the serum, the estimation of which may be used as clinical parameters of cell death. For example, in myocardial infarction, estimation of elevated serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), isoenzyme of creatine kinase (CK-MB), and cardiac troponins (cTn) are useful guides for death of heart muscle. Some of the common enzyme markers for different forms of cell death are given in **Table 2.1**.

While cell damage from oxygen deprivation by above mechanisms develops slowly, taking several minutes to hours, the cell injury may be accentuated after restoration of blood supply and subsequent events termed *ischaemic-reperfusion injury and liberation of toxic free radicals (or reactive oxygen species)*, discussed below.

Table 2.1 Common enzyme markers of cell death.

ENZYME	DISEASE
1. *Aspartate aminotransferase (AST, SGOT)*	Diffuse liver cell necrosis e.g. viral hepatitis, alcoholic liver disease Acute myocardial infarction
2. *Alanine aminotransferase (ALT, SGPT)*	More specific for diffuse liver cell damage than AST e.g. viral hepatitis
3. *Creatine kinase-MB (CK-MB)*	Acute myocardial infarction, myocarditis Skeletal muscle injury
4. *Lipase*	More specific for acute pancreatitis
5. *Amylase*	Acute pancreatitis Sialadenitis
6. *Lactic dehydrogenase (LDH)*	Acute myocardial infarction Myocarditis Skeletal muscle injury
7. *Cardiac troponin (CTn)*	Specific for acute myocardial infarction

Ischaemia-Reperfusion Injury and Free Radical-Mediated Cell Injury

Depending upon the duration of ischaemia/hypoxia, restoration of blood flow may result in the following 3 different consequences:

1. From ischaemia to reversible injury *When the period of ischaemia is of short duration,* reperfusion with resupply of oxygen restores the structural and functional state of the injured cell i.e. reversible cell injury.

2. From ischaemia to irreversible injury *Another extreme is when much longer period of ischaemia* has resulted in irreversible cell injury *during ischaemia itself* i.e. when so much time has elapsed that neither blood flow restoration is helpful nor reperfusion injury can develop. Cell death in such cases is not attributed to formation of activated oxygen species. But instead, on reperfusion there is further marked intracellular excess of sodium and calcium ions due to persistent cell membrane damage.

3. From ischaemia to reperfusion injury *When ischaemia is for somewhat longer duration,* then restoration of blood supply to injured but viable cells (i.e. reperfusion), rather than restoring structure and function of the cell, paradoxically deteriorates the already injured cell and leads it to cell death. This is termed ischaemia-reperfusion injury. The examples of such forms of cell injury are irreversible cell injury in myocardial and cerebral ischaemia.

Ischaemia-reperfusion injury occurs due to excessive accumulation of free radicals or reactive oxygen species. The mechanism of reperfusion injury by free radicals is complex but following three aspects are involved:

1. Calcium overload.
2. Excessive generation of free radicals (superoxide, H_2O_2, hydroxyl radical, pernitrite).
3. Subsequent inflammatory reaction.

These are discussed below:

1. CALCIUM OVERLOAD Upon restoration of blood supply, the ischaemic cell is further bathed by the blood fluid that has more calcium ions at a time when the ATP stores of the cell are low. This results in further calcium overload on the already injured cells, triggering lipid peroxidation of the membrane causing further membrane damage.

2. EXCESSIVE GENERATION OF FREE RADICALS Although oxygen is the lifeline of all cells and tissues, its molecular forms as reactive oxygen radicals or reactive oxygen species can be most devastating for the cells. Free radical-mediated cell injury has been extensively studied and a brief account is given below.

Oxygen free radical generation Normally, reduction-oxidation (redox) reaction in the metabolism of the cell involves generation of ATP by oxidative process in which biradical oxygen (O_2) combines with hydrogen atom (H), and in the process, water (H_2O) is formed. This normal reaction of O_2 to H_2O involves 'four electron donation' in four steps involving transfer of one electron at each step. Free radicals are intermediate chemical species having a single unpaired electron in its outer orbit. These are generated within mitochondrial inner membrane where cytochrome oxidase catalyses the O_2 to H_2O reaction. Three intermediate molecules of partially reduced species of oxygen are generated depending upon the number of electrons transferred **(Fig. 2.4)**:

i) Superoxide oxygen (O'_2): one electron
ii) Hydrogen peroxide (H_2O_2): two electrons
iii) Hydroxyl radical (OH^-): three electrons

These are generated from enzymatic and non-enzymatic reaction as under:

i) Superoxide (O'_2): Superoxide anion O'_2 may be generated by direct auto-oxidation of O_2 during mitochondrial electron transport reaction. Alternatively, O'_2 is produced enzymatically by xanthine oxidase and cytochrome P_{450} in the mitochondria or cytosol.

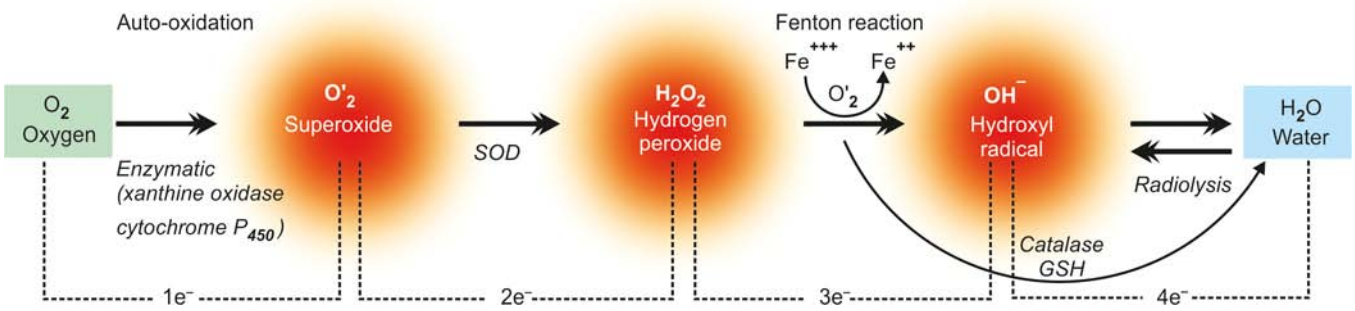


Figure 2.4 Mechanisms of generation of free radicals by four electron step reduction of oxygen. (SOD = *superoxide dismutase;* GSH = *glutathione peroxidase*).