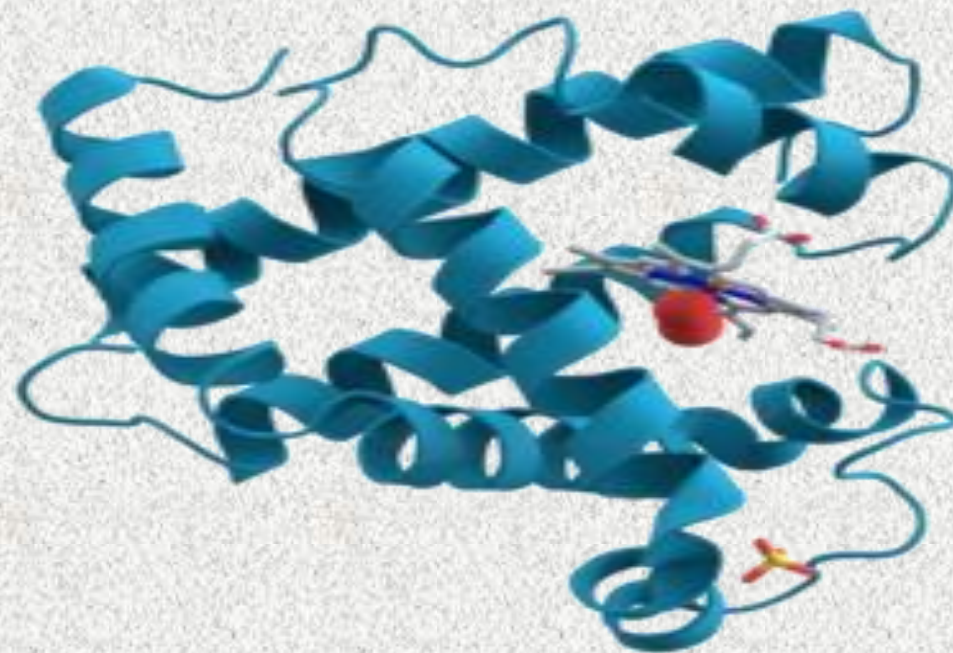


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TEXTBOOK OF BIOCHEMISTRY

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AUTHORS PROFILE

They have sufficient teaching and research experience with expertise in TEXTBOOK OF BIOCHEMISTRY. They have written this book for the benefits of undergraduate and postgraduate students.

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PREFACE

The study of chemical reactions that occur within and in relation to living things is known as biochemistry or biological chemistry. Structural biology, enzymology, and metabolism are the three subfields of biochemistry, which is a branch of both chemistry and biology. Through these three fields, biochemistry has been able to successfully describe life processes in the final decades of the 20th century. Biochemical methods and research are being used to discover and develop nearly every aspect of the biological sciences. In order to comprehend tissues and organs as well as the structure and function of organisms, biochemistry focuses on comprehending the chemical foundation that enables biological molecules to give rise to the events that take place within and between living cells. Molecular biology, the study of the molecular mechanisms underlying biological events, is closely associated with biochemistry.

The structures, roles, and interactions of biological macromolecules such as proteins, nucleic acids, polysaccharides, and lipids are the focus of a large portion of biochemistry. They provide cells their structure and carry out a lot of life-related tasks. The interactions of ions

and tiny molecules also affect the chemistry of the cell. These can be either organic (such the amino acids, which are utilized to make proteins) or inorganic (like water and metal ions). Metabolism is the process by which cells use chemical reactions to capture energy from their surroundings. The main fields in which biochemistry's discoveries are used are agriculture, nutrition, and medicine. Biochemists study the origins and treatments of illnesses in medicine. Nutrition investigates the impact of nutritional shortages as well as ways to preserve health and fitness.

We welcome comments by readers of **“TEXTBOOK OF BIOCHEMISTRY”** for ways to improve the book and to increase its value. Such suggestions will be seriously considered in the preparation of subsequent editions.

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CHAPTER I
BIOCHEMICAL ANALYSIS

Introduction

The study of chemical reactions that occur within and in relation to living things is known as biochemistry or biological chemistry. Structural biology, enzymology, and metabolism are the three subfields of biochemistry, which is a branch of both chemistry and biology. Through these three fields, biochemistry has been able to successfully describe life processes in the final decades of the 20th century. Biochemical methods and research are being used to discover and develop nearly every aspect of the biological sciences. comprehending the chemical underpinnings that enable biological molecules to produce the actions that take place within and between living cells is the main goal of biochemistry. This knowledge is closely related to comprehending tissues and organs as well as the structure and function of organisms. Molecular biology, the study of the molecular mechanisms underlying biological events, is closely associated with biochemistry.

The structures, roles, and interactions of biological macromolecules such proteins, nucleic acids, polysaccharides, and lipids are the focus of a large portion of biochemistry. They provide cells their structure and carry out a lot of life-related

tasks. The interactions of ions and tiny molecules also affect the chemistry of the cell. These can be either organic (such the amino acids, which are utilized to make proteins) or inorganic (like water and metal ions). Metabolism is the process by which cells use chemical reactions to capture energy from their surroundings. The main fields in which biochemistry's discoveries are used are agriculture, nutrition, and medicine. Biochemists study the origins and treatments of illnesses in medicine. Nutrition investigates the impact of nutritional shortages as well as ways to preserve health and fitness. Biochemists study soil and fertilizers in agriculture to enhance crop production, storage, and insect management. In recent decades, the field of biotechnology—which combines technical problem-solving techniques with biochemical concepts and methods—has been used to influence living systems to create practical instruments for industrial processes, research, and illness diagnosis and control.



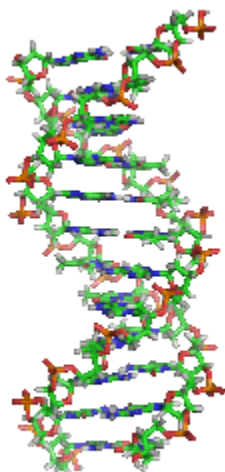
Gerty Cori and Carl Cori jointly won the Nobel Prize in 1947 for their discovery of the Cori cycle at RPMI.

At its most comprehensive definition, biochemistry can be seen as a study of the components and composition of living things and how they come together to become life. In this sense, the history of biochemistry may therefore go back as far as the ancient Greeks. However, biochemistry as a specific scientific discipline began sometime in the 19th century, or a little earlier, depending on which aspect of biochemistry is being focused on. Some argued that the beginning of biochemistry may have been the discovery of the first enzyme, diastase (now called amylase), in 1833 by Anselme Payen, while others considered Eduard Buchner's first demonstration of a complex biochemical process alcoholic

fermentation in cell-free extracts in 1897 to be the birth of biochemistry. Some might also point as its beginning to the influential 1842 work by Justus von Liebig, *Animal chemistry, or, Organic chemistry in its applications to physiology and pathology*, which presented a chemical theory of metabolism, or even earlier to the 18th century studies on fermentation and respiration by Antoine Lavoisier. Many other pioneers in the field who helped to uncover the layers of complexity of biochemistry have been proclaimed founders of modern biochemistry. Emil Fischer, who studied the chemistry of proteins, and F. Gowland Hopkins, who studied enzymes and the dynamic nature of biochemistry, represent two examples of early biochemists.

The term "biochemistry" was first used when Vinzenz Kletzinsky (1826–1882) had his "Compendium der Biochemie" printed in Vienna in 1858; it derived from a combination of biology and chemistry. In 1877, Felix Hoppe-Seyler used the term (*biochemie* in German) as a synonym for physiological chemistry in the foreword to the first issue of *Zeitschrift für Physiologische Chemie* (Journal of Physiological Chemistry) where he argued for the setting up of institutes dedicated to this

field of study. The German chemist Carl Neuberg however is often cited to have coined the word in 1903, while some credited it to Franz Hofmeister.



DNA structure (1D65)

It was once generally believed that life and its materials had some essential property or substance (often referred to as the "vital principle") distinct from any found in non-living matter, and it was thought that only living beings could produce the molecules of life. In 1828, Friedrich Wöhler published a paper on his serendipitous urea synthesis from potassium cyanate and ammonium sulfate; some regarded that as a direct overthrow of vitalism and the establishment of organic chemistry. However, the Wöhler synthesis has sparked controversy as some reject the

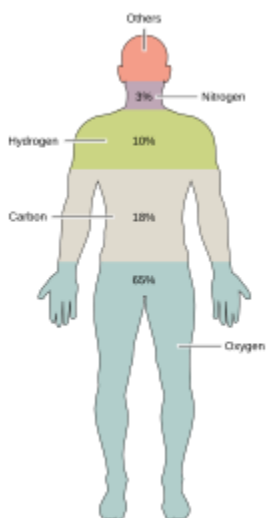
death of vitalism at his hands. Since then, biochemistry has advanced, especially since the mid-20th century, with the development of new techniques such as chromatography, X-ray diffraction, dual polarisation interferometry, NMR spectroscopy, radioisotopic labeling, electron microscopy and molecular dynamics simulations.

These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell, such as glycolysis and the Krebs cycle (citric acid cycle), and led to an understanding of biochemistry on a molecular level.

Another significant historic event in biochemistry is the discovery of the gene, and its role in the transfer of information in the cell. In the 1950s, James D. Watson, Francis Crick, Rosalind Franklin and Maurice Wilkins were instrumental in solving DNA structure and suggesting its relationship with the genetic transfer of information. In 1958, George Beadle and Edward Tatum received the Nobel Prize for work in fungi showing that one gene produces one enzyme. In 1988, Colin Pitchfork was the first person convicted of murder with DNA evidence, which led to the growth of forensic science. More recently, Andrew Z. Fire and Craig C.

Mello received the 2006 Nobel Prize for discovering the role of RNA interference (RNAi) in the silencing of gene expression.

Starting materials: the chemical elements of life



The main elements that compose the human body shown from most abundant (by mass) to least abundant

Around two dozen chemical elements are essential to various kinds of biological life. Most rare elements on Earth are not needed by life (exceptions being selenium and iodine),^[33] while a few common ones (aluminum and titanium) are not used. Most organisms share element needs, but there are a few differences between plants and animals. For example, ocean algae use bromine, but land plants and animals do not seem to need any. All animals require sodium, but is not an essential element

for plants. Plants need boron and silicon, but animals may not (or may need ultra-small amounts).

Just six elements

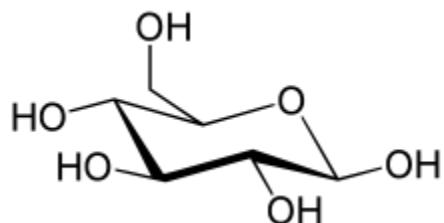
carbon, hydrogen, nitrogen, oxygen, calcium and phosphorus—make up almost 99% of the mass of living cells, including those in the human body (see composition of the human body for a complete list). In addition to the six major elements that compose most of the human body, humans require smaller amounts of possibly 18 more.

Biomolecules

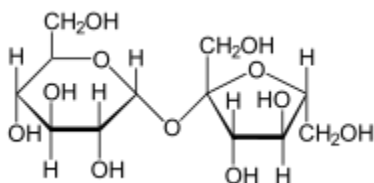
The 4 main classes of molecules in biochemistry (often called biomolecules) are carbohydrates, lipids, proteins, and nucleic acids. Many biological molecules are polymers: in this terminology, monomers are relatively small macromolecules that are linked together to create large macromolecules known as polymers. When monomers are linked together to synthesize a biological polymer, they undergo a process called dehydration synthesis. Different macromolecules can assemble in larger complexes, often needed for biological activity.

Carbohydrates

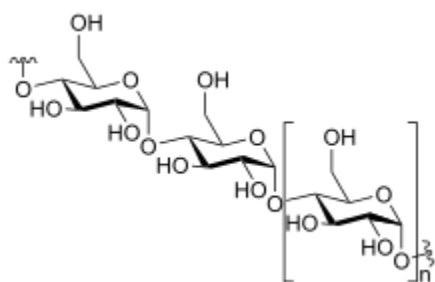
Carbohydrates



Glucose, a monosaccharide



A molecule of sucrose (glucose + fructose), a disaccharide



Amylose, a polysaccharide made up of several thousand glucose units

Two of the main functions of carbohydrates are energy storage and providing structure. One of the common sugars known as glucose is a carbohydrate, but not all carbohydrates are sugars. There are more carbohydrates on Earth than any other known type of biomolecule; they are used to store energy

and genetic information, as well as play important roles in cell to cell interactions and communications.

The simplest type of carbohydrate is a monosaccharide, which among other properties contains carbon, hydrogen, and oxygen, mostly in a ratio of 1:2:1 (generalized formula $C_nH_{2n}O_n$, where n is at least 3). Glucose ($C_6H_{12}O_6$) is one of the most important carbohydrates; others include fructose ($C_6H_{12}O_6$), the sugar commonly associated with the sweet taste of fruits, and deoxyribose ($C_5H_{10}O_4$), a component of DNA. A monosaccharide can switch between acyclic (open-chain) form and a cyclic form. The open-chain form can be turned into a ring of carbon atoms bridged by an oxygen atom created from the carbonyl group of one end and the hydroxyl group of another. The cyclic molecule has a hemiacetal or hemiketal group, depending on whether the linear form was an aldose or a ketose.

In these cyclic forms, the ring usually has 5 or 6 atoms. These forms are called furanoses and pyranoses, respectively—by analogy with furan and pyran, the simplest compounds with the same carbon-oxygen ring (although they lack the carbon-carbon double bonds of these two molecules). For example, the

aldohexose glucose may form a hemiacetal linkage between the hydroxyl on carbon 1 and the oxygen on carbon 4, yielding a molecule with a 5-membered ring, called glucofuranose. The same reaction can take place between carbons 1 and 5 to form a molecule with a 6-membered ring, called glucopyranose. Cyclic forms with a 7-atom ring called heptoses are rare.

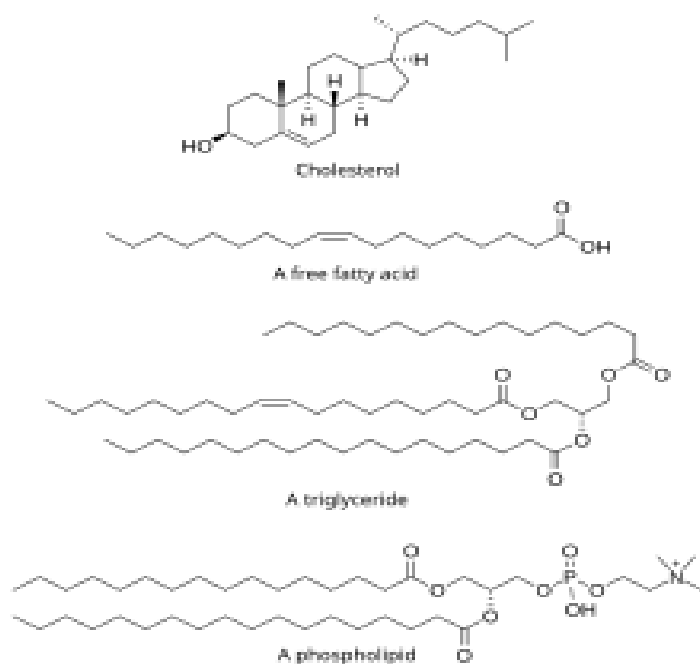
Two monosaccharides can be joined by a glycosidic or ester bond into a *disaccharide* through a dehydration reaction during which a molecule of water is released. The reverse reaction in which the glycosidic bond of a disaccharide is broken into two monosaccharides is termed *hydrolysis*. The best-known disaccharide is sucrose or ordinary sugar, which consists of a glucose molecule and a fructose molecule joined. Another important disaccharide is lactose found in milk, consisting of a glucose molecule and a galactose molecule. Lactose may be hydrolysed by lactase, and deficiency in this enzyme results in lactose intolerance.

When a few (around three to six) monosaccharides are joined, it is called an *oligosaccharide* (*oligo-* meaning "few"). These molecules tend to be used as markers and signals, as well as having some other uses. Many monosaccharides joined form

a polysaccharide. They can be joined in one long linear chain, or they may be branched. Two of the most common polysaccharides are cellulose and glycogen, both consisting of repeating glucose monomers. *Cellulose* is an important structural component of plant's cell walls and *glycogen* is used as a form of energy storage in animals.

Sugar can be characterized by having reducing or non-reducing ends. A reducing end of a carbohydrate is a carbon atom that can be in equilibrium with the open-chain aldehyde (aldose) or keto form (ketose). If the joining of monomers takes place at such a carbon atom, the free hydroxy group of the pyranose or furanose form is exchanged with an OH-side-chain of another sugar, yielding a full acetal. This prevents opening of the chain to the aldehyde or keto form and renders the modified residue non-reducing. Lactose contains a reducing end at its glucose moiety, whereas the galactose moiety forms a full acetal with the C4-OH group of glucose. Saccharose does not have a reducing end because of full acetal formation between the aldehyde carbon of glucose (C1) and the keto carbon of fructose (C2).

Lipids



Structures of some common lipids. At the top are cholesterol and oleic acid. The middle structure is a triglyceride composed of oleoyl, stearoyl, and palmitoyl chains attached to a glycerol backbone. At the bottom is the common phospholipid, phosphatidylcholine.

Lipids comprise a diverse range of molecules and to some extent is a catchall for relatively water-insoluble or nonpolar compounds of biological origin, including waxes, fatty acids, fatty-acid derived phospholipids, sphingolipids, glycolipids,

and terpenoids (e.g., retinoids and steroids). Some lipids are linear, open-chain aliphatic molecules, while others have ring structures. Some are aromatic (with a cyclic [ring] and planar [flat] structure) while others are not. Some are flexible, while others are rigid.

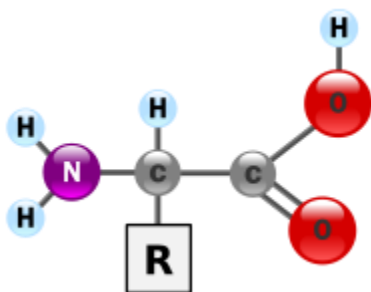
Lipids are usually made from one molecule of glycerol combined with other molecules. In triglycerides, the main group of bulk lipids, there is one molecule of glycerol and three fatty acids. Fatty acids are considered the monomer in that case, and maybe saturated (no double bonds in the carbon chain) or unsaturated (one or more double bonds in the carbon chain).

Most lipids have some polar character and are largely nonpolar. In general, the bulk of their structure is nonpolar or hydrophobic ("water-fearing"), meaning that it does not interact well with polar solvents like water. Another part of their structure is polar or hydrophilic ("water-loving") and will tend to associate with polar solvents like water. This makes them amphiphilic molecules (having both hydrophobic and hydrophilic portions). In the case of cholesterol, the polar group is a mere -OH (hydroxyl or alcohol).

In the case of phospholipids, the polar groups are considerably larger and more polar, as described below.

Lipids are an integral part of our daily diet. Most oils and milk products that we use for cooking and eating like butter, cheese, ghee etc. are composed of fats. Vegetable oils are rich in various polyunsaturated fatty acids (PUFA). Lipid-containing foods undergo digestion within the body and are broken into fatty acids and glycerol, the final degradation products of fats and lipids. Lipids, especially phospholipids, are also used in various pharmaceutical products, either as co-solubilizers (e.g. in parenteral infusions) or else as drug carrier components (e.g. in a liposome or transfersome).

Proteins

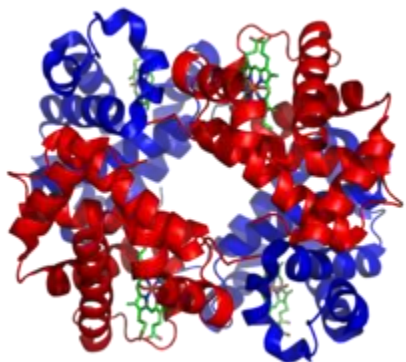


The general structure of an α -amino acid, with the amino group on the left and the carboxyl group on the right

Proteins are very large molecules—macro-biopolymers—made from monomers called amino acids. An amino acid consists of an alpha carbon atom attached to an amino group, $-\text{NH}_2$, a carboxylic acid group, $-\text{COOH}$ (although these exist as $-\text{NH}_3^+$ and $-\text{COO}^-$ under physiologic conditions), a simple hydrogen atom, and a side chain commonly denoted as " $-\text{R}$ ". The side chain " R " is different for each amino acid of which there are 20 standard ones. It is this " R " group that makes each amino acid different, and the properties of the side chains greatly influence the overall three-dimensional conformation of a protein. Some amino acids have functions by themselves or in a modified form; for instance, glutamate functions as an important neurotransmitter. Amino acids can be joined via a peptide bond. In this dehydration synthesis, a water molecule is removed and the peptide bond connects the nitrogen of one amino acid's amino group to the carbon of the other's carboxylic acid group. The resulting molecule is called a *dipeptide*, and short stretches of amino acids (usually, fewer than thirty) are called *peptides* or polypeptides. Longer stretches merit the title *proteins*. As an example, the important blood serum protein albumin contains 585 amino acid residues.



Generic amino acids (1) in neutral form, (2) as they exist physiologically, and (3) joined as a dipeptide



A schematic of hemoglobin. The red and blue ribbons represent the protein globin; the green structures are the heme groups.

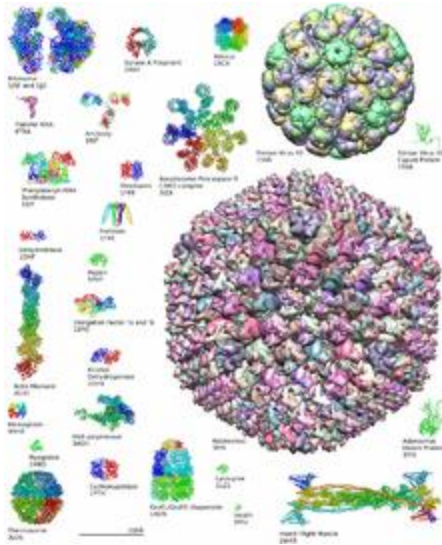
Proteins can have structural and/or functional roles. For instance, movements of the proteins actin and myosin ultimately are responsible for the contraction of skeletal muscle. One property many proteins have is that they specifically bind to a certain molecule or class of molecules—they may be *extremely* selective in what they bind. Antibodies are an example of proteins that attach to one specific type of molecule. Antibodies are composed of heavy and light chains. Two heavy chains would be linked to two light chains through disulfide

linkages between their amino acids. Antibodies are specific through variation based on differences in the N-terminal domain.

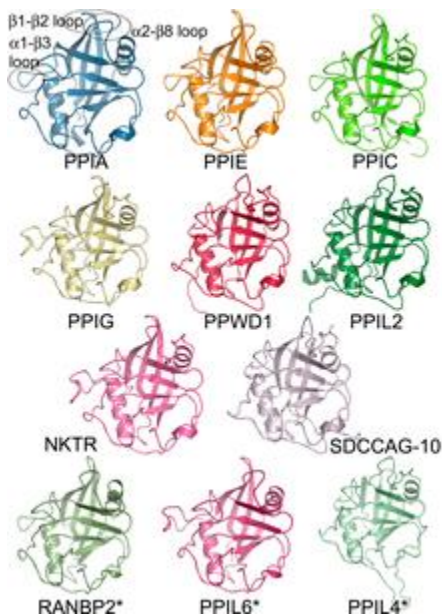
The enzyme-linked immunosorbent assay (ELISA), which uses antibodies, is one of the most sensitive tests modern medicine uses to detect various biomolecules. Probably the most important proteins, however, are the enzymes. Virtually every reaction in a living cell requires an enzyme to lower the activation energy of the reaction. These molecules recognize specific reactant molecules called *substrates*; they then catalyze the reaction between them. By lowering the activation energy, the enzyme speeds up that reaction by a rate of 10^{11} or more; a reaction that would normally take over 3,000 years to complete spontaneously might take less than a second with an enzyme. The enzyme itself is not used up in the process and is free to catalyze the same reaction with a new set of substrates. Using various modifiers, the activity of the enzyme can be regulated, enabling control of the biochemistry of the cell as a whole.

The structure of proteins is traditionally described in a hierarchy of four levels. The primary structure of a protein consists of its

linear sequence of amino acids; for instance, "alanine-glycine-tryptophan-serine-glutamate-asparagine-glycine-lysine-...". Secondary structure is concerned with local morphology (morphology being the study of structure). Some combinations of amino acids will tend to curl up in a coil called an α -helix or into a sheet called a β -sheet; some α -helixes can be seen in the hemoglobin schematic above. Tertiary structure is the entire three-dimensional shape of the protein. This shape is determined by the sequence of amino acids. In fact, a single change can change the entire structure. The alpha chain of hemoglobin contains 146 amino acid residues; substitution of the glutamate residue at position 6 with a valine residue changes the behavior of hemoglobin so much that it results in sickle-cell disease. Finally, quaternary structure is concerned with the structure of a protein with multiple peptide subunits, like hemoglobin with its four subunits. Not all proteins have more than one subunit.



Examples of protein structures from the Protein Data Bank



Members of a protein family, as represented by the structures of the isomerase domains

Ingested proteins are usually broken up into single amino acids or dipeptides in the small intestine and then absorbed. They can then be joined to form new proteins. Intermediate products of

glycolysis, the citric acid cycle, and the pentose phosphate pathway can be used to form all twenty amino acids, and most bacteria and plants possess all the necessary enzymes to synthesize them. Humans and other mammals, however, can synthesize only half of them. They cannot synthesize isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Because they must be ingested, these are the essential amino acids. Mammals do possess the enzymes to synthesize alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine, the nonessential amino acids. While they can synthesize arginine and histidine, they cannot produce it in sufficient amounts for young, growing animals, and so these are often considered essential amino acids. If the amino group is removed from an amino acid, it leaves behind a carbon skeleton called an α -keto acid. Enzymes called transaminases can easily transfer the amino group from one amino acid (making it an α -keto acid) to another α -keto acid (making it an amino acid). This is important in the biosynthesis of amino acids, as for many of the pathways, intermediates from other biochemical pathways are converted to the α -keto acid

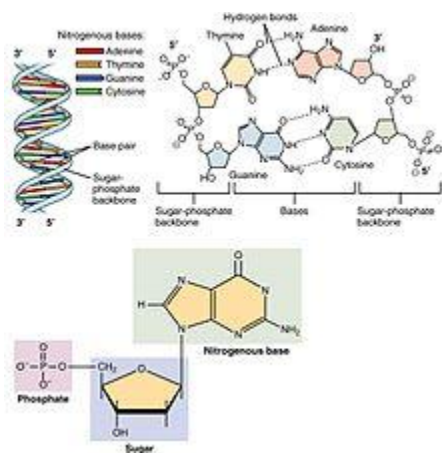
skeleton, and then an amino group is added, often via transamination. The amino acids may then be linked together to form a protein.

A similar process is used to break down proteins. It is first hydrolyzed into its component amino acids. Free ammonia (NH_3), existing as the ammonium ion (NH_4^+) in blood, is toxic to life forms. A suitable method for excreting it must therefore exist. Different tactics have evolved in different animals, depending on the animals' needs. Unicellular organisms release the ammonia into the environment. Likewise, bony fish can release ammonia into the water where it is quickly diluted. In general, mammals convert ammonia into urea, via the urea cycle.

In order to determine whether two proteins are related, or in other words to decide whether they are homologous or not, scientists use sequence-comparison methods. Methods like sequence alignments and structural alignments are powerful tools that help scientists identify homologies between related molecules. The relevance of finding homologies among proteins goes beyond forming an evolutionary pattern of protein families.

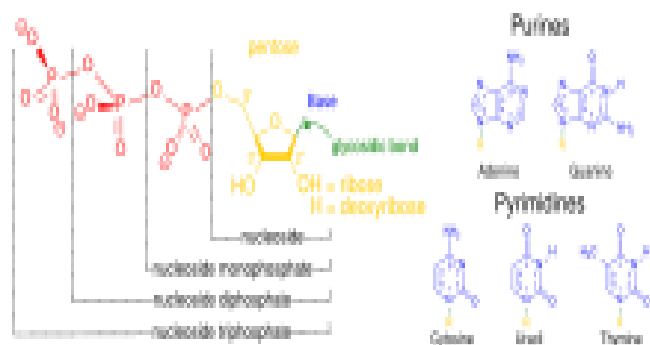
By finding how similar two protein sequences are, we acquire knowledge about their structure and therefore their function.

Nucleic acids



The structure of deoxyribonucleic acid (DNA); the picture shows the monomers being put together.

Nucleic acids, so-called because of their prevalence in cellular nuclei, is the generic name of the family of biopolymers. They are complex, high-molecular-weight biochemical macromolecules that can convey genetic information in all living cells and viruses. The monomers are called nucleotides, and each consists of three components: a nitrogenous heterocyclic base (either a purine or a pyrimidine), a pentose sugar, and a phosphate group.



Structural elements of common nucleic acid constituents. Because they contain at least one phosphate group, the compounds marked *nucleoside monophosphate*, *nucleoside diphosphate* and *nucleoside triphosphate* are all nucleotides (not phosphate-lacking nucleosides).

The most common nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The phosphate group and the sugar of each nucleotide bond with each other to form the backbone of the nucleic acid, while the sequence of nitrogenous bases stores the information. The most common nitrogenous bases are adenine, cytosine, guanine, thymine, and uracil. The nitrogenous bases of each strand of a nucleic acid will form hydrogen bonds with certain other nitrogenous bases in a complementary strand of nucleic acid. Adenine binds with thymine and uracil, thymine binds only with adenine, and

cytosine and guanine can bind only with one another. Adenine, thymine, and uracil contain two hydrogen bonds, while hydrogen bonds formed between cytosine and guanine are three. Aside from the genetic material of the cell, nucleic acids often play a role as second messengers, as well as forming the base molecule for adenosine triphosphate (ATP), the primary energy-carrier molecule found in all living organisms. Also, the nitrogenous bases possible in the two nucleic acids are different: adenine, cytosine, and guanine occur in both RNA and DNA, while thymine occurs only in DNA and uracil occurs in RNA.

Metabolism

Carbohydrates as energy source

Glucose is an energy source in most life forms. For instance, polysaccharides are broken down into their monomers by enzymes (glycogen phosphorylase removes glucose residues from glycogen, a polysaccharide). Disaccharides like lactose or sucrose are cleaved into their two component monosaccharides.

Glycolysis (anaerobic)

Glucose is mainly metabolized by a very important ten-step pathway called glycolysis, the net result of which is to break down one molecule of glucose into two molecules

of pyruvate. This also produces a net two molecules of ATP, the energy currency of cells, along with two reducing equivalents of converting NAD^+ (nicotinamide adenine dinucleotide: oxidized form) to NADH (nicotinamide adenine dinucleotide: reduced form). This does not require oxygen; if no oxygen is available (or the cell cannot use oxygen), the NAD is restored by converting the pyruvate to lactate (lactic acid) (e.g. in humans) or to ethanol plus carbon dioxide (e.g. in yeast). Other monosaccharides like galactose and fructose can be converted into intermediates of the glycolytic pathway.

Aerobic

In aerobic cells with sufficient oxygen, as in most human cells, the pyruvate is further metabolized. It is irreversibly converted to acetyl-CoA, giving off one carbon atom as the waste product carbon dioxide, generating another reducing equivalent as NADH. The two molecules acetyl-CoA (from one molecule of glucose) then enter the citric acid cycle, producing two molecules of ATP, six more NADH molecules and two reduced (ubi)quinones (via FADH_2 as enzyme-bound cofactor), and releasing the remaining carbon atoms as carbon dioxide. The produced NADH and quinol molecules then feed into the

enzyme complexes of the respiratory chain, an electron transport system transferring the electrons ultimately to oxygen and conserving the released energy in the form of a proton gradient over a membrane (inner mitochondrial membrane in eukaryotes). Thus, oxygen is reduced to water and the original electron acceptors NAD^+ and quinone are regenerated. This is why humans breathe in oxygen and breathe out carbon dioxide. The energy released from transferring the electrons from high-energy states in NADH and quinol is conserved first as proton gradient and converted to ATP via ATP synthase. This generates an additional 28 molecules of ATP (24 from the 8 NADH + 4 from the 2 quinols), totaling to 32 molecules of ATP conserved per degraded glucose (two from glycolysis + two from the citrate cycle). It is clear that using oxygen to completely oxidize glucose provides an organism with far more energy than any oxygen-independent metabolic feature, and this is thought to be the reason why complex life appeared only after Earth's atmosphere accumulated large amounts of oxygen.

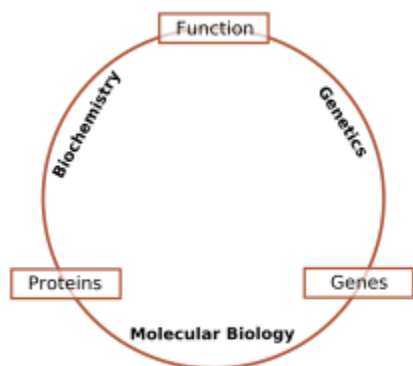
Gluconeogenesis

In vertebrates, vigorously contracting skeletal muscles (during weightlifting or sprinting, for example) do not receive enough

oxygen to meet the energy demand, and so they shift to anaerobic metabolism, converting glucose to lactate. The combination of glucose from noncarbohydrates origin, such as fat and proteins. This only happens when glycogen supplies in the liver are worn out. The pathway is a crucial reversal of glycolysis from pyruvate to glucose and can use many sources like amino acids, glycerol and Krebs Cycle. Large scale protein and fat catabolism usually occur when those suffer from starvation or certain endocrine disorders.

The liver regenerates the glucose, using a process called gluconeogenesis. This process is not quite the opposite of glycolysis, and actually requires three times the amount of energy gained from glycolysis (six molecules of ATP are used, compared to the two gained in glycolysis). Analogous to the above reactions, the glucose produced can then undergo glycolysis in tissues that need energy, be stored as glycogen (or starch in plants), or be converted to other monosaccharides or joined into di- or oligosaccharides. The combined pathways of glycolysis during exercise, lactate's crossing via the bloodstream to the liver, subsequent gluconeogenesis and release of glucose into the bloodstream is called the Cori cycle.

Relationship to other "molecular-scale" biological sciences



Schematic relationship between biochemistry, genetics, and molecular biology

Researchers in biochemistry use specific techniques native to biochemistry, but increasingly combine these with techniques and ideas developed in the fields of genetics, molecular biology, and biophysics. There is not a defined line between these disciplines. Biochemistry studies the chemistry required for biological activity of molecules, molecular biology studies their biological activity, genetics studies their heredity, which happens to be carried by their genome. This is shown in the following schematic that depicts one possible view of the relationships between the fields:

- *Biochemistry* is the study of the chemical substances and vital processes occurring in

live organisms. Biochemists focus heavily on the role, function, and structure of biomolecules. The study of the chemistry behind biological processes and the synthesis of biologically active molecules are applications of biochemistry. Biochemistry studies life at the atomic and molecular level.

- *Genetics* is the study of the effect of genetic differences in organisms. This can often be inferred by the absence of a normal component (e.g. one gene). The study of "mutants" – organisms that lack one or more functional components with respect to the so-called "wild type" or normal phenotype. Genetic interactions (epistasis) can often confound simple interpretations of such "knockout" studies.
- *Molecular biology* is the study of molecular underpinnings of the biological phenomena, focusing on molecular synthesis, modification, mechanisms and interactions. The central dogma of molecular biology, where genetic material is transcribed into RNA and then translated into protein, despite being oversimplified, still provides a good starting point for understanding the field. This

concept has been revised in light of emerging novel roles for RNA.

- *Chemical biology* seeks to develop new tools based on small molecules that allow minimal perturbation of biological systems while providing detailed information about their function. Further, chemical biology employs biological systems to create non-natural hybrids between biomolecules and synthetic devices (for example emptied viral capsids that can deliver gene therapy or drug molecules).

CHAPTER II
STRUCTURAL BIOLOGY

Introduction

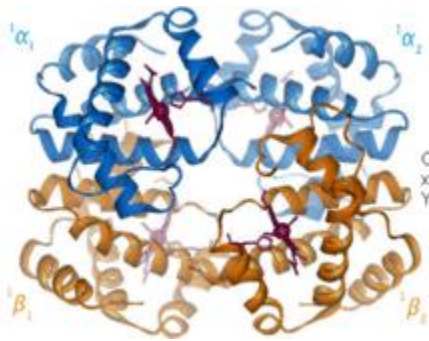
Structural biology, as defined by the *Journal of Structural Biology*, deals with structural analysis of living material (formed, composed of, and/or maintained and refined by living cells) at every level of organization.

Early structural biologists throughout the 19th and early 20th centuries were primarily only able to study structures to the limit of the naked eye's visual acuity and through magnifying glasses and light microscopes. In the 20th century, a variety of experimental techniques were developed to examine the 3D structures of biological molecules. The most prominent techniques are X-ray crystallography, nuclear magnetic resonance, and electron microscopy. Through the discovery of X-rays and its applications to protein crystals, structural biology was revolutionized, as now scientists could obtain the three-dimensional structures of biological molecules in atomic detail.^[2] Likewise, NMR spectroscopy allowed information about protein structure and dynamics to be obtained.^[3] Finally, in the 21st century, electron microscopy also saw a drastic revolution with the development of more coherent electron sources, aberration correction for electron microscopes, and

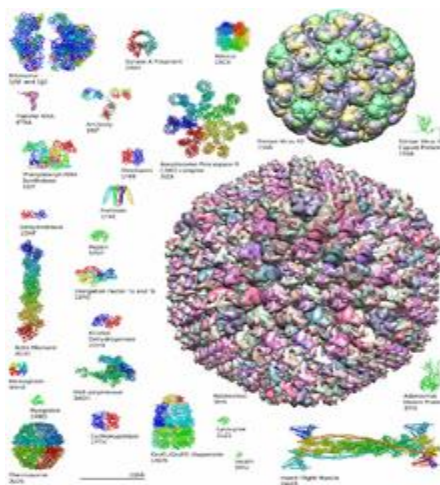
reconstruction software that enabled the successful implementation of high resolution cryo-electron microscopy, thereby permitting the study of individual proteins and molecular complexes in three-dimensions at angstrom resolution.

With the development of these three techniques, the field of structural biology expanded and also became a branch of molecular biology, biochemistry, and biophysics concerned with the molecular structure of biological macromolecules (especially proteins, made up of amino acids, RNA or DNA, made up of nucleotides, and membranes, made up of lipids), how they acquire the structures they have, and how alterations in their structures affect their function. This subject is of great interest to biologists because macromolecules carry out most of the functions of cells, and it is only by coiling into specific three-dimensional shapes that they are able to perform these functions. This architecture, the "tertiary structure" of molecules, depends in a complicated way on each molecule's basic composition, or "primary structure." At lower resolutions, tools such as FIB-SEM tomography have allowed for greater understanding of cells and

their organelles in 3-dimensions, and how each hierarchical level of various extracellular matrices contributes to function (for example in bone). In the past few years it has also become possible to predict highly accurate physical molecular models to complement the experimental study of biological structures. Computational techniques such as molecular dynamics simulations can be used in conjunction with empirical structure determination strategies to extend and study protein structure, conformation and function.



Hemoglobin, the oxygen transporting protein found in red blood cells



Examples of protein structures from the Protein Data Bank (PDB)

History

In 1912 Max Von Laue directed X-rays at crystallized copper sulfate generating a diffraction pattern. These experiments led to the development of X-ray crystallography, and its usage in exploring biological structures. In 1951, Rosalind Franklin and Maurice Wilkins used X-ray diffraction patterns to capture the first image of deoxyribonucleic acid (DNA). Francis Crick and James Watson modeled the double helical structure of DNA using this same technique in 1953 and received the Nobel Prize in Medicine along with Wilkins in 1962.

Pepsin crystals were the first proteins to be crystallized for use in X-ray diffraction, by Theodore Svedberg who received the 1962 Nobel Prize in Chemistry. The first tertiary protein structure, that of myoglobin, was published in 1958 by John Kendrew. During this time, modeling of protein structures was done using balsa wood or wire models. With the invention of modeling software such as CCP4 in the late 1970s, modeling is now done with computer assistance. Recent developments in the field have included the generation of X-ray free electron lasers,

allowing analysis of the dynamics and motion of biological molecules, and the use of structural biology in assisting synthetic biology.

In the late 1930s and early 1940s, the combination of work done by Isidor Rabi, Felix Bloch, and Edward Mills Purcell led to the development of nuclear magnetic resonance (NMR). Currently, solid-state NMR is widely used in the field of structural biology to determine the structure and dynamic nature of proteins (protein NMR).

In 1990, Richard Henderson produced the first three-dimensional, high resolution image of bacteriorhodopsin using cryogenic electron microscopy (cryo-EM).^[16] Since then, cryo-EM has emerged as an increasingly popular technique to determine three-dimensional, high resolution structures of biological images.

More recently, computational methods have been developed to model and study biological structures. For example, molecular dynamics (MD) is commonly used to analyze the dynamic movements of biological molecules. In 1975, the first simulation of a biological folding process using MD was published in Nature. Recently, protein structure prediction was significantly

improved by a new machine learning method called AlphaFold. Some claim that computational approaches are starting to lead the field of structural biology research.

Techniques

Biomolecules are too small to see in detail even with the most advanced light microscopes. The methods that structural biologists use to determine their structures generally involve measurements on vast numbers of identical molecules at the same time. These methods include:

- Mass spectrometry
- Macromolecular crystallography
- Neutron diffraction
- Proteolysis
- Nuclear magnetic resonance spectroscopy of proteins (NMR)
- Electron paramagnetic resonance (EPR)
- Cryogenic electron microscopy (cryoEM)
- Electron crystallography and microcrystal electron diffraction
- Multiangle light scattering
- Small angle scattering

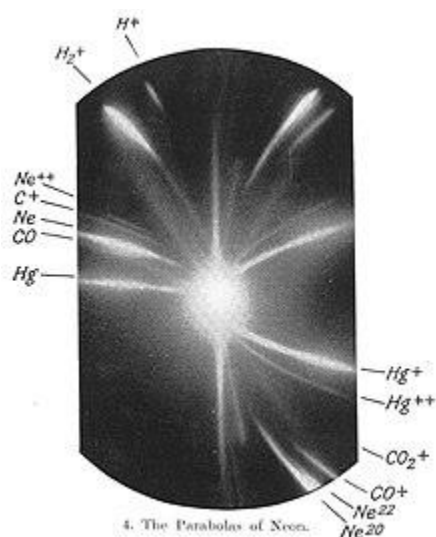
- Ultrafast laser spectroscopy
- Anisotropic terahertz microspectroscopy
- Two-dimensional infrared spectroscopy
- Dual-polarization interferometry and circular dichroism

Most often researchers use them to study the "native states" of macromolecules. But variations on these methods are also used to watch nascent or denatured molecules assume or reassume their native states. See protein folding.

A third approach that structural biologists take to understanding structure is bioinformatics to look for patterns among the diverse sequences that give rise to particular shapes. Researchers often can deduce aspects of the structure of integral membrane proteins based on the membrane topology predicted by hydrophobicity analysis. See protein structure prediction.

Mass spectrometry (MS) is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are presented as a *mass spectrum*, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.

A mass spectrum is a type of plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds.



Discovery of Neon Isotopes

In a typical MS procedure, a sample, which may be solid, liquid, or gaseous, is ionized, for example by bombarding it with a beam of electrons. This may cause some of the sample's molecules to break up into positively charged fragments or simply become positively charged without fragmenting. These ions (fragments) are then separated according to their mass-to-charge ratio, for example by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-

charge ratio will undergo the same amount of deflection.^[1] The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the signal intensity of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses (e.g. an entire molecule) to the identified masses or through a characteristic fragmentation pattern.

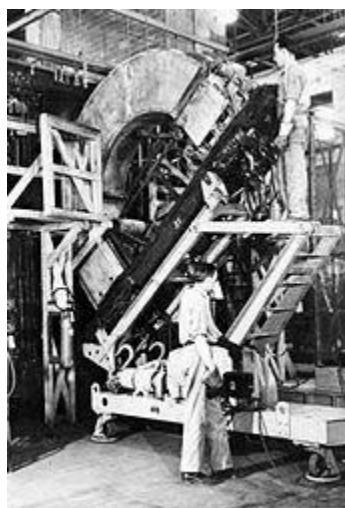
History of the mass spectrometer



Replica of F.W. Aston's third mass spectrometer

In 1886, Eugen Goldstein observed rays in gas discharges under low pressure that traveled away from the anode and through channels in a perforated cathode, opposite to the direction of negatively charged cathode rays (which travel from cathode to anode). Goldstein called these positively charged anode rays "Kanalstrahlen"; the standard translation of this term into English is "canal rays". Wilhelm Wien found that strong electric or magnetic fields deflected the canal rays and, in

1899, constructed a device with perpendicular electric and magnetic fields that separated the positive rays according to their charge-to-mass ratio (Q/m). Wien found that the charge-to-mass ratio depended on the nature of the gas in the discharge tube. English scientist J. J. Thomson later improved on the work of Wien by reducing the pressure to create the mass spectrograph.



Calutron mass spectrometers were used in the Manhattan Project for uranium enrichment.

The word *spectrograph* had become part of the international scientific vocabulary by 1884.^{[2][3]} Early *spectrometry* devices that measured the mass-to-charge ratio of ions were called *mass spectrographs* which consisted of instruments that recorded a spectrum of mass values on a photographic plate.^{[4][5]} A *mass*

spectroscope is similar to a *mass spectrograph* except that the beam of ions is directed onto a phosphor screen.^[6] A mass spectroscope configuration was used in early instruments when it was desired that the effects of adjustments be quickly observed. Once the instrument was properly adjusted, a photographic plate was inserted and exposed. The term mass spectroscope continued to be used even though the direct illumination of a phosphor screen was replaced by indirect measurements with an oscilloscope. The use of the term *mass spectroscopy* is now discouraged due to the possibility of confusion with light spectroscopy. Mass spectrometry is often abbreviated as *mass-spec* or simply as *MS*.

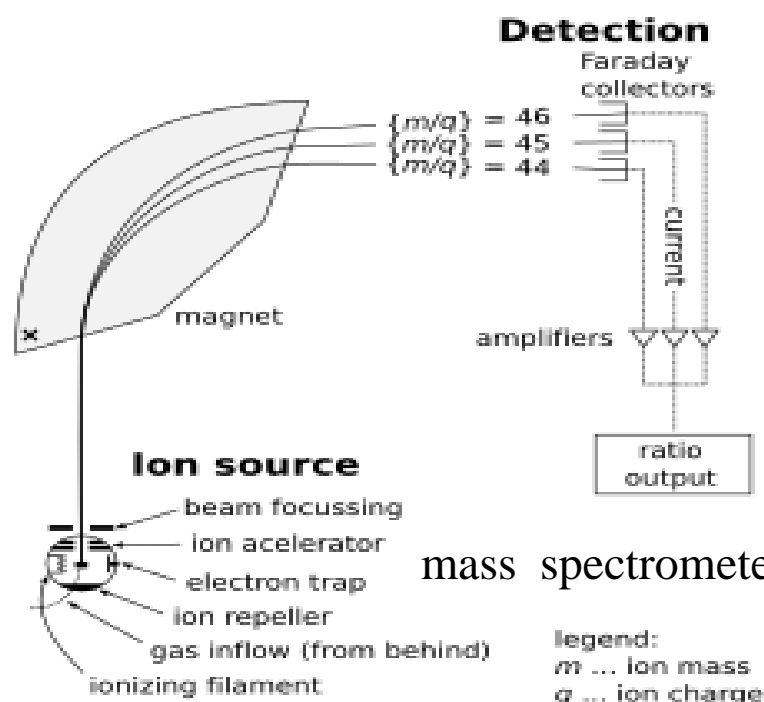
Modern techniques of mass spectrometry were devised by Arthur Jeffrey Dempster and F.W. Aston in 1918 and 1919 respectively.

Sector mass spectrometers known as calutrons were developed by Ernest O. Lawrence and used for separating the isotopes of uranium during the Manhattan Project. Calutron mass spectrometers were used for uranium enrichment at the Oak Ridge, Tennessee Y-12 plant established during World War II.

In 1989, half of the Nobel Prize in Physics was awarded to Hans Dehmelt and Wolfgang Paul for the development of the ion trap technique in the 1950s and 1960s.

In 2002, the Nobel Prize in Chemistry was awarded to John Bennett Fenn for the development of electrospray ionization (ESI) and Koichi Tanaka for the development of soft laser desorption (SLD) and their application to the ionization of biological macromolecules, especially proteins.

Parts of a mass spectrometer



Schematics of a simple mass spectrometer with sector type mass analyzer. This one is for measurement of carbon

the dioxide isotope ratios (IRMS) as in the carbon-13 urea breath test.

A mass spectrometer consists of three components: an ion source, a mass analyzer, and a detector. The *ionizer* converts a portion of the sample into ions. There is a wide variety of ionization techniques, depending on the phase (solid, liquid, gas) of the sample and the efficiency of various ionization mechanisms for the unknown species. An extraction system removes ions from the sample, which are then targeted through the mass analyzer and into the *detector*. The differences in masses of the fragments allows the mass analyzer to sort the ions by their mass-to-charge ratio. The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. Some detectors also give spatial information, e.g., a multichannel plate.

Theoretical example

The following describes the operation of a spectrometer mass analyzer, which is of the sector type. (Other analyzer types are treated below.) Consider a sample of sodium chloride (table salt). In the ion source, the sample is vaporized (turned into gas) and ionized (transformed into electrically charged particles) into sodium (Na^+) and chloride (Cl^-) ions. Sodium atoms and ions are monoisotopic, with a mass of about 23 daltons (symbol:

Da or older symbol: u). Chloride atoms and ions come in two stable isotopes with masses of approximately 35 u (at a natural abundance of about 75 percent) and approximately 37 u (at a natural abundance of about 25 percent). The analyzer part of the spectrometer contains electric and magnetic fields, which exert forces on ions traveling through these fields. The speed of a charged particle may be increased or decreased while passing through the electric field, and its direction may be altered by the magnetic field. The magnitude of the deflection of the moving ion's trajectory depends on its mass-to-charge ratio. Lighter ions are deflected by the magnetic force to a greater degree than heavier ions (based on Newton's second law of motion, $F = ma$). The streams of magnetically sorted ions pass from the analyzer to the detector, which records the relative abundance of each ion type. This information is used to determine the chemical element composition of the original sample (i.e. that both sodium and chlorine are present in the sample) and the isotopic composition of its constituents (the ratio of ^{35}Cl to ^{37}Cl).

Creating ions



Surface ionization source at the Argonne National Laboratory linear accelerator

The ion source is the part of the mass spectrometer that ionizes the material under analysis (the analyte). The ions are then transported by magnetic or electric fields to the mass analyzer.

Techniques for ionization have been key to determining what types of samples can be analyzed by mass spectrometry. Electron ionization and chemical ionization are used for gases and vapors. In chemical ionization sources, the analyte is ionized by chemical ion-molecule reactions during collisions in the source. Two techniques often used with liquid and solid biological samples include electrospray ionization (invented by John Fenn) and matrix-assisted laser desorption/ionization (MALDI, initially developed as a similar technique "Soft Laser Desorption (SLD)" by K. Tanaka for which a Nobel Prize was awarded and as MALDI by M. Karas and F. Hillenkamp).

Hard ionization and soft ionization



Quadrupole mass spectrometer and electrospray ion source used for Fenn's early work

In mass spectrometry, ionization refers to the production of gas phase ions suitable for resolution in the mass analyser or mass filter. Ionization occurs in the ion source. There are several ion sources available; each has advantages and disadvantages for particular applications. For example, electron ionization (EI) gives a high degree of fragmentation, yielding highly detailed mass spectra which when skilfully analysed can provide important information for structural elucidation/characterisation and facilitate identification of unknown compounds by comparison to mass spectral libraries obtained under identical operating conditions. However, EI is not suitable for coupling to HPLC, i.e. LC-MS, since at atmospheric pressure, the

filaments used to generate electrons burn out rapidly. Thus EI is coupled predominantly with GC, i.e. GC-MS, where the entire system is under high vacuum.

Hard ionization techniques are processes which impart high quantities of residual energy in the subject molecule invoking large degrees of fragmentation (i.e. the systematic rupturing of bonds acts to remove the excess energy, restoring stability to the resulting ion). Resultant ions tend to have m/z lower than the molecular ion (other than in the case of proton transfer and not including isotope peaks). The most common example of hard ionization is electron ionization (EI).

Soft ionization refers to the processes which impart little residual energy onto the subject molecule and as such result in little fragmentation. Examples include fast atom bombardment (FAB), chemical ionization (CI), atmospheric-pressure chemical ionization (APCI), atmospheric-pressure photoionization (APPI), electrospray ionization (ESI), desorption electrospray ionization (DESI), and matrix-assisted laser desorption/ionization (MALDI).

Inductively coupled plasma



Inductively coupled plasma ion source

Inductively coupled plasma (ICP) sources are used primarily for cation analysis of a wide array of sample types. In this source, a plasma that is electrically neutral overall, but that has had a substantial fraction of its atoms ionized by high temperature, is used to atomize introduced sample molecules and to further strip the outer electrons from those atoms. The plasma is usually generated from argon gas, since the first ionization energy of argon atoms is higher than the first of any other elements except He, F and Ne, but lower than the second ionization energy of all except the most electropositive metals. The heating is achieved by a radio-frequency current passed through a coil surrounding the plasma.

Photoionization mass spectrometry

Photoionization can be used in experiments which seek to use mass spectrometry as a means of resolving chemical kinetics

mechanisms and isomeric product branching. In such instances a high energy photon, either X-ray or uv, is used to dissociate stable gaseous molecules in a carrier gas of He or Ar. In instances where a synchrotron light source is utilized, a tuneable photon energy can be utilized to acquire a photoionization efficiency curve which can be used in conjunction with the charge ratio m/z to fingerprint molecular and ionic species. More recently atmospheric pressure photoionization (APPI) has been developed to ionize molecules mostly as effluents of LC-MS systems.

Ambient ionization

Some applications for ambient ionization include environmental applications as well as clinical applications. In these techniques, ions form in an ion source outside the mass spectrometer. Sampling becomes easy as the samples don't need previous separation nor preparation. Some examples of ambient ionization techniques are Direct Analysis in Real Time (DART), DESI, SESI, LAESI, desorption atmospheric-pressure chemical ionization (DAPCI), Soft Ionization by Chemical Reaction in Transfer (SICRT) and desorption atmospheric pressure photoionization DAPPI among others.

Other ionization techniques

Others include glow discharge, field desorption (FD), fast atom bombardment (FAB), thermospray, desorption/ionization on silicon (DIOS), atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS), spark ionization and thermal ionization (TIMS).

Mass selection

Mass analyzers separate the ions according to their mass-to-charge ratio. The following two laws govern the dynamics of charged particles in electric and magnetic fields in vacuum:

(Lorentz force law);

(Newton's second law of motion in the non-relativistic case, i.e. valid only at ion velocity much lower than the speed of light).

Here F is the force applied to the ion, m is the mass of the ion, a is the acceleration, Q is the ion charge, E is the electric field, and $v \times B$ is the vector cross product of the ion velocity and the magnetic field

Equating the above expressions for the force applied to the ion yields:

This differential equation is the classic equation of motion for charged particles. Together with the particle's initial conditions, it completely determines the particle's motion in space and time in terms of m/Q . Thus mass spectrometers could be thought of as "mass-to-charge spectrometers". When presenting data, it is common to use the (officially) dimensionless m/z , where z is the number of elementary charges (e) on the ion ($z=Q/e$). This quantity, although it is informally called the mass-to-charge ratio, more accurately speaking represents the ratio of the mass number and the charge number, z .

There are many types of mass analyzers, using either static or dynamic fields, and magnetic or electric fields, but all operate according to the above differential equation. Each analyzer type has its strengths and weaknesses. Many mass spectrometers use two or more mass analyzers for tandem mass spectrometry (MS/MS). In addition to the more common mass analyzers listed below, there are others designed for special situations.

There are several important analyzer characteristics. The mass resolving power is the measure of the ability to distinguish two peaks of slightly different m/z . The mass accuracy is the ratio of the m/z measurement error to the true m/z . Mass accuracy is usually measured in ppm or milli mass units. The mass range is the range of m/z amenable to analysis by a given analyzer. The linear dynamic range is the range over which ion signal is linear with analyte concentration. Speed refers to the time frame of the experiment and ultimately is used to determine the number of spectra per unit time that can be generated.

Sector instruments



ThermoQuest AvantGarde sector mass spectrometer

A sector field mass analyzer uses a static electric and/or magnetic field to affect the path and/or velocity of the charged particles in some way. As shown above, sector instruments bend the trajectories of the ions as they pass through the mass analyzer, according to their mass-to-charge ratios,

deflecting the more charged and faster-moving, lighter ions more. The analyzer can be used to select a narrow range of m/z or to scan through a range of m/z to catalog the ions present.

Time-of-flight

The time-of-flight (TOF) analyzer uses an electric field to accelerate the ions through the same potential, and then measures the time they take to reach the detector. If the particles all have the same charge, their kinetic energies will be identical, and their velocities will depend only on their masses. For example, ions with a lower mass will travel faster, reaching the detector first. Ions usually are moving prior to being accelerated by the electric field, this causes particles with the same m/z to arrive at different times at the detector. This difference in initial velocities is often not dependent on the mass of the ion, and will turn into a difference in the final velocity. This distribution in velocities broadens the peaks shown on the count vs m/z plot, but will generally not change the central location of the peaks, since the starting velocity of ions is generally centered at zero. To fix this problem, time-lag focusing/delayed extraction has been coupled with TOF-MS.

Quadrupole mass filter

Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (RF) quadrupole field created between four parallel rods. Only the ions in a certain range of mass/charge ratio are passed through the system at any time, but changes to the potentials on the rods allow a wide range of m/z values to be swept rapidly, either continuously or in a succession of discrete hops. A quadrupole mass analyzer acts as a mass-selective filter and is closely related to the quadrupole ion trap, particularly the linear quadrupole ion trap except that it is designed to pass the untrapped ions rather than collect the trapped ones, and is for that reason referred to as a transmission quadrupole. A magnetically enhanced quadrupole mass analyzer includes the addition of a magnetic field, either applied axially or transversely. This novel type of instrument leads to an additional performance enhancement in terms of resolution and/or sensitivity depending upon the magnitude and orientation of the applied magnetic field. A common variation of the transmission quadrupole is the triple quadrupole mass spectrometer. The "triple quad" has three consecutive

quadrupole stages, the first acting as a mass filter to transmit a particular incoming ion to the second quadrupole, a collision chamber, wherein that ion can be broken into fragments. The third quadrupole also acts as a mass filter, to transmit a particular fragment ion to the detector. If a quadrupole is made to rapidly and repetitively cycle through a range of mass filter settings, full spectra can be reported. Likewise, a triple quad can be made to perform various scan types characteristic of tandem mass spectrometry.

Ion traps

The quadrupole ion trap works on the same physical principles as the quadrupole mass analyzer, but the ions are trapped and sequentially ejected. Ions are trapped in a mainly quadrupole RF field, in a space defined by a ring electrode (usually connected to the main RF potential) between two endcap electrodes (typically connected to DC or auxiliary AC potentials). The sample is ionized either internally (e.g. with an electron or laser beam), or externally, in which case the ions are often introduced through an aperture in an endcap electrode.

There are many mass/charge separation and isolation methods but the most commonly used is the mass instability mode in

which the RF potential is ramped so that the orbit of ions with a mass $a > b$ are stable while ions with mass b become unstable and are ejected on the z -axis onto a detector. There are also non-destructive analysis methods.

Ions may also be ejected by the resonance excitation method, whereby a supplemental oscillatory excitation voltage is applied to the endcap electrodes, and the trapping voltage amplitude and/or excitation voltage frequency is varied to bring ions into a resonance condition in order of their mass/charge ratio.

Cylindrical ion trap

The cylindrical ion trap mass spectrometer (CIT) is a derivative of the quadrupole ion trap where the electrodes are formed from flat rings rather than hyperbolic shaped electrodes. The architecture lends itself well to miniaturization because as the size of a trap is reduced, the shape of the electric field near the center of the trap, the region where the ions are trapped, forms a shape similar to that of a hyperbolic trap.

Linear quadrupole ion trap

A linear quadrupole ion trap is similar to a quadrupole ion trap, but it traps ions in a two dimensional quadrupole field, instead of a three-dimensional quadrupole field as in a 3D quadrupole

ion trap. Thermo Fisher's LTQ ("linear trap quadrupole") is an example of the linear ion trap.

A toroidal ion trap can be visualized as a linear quadrupole curved around and connected at the ends or as a cross-section of a 3D ion trap rotated on edge to form the toroid, donut-shaped trap. The trap can store large volumes of ions by distributing them throughout the ring-like trap structure. This toroidal shaped trap is a configuration that allows the increased miniaturization of an ion trap mass analyzer. Additionally, all ions are stored in the same trapping field and ejected together simplifying detection that can be complicated with array configurations due to variations in detector alignment and machining of the arrays.

As with the toroidal trap, linear traps and 3D quadrupole ion traps are the most commonly miniaturized mass analyzers due to their high sensitivity, tolerance for mTorr pressure, and capabilities for single analyzer tandem mass spectrometry (e.g. product ion scans).

Orbitrap



Orbitrap mass analyzer

Orbitrap instruments are similar to Fourier-transform ion cyclotron resonance mass spectrometers (see text below). Ions are electrostatically trapped in an orbit around a central, spindle shaped electrode. The electrode confines the ions so that they both orbit around the central electrode and oscillate back and forth along the central electrode's long axis. This oscillation generates an image current in the detector plates which is recorded by the instrument. The frequencies of these image currents depend on the mass-to-charge ratios of the ions. Mass spectra are obtained by Fourier transformation of the recorded image currents.

Orbitraps have a high mass accuracy, high sensitivity and a good dynamic range.

Fourier-transform ion cyclotron resonance



Fourier-transform ion cyclotron resonance mass spectrometer

Fourier-transform mass spectrometry (FTMS), or more precisely Fourier-transform ion cyclotron resonance MS, measures mass by detecting the image current produced by ions cyclotroning in the presence of a magnetic field. Instead of measuring the deflection of ions with a detector such as an electron multiplier, the ions are injected into a Penning trap (a static electric/magnetic ion trap) where they effectively form part of a circuit. Detectors at fixed positions in space measure the electrical signal of ions which pass near them over time, producing a periodic signal. Since the frequency of an ion's cycling is determined by its mass-to-charge ratio, this can be deconvoluted by performing a Fourier transform on the signal. FTMS has the advantage of high sensitivity (since each ion is "counted" more than once) and much higher resolution and thus precision.

Ion cyclotron resonance (ICR) is an older mass analysis technique similar to FTMS except that ions are detected with a traditional detector. Ions trapped in a Penning trap are excited by an RF electric field until they impact the wall of the trap, where the detector is located. Ions of different mass are resolved according to impact time.

Detectors

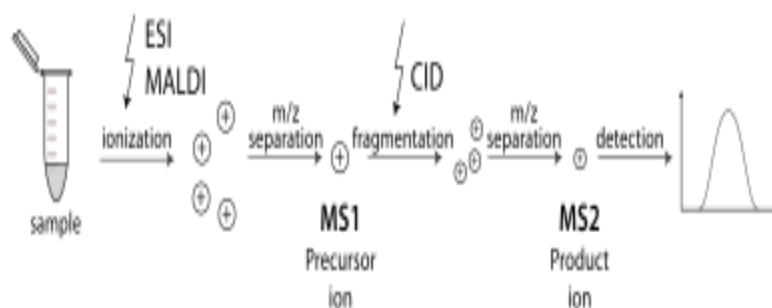


A continuous dynode particle multiplier detector

The final element of the mass spectrometer is the detector. The detector records either the charge induced or the current produced when an ion passes by or hits a surface. In a scanning instrument, the signal produced in the detector during the course of the scan versus where the instrument is in the scan (at what m/Q) will produce a mass spectrum, a record of ions as a function of m/Q .

Typically, some type of electron multiplier is used, though other detectors including Faraday cups and ion-to-photon detectors are also used. Because the number of ions leaving the mass analyzer at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. Microchannel plate detectors are commonly used in modern commercial instruments. In FTMS and Orbitraps, the detector consists of a pair of metal surfaces within the mass analyzer/ion trap region which the ions only pass near as they oscillate. No direct current is produced, only a weak AC image current is produced in a circuit between the electrodes. Other inductive detectors have also been used.

Tandem mass spectrometry



Tandem mass spectrometry for biological molecules using ESI or MALDI

A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry, usually separated by some form of molecule fragmentation. For example, one mass analyzer can isolate one peptide from many entering a mass spectrometer. A collision cell then stabilizes the peptide ions while they collide with a gas, causing them to fragment by collision-induced dissociation (CID). A further mass analyzer then sorts the fragments produced from the peptides. Tandem MS can also be done in a single mass analyzer over time, as in a quadrupole ion trap. There are various methods for fragmenting molecules for tandem MS, including collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD), blackbody infrared radiative dissociation (BIRD), electron-detachment dissociation (EDD) and surface-induced dissociation (SID). An important application using tandem mass spectrometry is in protein identification.

Tandem mass spectrometry enables a variety of experimental sequences. Many commercial mass spectrometers are designed to expedite the execution of such routine sequences as selected

reaction monitoring (SRM), precursor ion scanning, product ion scanning, and neutral loss scanning.

- In SRM, the first analyzer allows only a single mass through and the second analyzer monitors for multiple user-defined fragment ions over longer dwell-times than could be achieved in a full scan. This increases sensitivity.
- In product ion scans, the first mass analyzer is fixed to select a particular precursor ion ("parent"), while the second is scanned to find all the fragments ("products", or "daughter ions") to which it can be fragmented in the collision cell.
- In precursor ion scans, the second mass analyzer is fixed to select a particular fragment ion ("daughter"), while the first is scanned to find all possible precursor ions that could give rise to this fragment.
- In neutral loss scans, the two mass analyzers are scanned in parallel, but separated by the mass of a molecular subunit of interest to the analyst. Ions are detected if they lose that fixed mass during fragmentation. This can be used to look for any chemical that is capable of losing a particular neutral group, for example a sugar residue. Together,

neutral loss and precursor ion scans can be used to hunt for chemicals with particular motifs.

Another type of tandem mass spectrometry used for radiocarbon dating is accelerator mass spectrometry (AMS), which uses very high voltages, usually in the mega-volt range, to accelerate negative ions into a type of tandem mass spectrometer.

The METLIN Metabolite and Chemical Entity Database is the largest repository of experimental tandem mass spectrometry data acquired from standards. The tandem mass spectrometry data on over 930,000 molecular standards (as of January 2024) is provided to facilitate the identification of chemical entities from tandem mass spectrometry experiments. In addition to the identification of known molecules it is also useful for identifying unknowns using its similarity searching/analysis. All tandem mass spectrometry data comes from the experimental analysis of standards at multiple collision energies and in both positive and negative ionization modes.

Common mass spectrometer configurations and techniques

When a specific combination of source, analyzer, and detector becomes conventional in practice, a compound acronym may

arise to designate it succinctly. One example is MALDI-TOF, which refers to a combination of a matrix-assisted laser desorption/ionization source with a time-of-flight mass analyzer. Other examples include inductively coupled plasma-mass spectrometry (ICP-MS), accelerator mass spectrometry (AMS), thermal ionization-mass spectrometry (TIMS) and spark source mass spectrometry (SSMS).

Certain applications of mass spectrometry have developed monikers that although strictly speaking would seem to refer to a broad application, in practice have come instead to connote a specific or a limited number of instrument configurations. An example of this is isotope-ratio mass spectrometry (IRMS), which refers in practice to the use of a limited number of sector based mass analyzers; this name is used to refer to both the application and the instrument used for the application.

Separation techniques combined with mass spectrometry

An important enhancement to the mass resolving and mass determining capabilities of mass spectrometry is using it in tandem with chromatographic and other separation techniques.

Gas chromatography



A gas chromatograph (right) directly coupled to a mass spectrometer (left)

A common combination is gas chromatography-mass spectrometry (GC/MS or GC-MS). In this technique, a gas chromatograph is used to separate different compounds. This stream of separated compounds is fed online into the ion source, a metallic filament to which voltage is applied. This filament emits electrons which ionize the compounds. The ions can then further fragment, yielding predictable patterns. Intact ions and fragments pass into the mass spectrometer's analyzer and are eventually detected. However, the high temperatures (300°C) used in the GC-MS injection port (and oven) can result in thermal degradation of injected molecules, thus resulting in the measurement of degradation products instead of the actual molecule(s) of interest.

Liquid chromatography



Indianapolis Museum of Art conservation scientist performing liquid chromatography–mass spectrometry

Similarly to gas chromatography MS (GC-MS), liquid chromatography-mass spectrometry (LC/MS or LC-MS) separates compounds chromatographically before they are introduced to the ion source and mass spectrometer. It differs from GC-MS in that the mobile phase is liquid, usually a mixture of water and organic solvents, instead of gas. Most commonly, an electrospray ionization source is used in LC-MS. Other popular and commercially available LC-MS ion sources are atmospheric pressure chemical ionization and atmospheric pressure photoionization. There are also some newly developed ionization techniques like laser spray.

Capillary electrophoresis–mass spectrometry

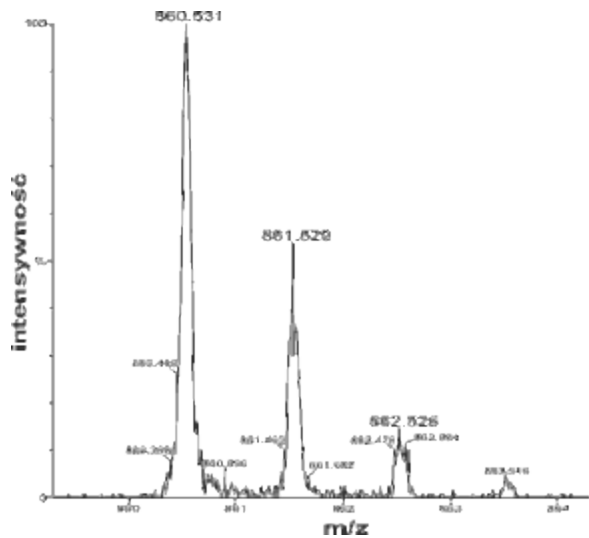
Capillary electrophoresis–mass spectrometry (CE-MS) is a technique that combines the liquid separation process of capillary electrophoresis with mass spectrometry. CE-MS is typically coupled to electrospray ionization.

Ion mobility

Ion mobility spectrometry-mass spectrometry (IMS/MS or IMMS) is a technique where ions are first separated by drift time through some neutral gas under an applied electrical potential gradient before being introduced into a mass spectrometer. Drift time is a measure of the collisional cross section relative to the charge of the ion. The duty cycle of IMS (the time over which the experiment takes place) is longer than most mass spectrometric techniques, such that the mass spectrometer can sample along the course of the IMS separation. This produces data about the IMS separation and the mass-to-charge ratio of the ions in a manner similar to LC-MS.

The duty cycle of IMS is short relative to liquid chromatography or gas chromatography separations and can thus be coupled to such techniques, producing triple modalities such as LC/IMS/MS.

Data and analysis



Mass spectrum of a peptide

showing the isotopic distribution

Data representations

Mass spectrometry produces various types of data. The most common data representation is the mass spectrum.

Certain types of mass spectrometry data are best represented as a mass chromatogram. Types of chromatograms include selected ion monitoring (SIM), total ion current (TIC), and selected reaction monitoring (SRM), among many others.

Other types of mass spectrometry data are well represented as a three-dimensional contour map. In this form, the mass-to-charge, m/z is on the x -axis, intensity the y -axis, and an additional experimental parameter, such as time, is recorded on the z -axis.

Data analysis

Mass spectrometry data analysis is specific to the type of experiment producing the data. General subdivisions of data are fundamental to understanding any data.

Many mass spectrometers work in either *negative ion mode* or *positive ion mode*. It is very important to know whether the observed ions are negatively or positively charged. This is often important in determining the neutral mass but it also indicates something about the nature of the molecules.

Different types of ion source result in different arrays of fragments produced from the original molecules. An electron ionization source produces many fragments and mostly single-charged (1-) radicals (odd number of electrons), whereas an electrospray source usually produces non-radical quasimolecular ions that are frequently multiply charged. Tandem mass spectrometry purposely produces fragment ions post-source and can drastically change the sort of data achieved by an experiment.

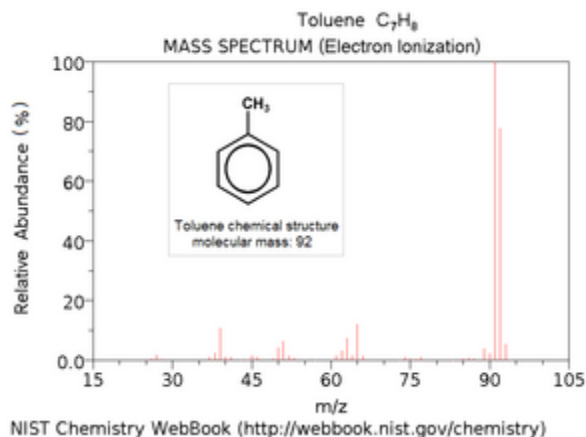
Knowledge of the origin of a sample can provide insight into the component molecules of the sample and their fragmentations. A sample from a synthesis/manufacturing process will probably contain impurities chemically related to the target component. A

crudely prepared biological sample will probably contain a certain amount of salt, which may form adducts with the analyte molecules in certain analyses.

Results can also depend heavily on sample preparation and how it was run/introduced. An important example is the issue of which matrix is used for MALDI spotting, since much of the energetics of the desorption/ionization event is controlled by the matrix rather than the laser power. Sometimes samples are spiked with sodium or another ion-carrying species to produce adducts rather than a protonated species.

Mass spectrometry can measure molar mass, molecular structure, and sample purity. Each of these questions requires a different experimental procedure; therefore, adequate definition of the experimental goal is a prerequisite for collecting the proper data and successfully interpreting it.

Interpretation of mass spectra



Toluene electron ionization mass spectrum

Since the precise structure or peptide sequence of a molecule is deciphered through the set of fragment masses, the interpretation of mass spectra requires combined use of various techniques. Usually the first strategy for identifying an unknown compound is to compare its experimental mass spectrum against a library of mass spectra. If no matches result from the search, then manual interpretation or software assisted interpretation of mass spectra must be performed. Computer simulation of ionization and fragmentation processes occurring in mass spectrometer is the primary tool for assigning structure or peptide sequence to a molecule. An *a priori* structural information is fragmented *in silico* and the resulting pattern is compared with observed spectrum. Such simulation is often supported by a fragmentation library that contains published

patterns of known decomposition reactions. Software taking advantage of this idea has been developed for both small molecules and proteins.

Analysis of mass spectra can also be spectra with accurate mass. A mass-to-charge ratio value (m/z) with only integer precision can represent an immense number of theoretically possible ion structures; however, more precise mass figures significantly reduce the number of candidate molecular formulas. A computer algorithm called formula generator calculates all molecular formulas that theoretically fit a given mass with specified tolerance.

A recent technique for structure elucidation in mass spectrometry, called precursor ion fingerprinting, identifies individual pieces of structural information by conducting a search of the tandem spectra of the molecule under investigation against a library of the product-ion spectra of structurally characterized precursor ions.

Applications



NOAA Particle Analysis by Laser Mass Spectrometry aerosol mass spectrometer aboard a NASA WB-57 high-altitude research aircraft

Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now commonly used in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds. Quantification can be relative (analyzed relative to a reference sample) or absolute (analyzed using a standard curve method).

As an analytical technique it possesses distinct advantages such as: Increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduces background interference, Excellent specificity from characteristic fragmentation patterns to identify unknowns or confirm the presence of suspected compounds, Information about molecular weight, Information about the isotopic abundance of elements, Temporally resolved chemical data.

A few of the disadvantages of the method is that it often fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p- positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.

Isotope ratio MS: isotope dating and tracing



Mass spectrometer to determine the $^{16}\text{O}/^{18}\text{O}$ and $^{12}\text{C}/^{13}\text{C}$ isotope ratio on biogenous carbonate

Mass spectrometry is also used to determine the isotopic composition of elements within a sample. Differences in mass among isotopes of an element are very small, and the less abundant isotopes of an element are typically very rare, so a very sensitive instrument is required. These instruments, sometimes referred to as isotope ratio mass spectrometers (IR-MS), usually use a single magnet to bend a beam of ionized particles towards a series of Faraday cups which convert particle impacts to electric current. A fast on-line analysis of deuterium content of water can be done using flowing afterglow mass spectrometry, FA-MS. Probably the most sensitive and accurate mass spectrometer for this purpose is the accelerator mass spectrometer (AMS). This is because it provides ultimate sensitivity, capable of measuring individual atoms and measuring nuclides with a dynamic range of $\sim 10^{15}$ relative to the major stable isotope.^[51] Isotope ratios are important markers of a variety of processes. Some isotope ratios are used to determine the age of materials for example as in carbon dating. Labeling with stable isotopes is also used for protein quantification. (see protein characterization below)

Membrane-introduction mass spectrometry: measuring gases in solution

Membrane-introduction mass spectrometry combines the isotope ratio MS with a reaction chamber/cell separated by a gas-permeable membrane. This method allows the study of gases as they evolve in solution. This method has been extensively used for the study of the production of oxygen by Photosystem II.^[52]

Trace gas analysis

Several techniques use ions created in a dedicated ion source injected into a flow tube or a drift tube: selected ion flow tube (SIFT-MS), and proton transfer reaction (PTR-MS), are variants of chemical ionization dedicated for trace gas analysis of air, breath or liquid headspace using well defined reaction time allowing calculations of analyte concentrations from the known reaction kinetics without the need for internal standard or calibration.

Another technique with applications in trace gas analysis field is secondary electrospray ionization (SESI-MS), which is a variant of electrospray ionization. SESI consist of an electrospray plume of pure acidified solvent that interacts with neutral vapors. Vapor molecules get ionized at atmospheric

pressure when charge is transferred from the ions formed in the electrospray to the molecules. One advantage of this approach is that it is compatible with most ESI-MS systems.

Residual gas analysis



Residual gas analyzer installed on a laboratory-scale freeze dryer

A residual gas analyzer (RGA) is a small and usually rugged mass spectrometer, typically designed for process control and contamination monitoring in vacuum systems. When constructed as a quadrupole mass analyzer, there exist two implementations, utilizing either an open ion source (OIS) or a closed ion source (CIS). RGAs may be found in high vacuum applications such as research chambers, surface

science setups, accelerators, scanning microscopes, etc. RGAs are used in most cases to monitor the quality of the vacuum and easily detect minute traces of impurities in the low-pressure gas environment. These impurities can be measured down to Torr levels, possessing sub-ppm detectability in the absence of background interferences.

RGAs would also be used as sensitive in-situ leak detectors commonly using helium, isopropyl alcohol or other tracer molecules. With vacuum systems pumped down to lower than Torr—checking of the integrity of the vacuum seals and the quality of the vacuum—air leaks, virtual leaks and other contaminants at low levels may be detected before a process is initiated.

Atom probe

An atom probe is an instrument that combines time-of-flight mass spectrometry and field-evaporation microscopy to map the location of individual atoms.

Pharmacokinetics

Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix (often blood or

urine) and the need for high sensitivity to observe low dose and long time point data. The most common instrumentation used in this application is LC-MS with a triple quadrupole mass spectrometer. Tandem mass spectrometry is usually employed for added specificity. Standard curves and internal standards are used for quantitation of usually a single pharmaceutical in the samples. The samples represent different time points as a pharmaceutical is administered and then metabolized or cleared from the body. Blank or $t=0$ samples taken before administration are important in determining background and ensuring data integrity with such complex sample matrices. Much attention is paid to the linearity of the standard curve; however it is not uncommon to use curve fitting with more complex functions such as quadratics since the response of most mass spectrometers is less than linear across large concentration ranges.

There is currently considerable interest in the use of very high sensitivity mass spectrometry for microdosing studies, which are seen as a promising alternative to animal experimentation.

Recent studies show that secondary electrospray ionization (SESI) is a powerful technique to monitor drug

kinetics via breath analysis. Because breath is naturally produced, several datapoints can be readily collected. This allows for the number of collected data-points to be greatly increased. In animal studies, this approach SEI can reduce animal sacrifice. In humans, SEI-MS non-invasive analysis of breath can help study the kinetics of drugs at a personalized level.

Protein characterization

Mass spectrometry is an important method for the characterization and sequencing of proteins. The two primary methods for ionization of whole proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). In keeping with the performance and mass range of available mass spectrometers, two approaches are used for characterizing proteins. In the first, intact proteins are ionized by either of the two techniques described above, and then introduced to a mass analyzer. This approach is referred to as "top-down" strategy of protein analysis. The top-down approach however is largely limited to low-throughput single-protein studies. In the second, proteins are enzymatically digested into

smaller peptides using proteases such as trypsin or pepsin, either in solution or in gel after electrophoretic separation. Other proteolytic agents are also used. The collection of peptide products are often separated by chromatography prior to introduction to the mass analyzer. When the characteristic pattern of peptides is used for the identification of the protein the method is called peptide mass fingerprinting (PMF), if the identification is performed using the sequence data determined in tandem MS analysis it is called de novo peptide sequencing. These procedures of protein analysis are also referred to as the "bottom-up" approach, and have also been used to analyse the distribution and position of post-translational modifications such as phosphorylation on proteins. A third approach is also beginning to be used, this intermediate "middle-down" approach involves analyzing proteolytic peptides that are larger than the typical tryptic peptide.

Space exploration



NASA's Phoenix Mars Lander analyzing a soil sample from the "Rosy Red" trench with the TEGA mass spectrometer

As a standard method for analysis, mass spectrometers have reached other planets and moons. Two were taken to Mars by the Viking program. In early 2005 the Cassini–Huygens mission delivered a specialized GC-MS instrument aboard the Huygens probe through the atmosphere of Titan, the largest moon of the planet Saturn. This instrument analyzed atmospheric samples along its descent trajectory and was able to vaporize and analyze samples of Titan's frozen, hydrocarbon covered surface once the probe had landed. These measurements compare the abundance of isotope(s) of each particle comparatively to earth's natural

abundance. Also on board the Cassini–Huygens spacecraft was an ion and neutral mass spectrometer which had been taking measurements of Titan's atmospheric composition as well as the composition of Enceladus' plumes. A Thermal and Evolved Gas Analyzer mass spectrometer was carried by the Mars Phoenix Lander launched in 2007.

Mass spectrometers are also widely used in space missions to measure the composition of plasmas. For example, the Cassini spacecraft carried the Cassini Plasma Spectrometer (CAPS), which measured the mass of ions in Saturn's magnetosphere.

Respired gas monitor

Mass spectrometers were used in hospitals for respiratory gas analysis beginning around 1975 through the end of the century. Some are probably still in use but none are currently being manufactured.

Found mostly in the operating room, they were a part of a complex system, in which respired gas samples from patients undergoing anesthesia were drawn into the instrument through a valve mechanism designed to sequentially connect up to 32 rooms to the mass spectrometer. A computer directed all operations of the system. The data collected from the mass

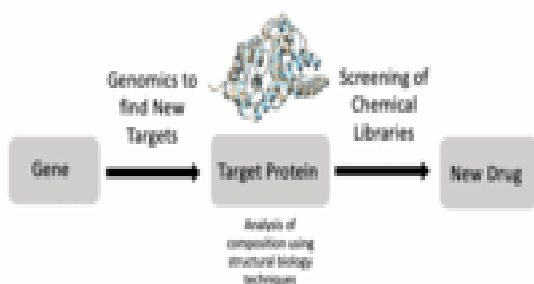
spectrometer was delivered to the individual rooms for the anesthesiologist to use.

The uniqueness of this magnetic sector mass spectrometer may have been the fact that a plane of detectors, each purposely positioned to collect all of the ion species expected to be in the samples, allowed the instrument to simultaneously report all of the gases respired by the patient. Although the mass range was limited to slightly over 120 u, fragmentation of some of the heavier molecules negated the need for a higher detection limit.

Preparative mass spectrometry

The primary function of mass spectrometry is as a tool for chemical analyses based on detection and quantification of ions according to their mass-to-charge ratio. However, mass spectrometry also shows promise for material synthesis. Ion soft landing is characterized by deposition of intact species on surfaces at low kinetic energies which precludes the fragmentation of the incident species. The soft landing technique was first reported in 1977 for the reaction of low energy sulfur containing ions on a lead surface.

Applications



Flowchart of how structural biology plays a role in drug discovery

Structural biologists have made significant contributions towards understanding the molecular components and mechanisms underlying human diseases. For example, cryo-EM and ssNMR have been used to study the aggregation of amyloid fibrils, which are associated with Alzheimer's disease, Parkinson's disease, and type II diabetes. In addition to amyloid proteins, scientists have used cryo-EM to produce high resolution models of tau filaments in the brain of Alzheimer's patients which may help develop better treatments in the future. Structural biology tools can also be used to explain interactions between pathogens and hosts. For example, structural biology tools have enabled virologists to understand how the HIV envelope allows the virus to evade human immune responses.

Structural biology is also an important component of drug discovery. Scientists can identify targets using genomics, study

those targets using structural biology, and develop drugs that are suited for those targets. Specifically, ligand-NMR, mass spectrometry, and X-ray crystallography are commonly used techniques in the drug discovery process. For example, researchers have used structural biology to better understand Met, a protein encoded by a protooncogene that is an important drug target in cancer. Similar research has been conducted for HIV targets to treat people with AIDS. Researchers are also developing new antimicrobials for mycobacterial infections using structure-driven drug discovery.

CHAPTER III
CANCER BIOLOGY

Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumors, which do not spread. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they can also have other causes. Over 100 types of cancers affect humans.

Tobacco use is the cause of about 22% of cancer deaths. Another 10% are due to obesity, poor diet, lack of physical activity or excessive alcohol consumption. Other factors include certain infections, exposure to ionizing radiation, and environmental pollutants. Infection with specific viruses, bacteria and parasites is an environmental factor causing approximately 16–18% of cancers worldwide. These infectious agents include *Helicobacter pylori*, hepatitis B, hepatitis C, human papillomavirus infection, Epstein–Barr virus, Human T-lymphotropic virus 1, Kaposi's sarcoma-associated herpesvirus and Merkel cell polyomavirus. Human immunodeficiency virus (HIV) does not directly cause cancer

but it causes immune deficiency that can magnify the risk due to other infections, sometimes up to several thousand fold (in the case of Kaposi's sarcoma). Importantly, vaccination against hepatitis B and human papillomavirus have been shown to nearly eliminate risk of cancers caused by these viruses in persons successfully vaccinated prior to infection.

These environmental factors act, at least partly, by changing the genes of a cell. Typically, many genetic changes are required before cancer develops. Approximately 5–10% of cancers are due to inherited genetic defects. Cancer can be detected by certain signs and symptoms or screening tests. It is then typically further investigated by medical imaging and confirmed by biopsy.

The risk of developing certain cancers can be reduced by not smoking, maintaining a healthy weight, limiting alcohol intake, eating plenty of vegetables, fruits, and whole grains, vaccination against certain infectious diseases, limiting consumption of processed meat and red meat, and limiting exposure to direct sunlight. Early detection through screening is useful for cervical and colorectal cancer. The benefits of screening for breast cancer are controversial. Cancer is often

treated with some combination of radiation therapy, surgery, chemotherapy and targeted therapy. Pain and symptom management are an important part of care. Palliative care is particularly important in people with advanced disease. The chance of survival depends on the type of cancer and extent of disease at the start of treatment. In children under 15 at diagnosis, the five-year survival rate in the developed world is on average 80%. For cancer in the United States, the average five-year survival rate is 66% for all ages.

In 2015, about 90.5 million people worldwide had cancer. In 2019, annual cancer cases grew by 23.6 million people, and there were 10 million deaths worldwide, representing over the previous decade increases of 26% and 21%, respectively.

The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer, and stomach cancer. In females, the most common types are breast cancer, colorectal cancer, lung cancer, and cervical cancer. If skin cancer other than melanoma were included in total new cancer cases each year, it would account for around 40% of cases. In children, acute lymphoblastic leukemia and brain tumors are most common, except in Africa, where non-Hodgkin

lymphoma occurs more often. In 2012, about 165,000 children under 15 years of age were diagnosed with cancer. The risk of cancer increases significantly with age, and many cancers occur more commonly in developed countries. Rates are increasing as more people live to an old age and as lifestyle changes occur in the developing world. The global total economic costs of cancer were estimated at US\$1.16 trillion (equivalent to \$1.62 trillion in 2023) per year as of 2010.

Etymology and definitions

The word comes from the ancient Greek *καρκίνος*, meaning 'crab' and 'tumor'. Greek physicians Hippocrates and Galen, among others, noted the similarity of crabs to some tumors with swollen veins. The word was introduced in English in the modern medical sense around 1600.

Cancers comprise a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely.

All tumor cells show the six hallmarks of cancer. These characteristics are required to produce a malignant tumor. They include:

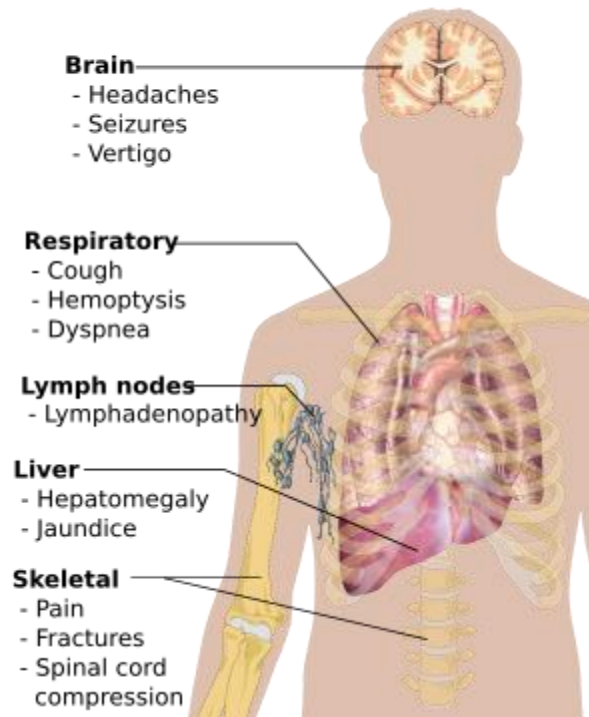
- Cell growth and division absent the proper signals
- Continuous growth and division even given contrary signals
- Avoidance of programmed cell death
- Limitless number of cell divisions
- Promoting blood vessel construction
- Invasion of tissue and formation of metastases

The progression from normal cells to cells that can form a detectable mass to cancer involves multiple steps known as malignant progression.

Signs and symptoms

Main article: Signs and symptoms of cancer

Common sites and symptoms of Cancer metastasis



Symptoms of cancer metastasis depend on the location of the tumor.

When cancer begins, it produces no symptoms. Signs and symptoms appear as the mass grows or ulcerates. The findings that result depend on cancer's type and location. Few symptoms are specific. Many frequently occur in individuals who have other conditions. Cancer can be difficult to diagnose and can be considered a "great imitator".

People may become anxious or depressed post-diagnosis. The risk of suicide in people with cancer is approximately double.

Local symptoms

Local symptoms may occur due to the mass of the tumor or its ulceration. For example, mass effects from lung cancer can block the bronchus resulting in cough or pneumonia; esophageal cancer can cause narrowing of the esophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, affecting bowel habits. Masses in breasts or testicles may produce observable lumps. Ulceration can cause bleeding that can lead to symptoms such as coughing up blood (lung cancer), anemia or rectal bleeding (colon cancer), blood in the urine (bladder cancer), or abnormal vaginal bleeding (endometrial or cervical cancer). Although localized pain may occur in advanced cancer, the initial tumor is usually painless. Some cancers can cause a buildup of fluid within the chest or abdomen.

Systemic symptoms

Systemic symptoms may occur due to the body's response to the cancer. This may include fatigue, unintentional weight loss, or skin changes. Some cancers can cause a systemic inflammatory

state that leads to ongoing muscle loss and weakness, known as cachexia.

Some cancers, such as Hodgkin's disease, leukemias, and liver or kidney cancers, can cause a persistent fever.

Shortness of breath, called dyspnea, is a common symptom of cancer and its treatment. The causes of cancer-related dyspnea can include tumors in or around the lung, blocked airways, fluid in the lungs, pneumonia, or treatment reactions including an allergic response. Treatment for dyspnea in patients with advanced cancer can include fans, bilevel ventilation, acupuncture/reflexology and multicomponent nonpharmacological interventions.

Some systemic symptoms of cancer are caused by hormones or other molecules produced by the tumor, known as paraneoplastic syndromes. Common paraneoplastic syndromes include hypercalcemia, which can cause altered mental state, constipation and dehydration, or hyponatremia, which can also cause altered mental status, vomiting, headaches, or seizures.

Metastasis

Metastasis is the spread of cancer to other locations in the body. The dispersed tumors are called metastatic tumors, while the original is called the primary tumor. Almost all cancers can metastasize. Most cancer deaths are due to cancer that has metastasized.

Metastasis is common in the late stages of cancer and it can occur via the blood or the lymphatic system or both. The typical steps in metastasis are:

1. Local invasion
2. Intravasation into the blood or lymph.
3. Circulation through the body.
4. Extravasation into the new tissue.
5. Proliferation
6. Angiogenesis

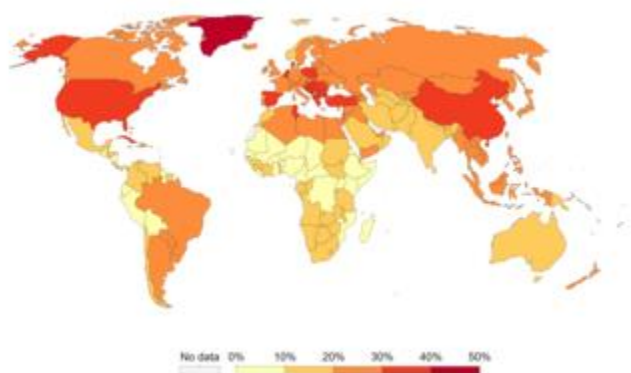
Different types of cancers tend to metastasize to particular organs. Overall, the most common places for metastases to occur are the lungs, liver, brain, and the bones.

While some cancers can be cured if detected early, metastatic cancer is more difficult to treat and control. Nevertheless, some recent treatments are demonstrating encouraging results.

Causes



The GHS Hazard pictogram for carcinogenic substances



Share of cancer deaths attributed to tobacco in 2016.

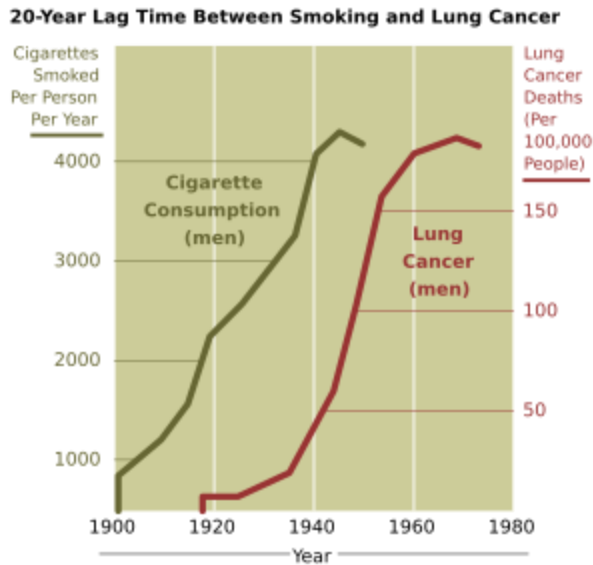
The majority of cancers, some 90–95% of cases, are due to genetic mutations from environmental and lifestyle factors. The remaining 5–10% are due to inherited genetics. *Environmental* refers to any cause that is not inherited, such as lifestyle, economic, and behavioral factors and not merely pollution. Common environmental factors that contribute to cancer death include tobacco use (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both

ionizing and non-ionizing, up to 10%), lack of physical activity, and pollution. Psychological stress does not appear to be a risk factor for the onset of cancer, though it may worsen outcomes in those who already have cancer.

Environmental or lifestyle factors that caused cancer to develop in an individual can be identified by analyzing mutational signatures from genomic sequencing of tumor DNA. For example, this can reveal if lung cancer was caused by tobacco smoke, if skin cancer was caused by UV radiation, or if secondary cancers were caused by previous chemotherapy treatment.

Cancer is generally not a transmissible disease. Exceptions include rare transmissions that occur with pregnancies and occasional organ donors. However, transmissible infectious diseases such as hepatitis B, Epstein-Barr virus, Human Papilloma Virus and HIV, can contribute to the development of cancer.

Chemicals



The incidence of lung cancer is highly correlated with smoking.

Exposure to particular substances have been linked to specific types of cancer. These substances are called *carcinogens*.

Tobacco smoke, for example, causes 90% of lung cancer. Tobacco use can cause cancer throughout the body including in the mouth and throat, larynx, esophagus, stomach, bladder, kidney, cervix, colon/rectum, liver and pancreas. Tobacco smoke contains over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons.

Tobacco is responsible for about one in five cancer deaths worldwide and about one in three in the developed world. Lung cancer death rates in the United States have mirrored smoking patterns, with increases in smoking followed

by dramatic increases in lung cancer death rates and, more recently, decreases in smoking rates since the 1950s followed by decreases in lung cancer death rates in men since 1990.

In Western Europe, 10% of cancers in males and 3% of cancers in females are attributed to alcohol exposure, especially liver and digestive tract cancers. Cancer from work-related substance exposures may cause between 2 and 20% of cases, causing at least 200,000 deaths. Cancers such as lung cancer and mesothelioma can come from inhaling tobacco smoke or asbestos fibers, or leukemia from exposure to benzene.

Exposure to perfluorooctanoic acid (PFOA), which is predominantly used in the production of Teflon, is known to cause two kinds of cancer.

Chemotherapy drugs such as platinum-based compounds are carcinogens that increase the risk of secondary cancers

Azathioprine, an immunosuppressive medication, is a carcinogen that can cause primary tumors to develop.

Diet and exercise

Diet, physical inactivity, and obesity are related to up to 30–35% of cancer deaths. In the United States, excess body weight is associated with the development of many types of cancer and is

a factor in 14–20% of cancer deaths. A UK study including data on over 5 million people showed higher body mass index to be related to at least 10 types of cancer and responsible for around 12,000 cases each year in that country. Physical inactivity is believed to contribute to cancer risk, not only through its effect on body weight but also through negative effects on the immune system and endocrine system. More than half of the effect from the diet is due to overnutrition (eating too much), rather than from eating too few vegetables or other healthful foods.

Some specific foods are linked to specific cancers. A high-salt diet is linked to gastric cancer. Aflatoxin B₁, a frequent food contaminant, causes liver cancer. Betel nut chewing can cause oral cancer. National differences in dietary practices may partly explain differences in cancer incidence. For example, gastric cancer is more common in Japan due to its high-salt diet while colon cancer is more common in the United States. Immigrant cancer profiles mirror those of their new country, often within one generation.

Infection

Worldwide, approximately 18% of cancer deaths are related to infectious diseases. This proportion ranges from a high of

25% in Africa to less than 10% in the developed world. Viruses are the usual infectious agents that cause cancer but bacteria and parasites may also play a role. Oncoviruses (viruses that can cause human cancer) include:

- Human papillomavirus (cervical cancer),
- Epstein–Barr virus (B-cell lymphoproliferative disease and nasopharyngeal carcinoma),
- Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas),
- Hepatitis B and hepatitis C viruses (hepatocellular carcinoma)
- Human T-cell leukemia virus-1 (T-cell leukemias).
- Merkel cell polyomavirus (Merkel cell carcinoma)

Bacterial infection may also increase the risk of cancer, as seen in

- *Helicobacter pylori*-induced gastric carcinoma.
- Colibactin, a genotoxin associated with *Escherichia coli* infection (colorectal cancer)

Parasitic infections associated with cancer include:

- *Schistosoma haematobium* (squamous cell carcinoma of the bladder)

- The liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis* (cholangiocarcinoma).

Radiation

Radiation exposure such as ultraviolet radiation and radioactive material is a risk factor for cancer. Many non-melanoma skin cancers are due to ultraviolet radiation, mostly from sunlight. Sources of ionizing radiation include medical imaging and radon gas. Ionizing radiation is not a particularly strong mutagen. Residential exposure to radon gas, for example, has similar cancer risks as passive smoking. Radiation is a more potent source of cancer when combined with other cancer-causing agents, such as radon plus tobacco smoke. Radiation can cause cancer in most parts of the body, in all animals and at any age. Children are twice as likely to develop radiation-induced leukemia as adults; radiation exposure before birth has ten times the effect.

Medical use of ionizing radiation is a small but growing source of radiation-induced cancers. Ionizing radiation may be used to treat other cancers, but this may, in some cases, induce a second form of cancer. It is also used in some kinds of medical imaging.

Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies. Clear evidence establishes ultraviolet radiation, especially the non-ionizing medium wave UVB, as the cause of most non-melanoma skin cancers, which are the most common forms of cancer in the world.

Non-ionizing radio frequency radiation from mobile phones, electric power transmission and other similar sources has been described as a possible carcinogen by the World Health Organization's International Agency for Research on Cancer. Evidence, however, has not supported a concern. This includes that studies have not found a consistent link between mobile phone radiation and cancer risk.

Heredity

The vast majority of cancers are non-hereditary (sporadic). Hereditary cancers are primarily caused by an inherited genetic defect. Less than 0.3% of the population are carriers of a genetic mutation that has a large effect on cancer risk and these causes less than 3–10% of cancer. Some of these syndromes include: certain inherited mutations in the genes *BRCA1* and *BRCA2* with a more than 75% risk of breast

cancer and ovarian cancer, and hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome), which is present in about 3% of people with colorectal cancer, among others.

Statistically for cancers causing most mortality, the relative risk of developing colorectal cancer when a first-degree relative (parent, sibling or child) has been diagnosed with it is about 2. The corresponding relative risk is 1.5 for lung cancer, and 1.9 for prostate cancer. For breast cancer, the relative risk is 1.8 with a first-degree relative having developed it at 50 years of age or older, and 3.3 when the relative developed it when being younger than 50 years of age.

Taller people have an increased risk of cancer because they have more cells than shorter people. Since height is genetically determined to a large extent, taller people have a heritable increase of cancer risk.

Physical agents

Some substances cause cancer primarily through their physical, rather than chemical, effects. A prominent example of this is prolonged exposure to asbestos, naturally occurring mineral fibers that are a major cause of mesothelioma (cancer of

the serous membrane) usually the serous membrane surrounding the lungs. Other substances in this category, including both naturally occurring and synthetic asbestos-like fibers, such as wollastonite, attapulgite, glass wool and rock wool, are believed to have similar effects. Non-fibrous particulate materials that cause cancer include powdered metallic cobalt and nickel and crystalline silica (quartz, cristobalite and tridymite). Usually, physical carcinogens must get inside the body (such as through inhalation) and require years of exposure to produce cancer.

Physical trauma resulting in cancer is relatively rare. Claims that breaking bones resulted in bone cancer, for example, have not been proven. Similarly, physical trauma is not accepted as a cause for cervical cancer, breast cancer or brain cancer. One accepted source is frequent, long-term application of hot objects to the body. It is possible that repeated burns on the same part of the body, such as those produced by kanger and kairo heaters (charcoal hand warmers), may produce skin cancer, especially if carcinogenic chemicals are also present. Frequent consumption of scalding hot tea may produce esophageal cancer. Generally, it is believed that cancer arises, or a pre-existing cancer is

encouraged, during the process of healing, rather than directly by the trauma. However, repeated injuries to the same tissues might promote excessive cell proliferation, which could then increase the odds of a cancerous mutation.

Chronic inflammation has been hypothesized to directly cause mutation. Inflammation can contribute to proliferation, survival, angiogenesis and migration of cancer cells by influencing the tumor microenvironment. Oncogenes build up an inflammatory pro-tumorigenic microenvironment.

Hormones

Hormones also play a role in the development of cancer by promoting cell proliferation. Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis.

Hormones are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary and testis and also of thyroid cancer and bone cancer. For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may

explain their higher risk of breast cancer, even in the absence of a breast-cancer gene. Similarly, men of African ancestry have significantly higher levels of testosterone than men of European ancestry and have a correspondingly higher level of prostate cancer. Men of Asian ancestry, with the lowest levels of testosterone-activating androstenediol glucuronide, have the lowest levels of prostate cancer.

Other factors are relevant: obese people have higher levels of some hormones associated with cancer and a higher rate of those cancers. Women who take hormone replacement therapy have a higher risk of developing cancers associated with those hormones. On the other hand, people who exercise far more than average have lower levels of these hormones and lower risk of cancer. Osteosarcoma may be promoted by growth hormones. Some treatments and prevention approaches leverage this cause by artificially reducing hormone levels and thus discouraging hormone-sensitive cancers.

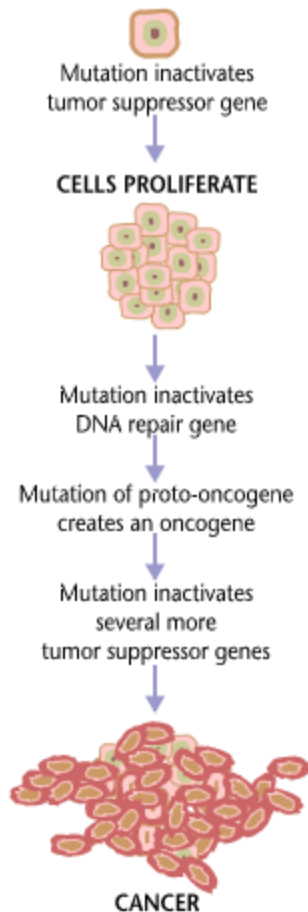
Autoimmune diseases

There is an association between celiac disease and an increased risk of all cancers. People with untreated celiac disease have a higher risk, but this risk decreases with time after diagnosis and

strict treatment. This may be due to the adoption of a gluten-free diet, which seems to have a protective role against development of malignancy in people with celiac disease. However, the delay in diagnosis and initiation of a gluten-free diet seems to increase the risk of malignancies. Rates of gastrointestinal cancers are increased in people with Crohn's disease and ulcerative colitis, due to chronic inflammation. Immunomodulators and biologic agents used to treat these diseases may promote developing extra-intestinal malignancies.

Pathophysiology

Genetics



Cancers are caused by a series of mutations. Each mutation alters the behavior of the cell somewhat.

Cancer is fundamentally a disease of tissue growth regulation. For a normal cell to transform into a cancer cell, the genes that regulate cell growth and differentiation must be altered.

The affected genes are divided into two broad categories. Oncogenes are genes that promote cell growth and reproduction. Tumor suppressor genes are genes that inhibit cell division and survival. Malignant transformation can occur

through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in multiple genes are required to transform a normal cell into a cancer cell.

Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA. Large-scale mutations involve the deletion or gain of a portion of a chromosome. Genomic amplification occurs when a cell gains copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location. A well-known example of this is the Philadelphia chromosome, or translocation of chromosomes 9 and 22, which occurs in chronic myelogenous leukemia and results in production of the BCR-abl fusion protein, an oncogenic tyrosine kinase.

Small-scale mutations include point mutations, deletions, and insertions, which may occur in the promoter region of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, leading to the expression of *viral* oncogenes in the affected cell and its descendants.

Replication of the data contained within the DNA of living cells will probabilistically result in some errors (mutations). Complex error correction and prevention are built into the process and safeguard the cell against cancer. If a significant error occurs, the damaged cell can self-destruct through programmed cell death, termed apoptosis. If the error control processes fail, then the mutations will survive and be passed along to daughter cells. Some environments make errors more likely to arise and propagate. Such environments can include the presence of disruptive substances called carcinogens, repeated physical injury, heat, ionising radiation, or hypoxia.

The errors that cause cancer are self-amplifying and compounding, for example:

- A mutation in the error-correcting machinery of a cell might cause that cell and its children to accumulate errors more rapidly.
- A further mutation in an oncogene might cause the cell to reproduce more rapidly and more frequently than its normal counterparts.
- A further mutation may cause loss of a tumor suppressor gene, disrupting the apoptosis signaling pathway and immortalizing the cell.
- A further mutation in the signaling machinery of the cell might send error-causing signals to nearby cells.

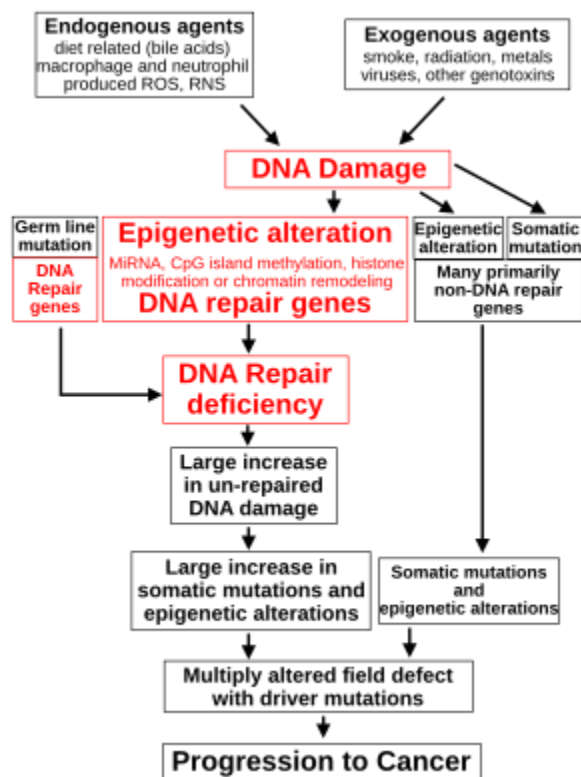
The transformation of a normal cell into cancer is akin to a chain reaction caused by initial errors, which compound into more severe errors, each progressively allowing the cell to escape more controls that limit normal tissue growth. This rebellion-like scenario is an undesirable survival of the fittest, where the driving forces of evolution work against the body's design and enforcement of order. Once cancer has begun to develop, this ongoing process, termed *clonal evolution*, drives progression towards more invasive stages. Clonal evolution leads to intra-

tumour heterogeneity (cancer cells with heterogeneous

mutations) that complicates designing effective treatment strategies and requires an evolutionary approach to designing treatment.

Characteristic abilities developed by cancers are divided into categories, specifically evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, limitless replicative potential, metastasis, reprogramming of energy metabolism and evasion of immune destruction.

Epigenetics



The central role of DNA damage and epigenetic defects in DNA repair genes in carcinogenesis

The classical view of cancer is a set of diseases driven by progressive genetic abnormalities that include mutations in tumor-suppressor genes and oncogenes, and in chromosomal abnormalities. A role for epigenetic alterations was identified in the early 21st century.

Epigenetic alterations are functionally relevant modifications to the genome that do not change the nucleotide sequence. Examples of such modifications are changes in DNA methylation (hypermethylation and hypomethylation), histone modification and changes in chromosomal architecture (caused by inappropriate expression of proteins such as HMGA2 or HMGA1). Each of these alterations regulates gene expression without altering the underlying DNA sequence. These changes may remain through cell divisions, endure for multiple generations, and can be considered as equivalent to mutations.

Epigenetic alterations occur frequently in cancers. As an example, one study listed protein coding genes that were frequently altered in their methylation in association with colon

cancer. These included 147 hypermethylated and 27 hypomethylated genes. Of the hypermethylated genes, 10 were hypermethylated in 100% of colon cancers and many others were hypermethylated in more than 50% of colon cancers.

While epigenetic alterations are found in cancers, the epigenetic alterations in DNA repair genes, causing reduced expression of DNA repair proteins, may be of particular importance. Such alterations may occur early in progression to cancer and are a possible cause of the genetic instability characteristic of cancers. Reduced expression of DNA repair genes disrupts DNA repair. This is shown in the figure at the 4th level from the top. (In the figure, red wording indicates the central role of DNA damage and defects in DNA repair in progression to cancer.) When DNA repair is deficient DNA damage remains in cells at a higher than usual level (5th level) and causes increased frequencies of mutation and/or epimutation (6th level). Mutation rates increase substantially in cells defective in DNA mismatch repair or in homologous recombinational repair (HRR). Chromosomal rearrangements and aneuploidy also increase in HRR defective cells.

Higher levels of DNA damage cause increased mutation (right side of figure) and increased epimutation. During repair of DNA double strand breaks, or repair of other DNA damage, incompletely cleared repair sites can cause epigenetic gene silencing.

Deficient expression of DNA repair proteins due to an inherited mutation can increase cancer risks. Individuals with an inherited impairment in any of 34 DNA repair genes (see article DNA repair-deficiency disorder) have increased cancer risk, with some defects ensuring a 100% lifetime chance of cancer (e.g. p53 mutations). Germ line DNA repair mutations are noted on the figure's left side. However, such germline mutations (which cause highly penetrant cancer syndromes) are the cause of only about 1 percent of cancers.

In sporadic cancers, deficiencies in DNA repair are occasionally caused by a mutation in a DNA repair gene but are much more frequently caused by epigenetic alterations that reduce or silence expression of DNA repair genes. This is indicated in the figure at the 3rd level. Many studies of heavy metal-induced carcinogenesis show that such heavy metals cause a reduction in expression of DNA repair enzymes, some through epigenetic

mechanisms. DNA repair inhibition is proposed to be a predominant mechanism in heavy metal-induced carcinogenicity. In addition, frequent epigenetic alterations of the DNA sequences code for small RNAs called microRNAs (or miRNAs). miRNAs do not code for proteins, but can "target" protein-coding genes and reduce their expression.

Cancers usually arise from an assemblage of mutations and epimutations that confer a selective advantage leading to clonal expansion (see Field defects in progression to cancer). Mutations, however, may not be as frequent in cancers as epigenetic alterations. An average cancer of the breast or colon can have about 60 to 70 protein-altering mutations, of which about three or four may be "driver" mutations and the remaining ones may be "passenger" mutations.

Metastasis

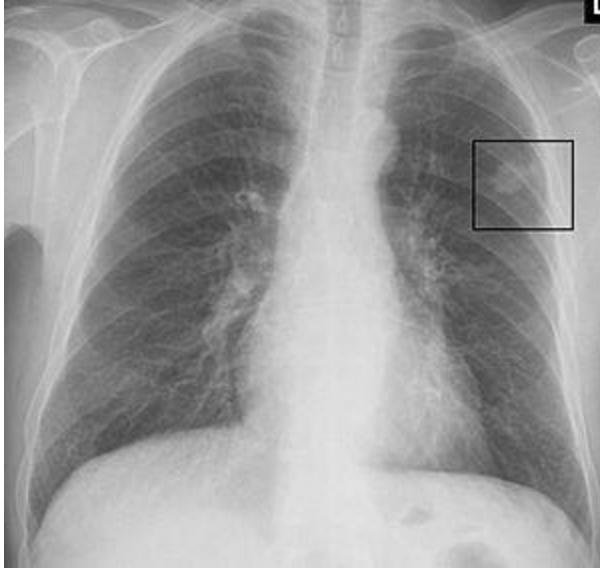
Metastasis is the spread of cancer to other locations in the body. The dispersed tumors are called metastatic tumors, while the original is called the primary tumor. Almost all cancers can metastasize. Most cancer deaths are due to cancer that has metastasized.

Metastasis is common in the late stages of cancer and it can occur via the blood or the lymphatic system or both. The typical steps in metastasis are local invasion, intravasation into the blood or lymph, circulation through the body, extravasation into the new tissue, proliferation and angiogenesis. Different types of cancers tend to metastasize to particular organs, but overall the most common places for metastases to occur are the lungs, liver, brain and the bones.

Metabolism

Normal cells typically generate only about 30% of energy from glycolysis, whereas most cancers rely on glycolysis for energy production (Warburg effect). But a minority of cancer types rely on oxidative phosphorylation as the primary energy source, including lymphoma, leukemia, and endometrial cancer. Even in these cases, however, the use of glycolysis as an energy source rarely exceeds 60%. A few cancers use glutamine as the major energy source, partly because it provides nitrogen required for nucleotide (DNA, RNA) synthesis. Cancer stem cells often use oxidative phosphorylation or glutamine as a primary energy source.

Diagnosis



Chest X-ray showing lung cancer in the left lung

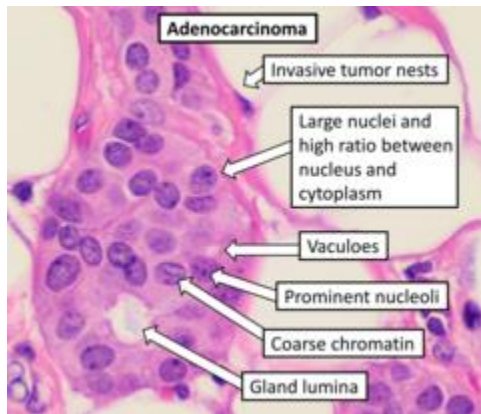
Most cancers are initially recognized either because of the appearance of signs or symptoms or through screening. Neither of these leads to a definitive diagnosis, which requires the examination of a tissue sample by a pathologist. People with suspected cancer are investigated with medical tests. These commonly include blood tests, X-rays, (contrast) CT scans and endoscopy.

The tissue diagnosis from the biopsy indicates the type of cell that is proliferating, its histological grade, genetic abnormalities and other features. Together, this information is useful to evaluate the prognosis and to choose the best treatment.

Cytogenetics and immunohistochemistry are other types of tissue tests. These tests provide information about molecular changes (such as mutations, fusion genes and numerical chromosome changes) and may thus also indicate the prognosis and best treatment.

Cancer diagnosis can cause psychological distress and psychosocial interventions, such as talking therapy, may help people with this. Some people choose to disclose the diagnosis widely; others prefer to keep the information private, especially shortly after the diagnosis, or to disclose it only partially or to selected people.

Classification



A large proportion of cancers are adenocarcinomas, with typical histopathology features shown, although they vary substantially from case to case.

Cancers are classified by the type of cell that the tumor cells resemble and is therefore presumed to be the origin of the tumor.

These types include:

- **Carcinoma:** Cancers derived from epithelial cells. This group includes many of the most common cancers and include nearly all those in the breast, prostate, lung, pancreas and colon. Most of these are of the adenocarcinoma type, which means that the cancer has gland-like differentiation.
- **Sarcoma:** Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating in mesenchymal cells outside the bone marrow.
- **Lymphoma and leukemia:** These two classes arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively.
- **Germ cell tumor:** Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).

- Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue.

Cancers are usually named using *-carcinoma*, *-sarcoma* or *-blastoma* as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root. For example, cancers of the liver parenchyma arising from malignant epithelial cells is called *hepatocarcinoma*, while a malignancy arising from primitive liver precursor cells is called a hepatoblastoma and a cancer arising from fat cells is called a *liposarcoma*. For some common cancers, the English organ name is used. For example, the most common type of breast cancer is called *ductal carcinoma of the breast*. Here, the adjective *ductal* refers to the appearance of cancer under the microscope, which suggests that it has originated in the milk ducts.

Benign tumors (which are not cancers) are named using *-oma* as a suffix with the organ name as the root. For example, a benign tumor of smooth muscle cells is called a *leiomyoma* (the common name of this frequently occurring benign tumor in the uterus is *fibroid*). Confusingly, some types of cancer use the *-noma* suffix, examples including melanoma and seminoma.

Some types of cancer are named for the size and shape of the cells under a microscope, such as giant cell carcinoma, spindle cell carcinoma and small-cell carcinoma.



An invasive ductal carcinoma of the breast (pale area at the center) surrounded by spikes of whitish scar tissue and yellow fatty tissue



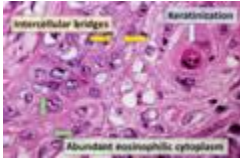
An invasive colorectal carcinoma (top center) in a colectomy specimen



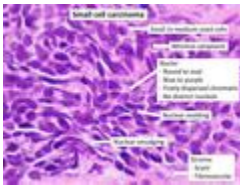
A squamous-cell carcinoma (the whitish tumor) near the bronchi in a lung specimen



• A large invasive ductal carcinoma in a mastectomy specimen



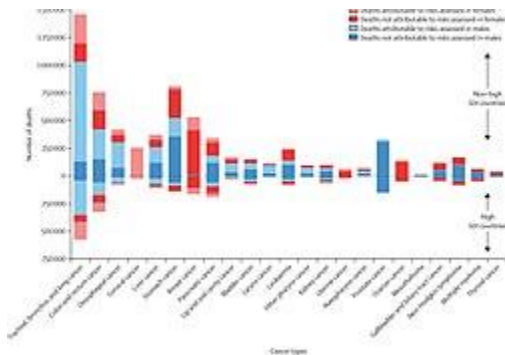
• Squamous cell carcinoma with typical histopathology features.



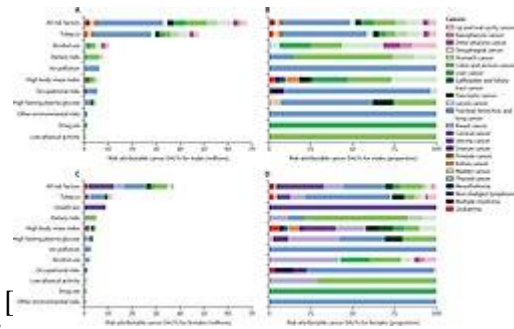
• Histopathology of small-cell carcinoma, with typical findings.

Prevention

Main article: Cancer prevention



Global deaths from cancers attributable to risk factors in 2019



by sex and Socio-demographic Index.^[1]

Cancer DALYs attributable to 11 Level 2 risk factors globally in 2019.

Cancer prevention is defined as active measures to decrease cancer risk. The vast majority of cancer cases are due to environmental risk factors. Many of these environmental factors are controllable lifestyle choices. Thus, cancer is generally preventable. Between 70% and 90% of common cancers are due to environmental factors and therefore potentially preventable.

Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, excess weight/obesity, poor diet, physical inactivity, alcohol, sexually transmitted infections and air pollution. Further, poverty could be considered as an indirect risk factor in human cancers. Not all environmental causes are controllable, such as naturally occurring background radiation and cancers caused through

hereditary genetic disorders and thus are not preventable via personal behavior.

In 2019, ~44% of all cancer deaths – or ~4.5 M deaths or ~105 million lost disability-adjusted life years – were due to known clearly preventable risk factors, led by smoking, alcohol use and high BMI, according to a GBD systematic analysis.

Dietary

While many dietary recommendations have been proposed to reduce cancer risks, the evidence to support them is not definitive. The primary dietary factors that increase risk are obesity and alcohol consumption. Diets low in fruits and vegetables and high in red meat have been implicated but reviews and meta-analyses do not come to a consistent conclusion. A 2014 meta-analysis found no relationship between fruits and vegetables and cancer. Coffee is associated with a reduced risk of liver cancer. Studies have linked excessive consumption of red or processed meat to an increased risk of breast cancer, colon cancer and pancreatic cancer, a phenomenon that could be due to the presence of carcinogens in meats cooked at high temperatures. In 2015 the IARC reported

that eating processed meat (e.g., bacon, ham, hot dogs, sausages) and, to a lesser degree, red meat was linked to some cancers.

Dietary recommendations for cancer prevention typically include an emphasis on vegetables, fruit, whole grains and fish and an avoidance of processed and red meat (beef, pork, lamb), animal fats, pickled foods and refined carbohydrates.

Medication

Medications can be used to prevent cancer in a few circumstances. In the general population, NSAIDs reduce the risk of colorectal cancer; however, due to cardiovascular and gastrointestinal side effects, they cause overall harm when used for prevention. Aspirin has been found to reduce the risk of death from cancer by about 7%. COX-2 inhibitors may decrease the rate of polyp formation in people with familial adenomatous polyposis; however, it is associated with the same adverse effects as NSAIDs. Daily use of tamoxifen or raloxifene reduce the risk of breast cancer in high-risk women. The benefit versus harm for 5-alpha-reductase inhibitor such as finasteride is not clear.

Vitamin supplementation does not appear to be effective at preventing cancer. While low blood levels of vitamin D are

correlated with increased cancer risk, whether this relationship is causal and vitamin D supplementation is protective is not determined. One 2014 review found that supplements had no significant effect on cancer risk. Another 2014 review concluded that vitamin D₃ may decrease the risk of death from cancer (one fewer death in 150 people treated over 5 years), but concerns with the quality of the data were noted.

Beta-Carotene supplementation increases lung cancer rates in those who are high risk. Folic acid supplementation is not effective in preventing colon cancer and may increase colon polyps. Selenium supplementation has not been shown to reduce the risk of cancer.

Vaccination

Vaccines have been developed that prevent infection by some carcinogenic viruses. Human papillomavirus vaccine (Gardasil and Cervarix) decrease the risk of developing cervical cancer. The hepatitis B vaccine prevents infection with hepatitis B virus and thus decreases the risk of liver cancer. The administration of human papillomavirus and hepatitis B vaccinations is recommended where resources allow.

Screening

Unlike diagnostic efforts prompted by symptoms and medical signs, cancer screening involves efforts to detect cancer after it has formed, but before any noticeable symptoms appear. This may involve physical examination, blood or urine tests or medical imaging.

Cancer screening is not available for many types of cancers. Even when tests are available, they may not be recommended for everyone. *Universal screening* or *mass screening* involves screening everyone. *Selective screening* identifies people who are at higher risk, such as people with a family history. Several factors are considered to determine whether the benefits of screening outweigh the risks and the costs of screening. These factors include:

- Possible harms from the screening test: for example, X-ray images involve exposure to potentially harmful ionizing radiation
- The likelihood of the test correctly identifying cancer
- The likelihood that cancer is present: Screening is not normally useful for rare cancers.
- Possible harms from follow-up procedures
- Whether suitable treatment is available

- Whether early detection improves treatment outcomes
- Whether cancer will ever need treatment
- Whether the test is acceptable to the people: If a screening test is too burdensome (for example, extremely painful), then people will refuse to participate.
- Cost

Recommendations

U.S. Preventive Services Task Force

The U.S. Preventive Services Task Force (USPSTF) issues recommendations for various cancers:

- Strongly recommends cervical cancer screening in women who are sexually active and have a cervix at least until the age of 65.
- Recommend that Americans be screened for colorectal cancer via fecal occult blood testing, sigmoidoscopy, or colonoscopy starting at age 50 until age 75.
- Evidence is insufficient to recommend for or against screening for skin cancer, oral cancer, lung cancer,¹ or prostate cancer in men under 75.

- Routine screening is not recommended for bladder cancer, testicular cancer, ovarian cancer, pancreatic cancer, or prostate cancer.
- Recommends mammography for breast cancer screening every two years from ages 50–74, but does not recommend either breast self-examination or clinical breast examination. A 2013 Cochrane review concluded that breast cancer screening by mammography had no effect in reducing mortality because of overdiagnosis and overtreatment.

Japan

Screens for gastric cancer using photofluorography due to the high incidence there.^[25]

Genetic testing

See also: Cancer syndrome

Gene	Cancer types
BRCA1, BRCA2	Breast, ovarian, pancreatic
HNPCC, MLH1, MSH2, MSH6, PMS1, PMS2	Colon,

	uterine, small bowel, stomach, urinary tract
--	-------------------------------------------------------

Genetic testing for individuals at high-risk of certain cancers is recommended by unofficial groups.^{[160][176]} Carriers of these mutations may then undergo enhanced surveillance, chemoprevention, or preventative surgery to reduce their subsequent risk.

Management

Many treatment options for cancer exist. The primary ones include surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and palliative care. Which treatments are used depends on the type, location and grade of the cancer as well as the patient's health and preferences. The treatment intent may or may not be curative.

Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. The term encompasses a variety of drugs, which are divided into broad categories such

as alkylating agents and antimetabolites. Traditional chemotherapeutic agents act by killing cells that divide rapidly, a critical property of most cancer cells.

It was found that providing combined cytotoxic drugs is better than a single drug, a process called the combination therapy, which has an advantage in the statistics of survival and response to the tumor and in the progress of the disease. A Cochrane review concluded that combined therapy was more effective to treat metastasized breast cancer. However, generally it is not certain whether combination chemotherapy leads to better health outcomes, when both survival and toxicity are considered.

Targeted therapy is a form of chemotherapy that targets specific molecular differences between cancer and normal cells. The first targeted therapies blocked the estrogen receptor molecule, inhibiting the growth of breast cancer. Another common example is the class of Bcr-Abl inhibitors, which are used to treat chronic myelogenous leukemia (CML). Currently, targeted therapies exist for many of the most common cancer types, including bladder cancer, breast cancer, colorectal cancer, kidney cancer, leukemia, liver cancer, lung

cancer, lymphoma, pancreatic cancer, prostate cancer, skin cancer, and thyroid cancer as well as other cancer types.

The efficacy of chemotherapy depends on the type of cancer and the stage. In combination with surgery, chemotherapy has proven useful in cancer types including breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer and certain lung cancers. Chemotherapy is curative for some cancers, such as some leukemias, ineffective in some brain tumors, and needless in others, such as most non-melanoma skin cancers. The effectiveness of chemotherapy is often limited by its toxicity to other tissues in the body. Even when chemotherapy does not provide a permanent cure, it may be useful to reduce symptoms such as pain or to reduce the size of an inoperable tumor in the hope that surgery will become possible in the future.

Radiation

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve symptoms. It works by damaging the DNA of cancerous tissue, causing mitotic catastrophe resulting in the death of the cancer cells. To spare normal tissues (such as skin or organs, which radiation must

pass through to treat the tumor), shaped radiation beams are aimed from multiple exposure angles to intersect at the tumor, providing a much larger dose there than in the surrounding, healthy tissue. As with chemotherapy, cancers vary in their response to radiation therapy.

Radiation therapy is used in about half of cases. The radiation can be either from internal sources (brachytherapy) or external sources. The radiation is most commonly low energy X-rays for treating skin cancers, while higher energy X-rays are used for cancers within the body. Radiation is typically used in addition to surgery and or chemotherapy. For certain types of cancer, such as early head and neck cancer, it may be used alone. Radiation therapy after surgery for brain metastases has been shown to not improve overall survival in patients compared to surgery alone. For painful bone metastasis, radiation therapy has been found to be effective in about 70% of patients.

Surgery

Surgery is the primary method of treatment for most isolated, solid cancers and may play a role in palliation and prolongation of survival. It is typically an important part of definitive diagnosis and staging of tumors, as biopsies are usually

required. In localized cancer, surgery typically attempts to remove the entire mass along with, in certain cases, the lymph nodes in the area. For some types of cancer this is sufficient to eliminate the cancer.

Palliative care

Palliative care is treatment that attempts to help the patient feel better and may be combined with an attempt to treat the cancer. Palliative care includes action to reduce physical, emotional, spiritual and psycho-social distress. Unlike treatment that is aimed at directly killing cancer cells, the primary goal of palliative care is to improve quality of life.

People at all stages of cancer treatment typically receive some kind of palliative care. In some cases, medical specialty professional organizations recommend that patients and physicians respond to cancer only with palliative care. This applies to patients who:

- Display low performance status, implying limited ability to care for themselves
- Received no benefit from prior evidence-based treatments
- Are not eligible to participate in any appropriate clinical trial

- No strong evidence implies that treatment would be effective

Palliative care may be confused with hospice and therefore only indicated when people approach end of life. Like hospice care, palliative care attempts to help the patient cope with their immediate needs and to increase comfort. Unlike hospice care, palliative care does not require people to stop treatment aimed at the cancer.

Multiple national medical guidelines recommend early palliative care for patients whose cancer has produced distressing symptoms or who need help coping with their illness. In patients first diagnosed with metastatic disease, palliative care may be immediately indicated. Palliative care is indicated for patients with a prognosis of less than 12 months of life even given aggressive treatment.

Immunotherapy

A variety of therapies using immunotherapy, stimulating or helping the immune system to fight cancer, have come into use since 1997. Approaches include:

- Monoclonal antibody therapy

- Checkpoint therapy (therapy that targets the immune checkpoints or regulators of the immune system)
- Adoptive cell transfer

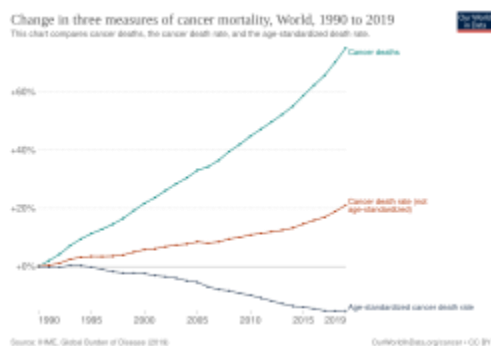
Laser therapy

Laser therapy uses high-intensity light to treat cancer by shrinking or destroying tumors or precancerous growths. Lasers are most commonly used to treat superficial cancers that are on the surface of the body or the lining of internal organs. It is used to treat basal cell skin cancer and the very early stages of others like cervical, penile, vaginal, vulvar, and non-small cell lung cancer. It is often combined with other treatments, such as surgery, chemotherapy, or radiation therapy. Laser-induced interstitial thermotherapy (LITT), or interstitial laser photocoagulation, uses lasers to treat some cancers using hyperthermia, which uses heat to shrink tumors by damaging or killing cancer cells. Lasers are more precise than surgery and cause less damage, pain, bleeding, swelling, and scarring. A disadvantage is surgeons must have specialized training. It may be more expensive than other treatments.

Alternative medicine

Complementary and alternative cancer treatments are a diverse group of therapies, practices and products that are not part of conventional medicine. "Complementary medicine" refers to methods and substances used along with conventional medicine, while "alternative medicine" refers to compounds used instead of conventional medicine. Most complementary and alternative medicines for cancer have not been studied or tested using conventional techniques such as clinical trials. Some alternative treatments have been investigated and shown to be ineffective but still continue to be marketed and promoted. Cancer researcher Andrew J. Vickers stated, "The label 'unproven' is inappropriate for such therapies; it is time to assert that many alternative cancer therapies have been 'disproven'."

Prognosis



Three measures of global cancer mortality from 1990 to 2017.

Survival rates vary by cancer type and by the stage at which it is diagnosed, ranging from majority survival to complete mortality five years after diagnosis. Once a cancer has metastasized, prognosis normally becomes much worse. About half of patients receiving treatment for invasive cancer (excluding carcinoma *in situ* and non-melanoma skin cancers) die from that cancer or its treatment. A majority of cancer deaths are due to metastases of the primary tumor.

Survival is worse in the developing world, partly because the types of cancer that are most common there are harder to treat than those associated with developed countries.

Those who survive cancer develop a second primary cancer at about twice the rate of those never diagnosed. The increased risk is believed to be due to the random chance of developing any cancer, the likelihood of surviving the first cancer, the same risk factors that produced the first cancer, unwanted side effects of treating the first cancer (particularly radiation therapy), and better compliance with screening.

Predicting short- or long-term survival depends on many factors. The most important are the cancer type and the patient's age and overall health. Those who are frail with other health problems

have lower survival rates than otherwise healthy people. Centenarians are unlikely to survive for five years even if treatment is successful. People who report a higher quality of life tend to survive longer. People with lower quality of life may be affected by depression and other complications and/or disease progression that both impairs quality and quantity of life. Additionally, patients with worse prognoses may be depressed or report poorer quality of life because they perceive that their condition is likely to be fatal.

People with cancer have an increased risk of blood clots in their veins which can be life-threatening. The use of blood thinners such as heparin decrease the risk of blood clots but have not been shown to increase survival in people with cancer. People who take blood thinners also have an increased risk of bleeding.

Although extremely rare, some forms of cancer, even from an advanced stage, can heal spontaneously. This phenomenon is known as the spontaneous remission.

Epidemiology

Age-standardized death rate from cancer per 10,000 people.

Estimates are that in 2018, 18.1 million new cases of cancer and 9.6 million deaths occur globally. About 20% of males and 17% of females will get cancer at some point in time while 13% of males and 9% of females will die from it.

In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers) and in 2010 nearly 7.98 million people died. Cancers account for approximately 16% of deaths. The most common as of 2018 are lung cancer (1.76 million deaths), colorectal cancer (860,000) stomach cancer (780,000), liver cancer (780,000), and breast cancer (620,000). This makes invasive cancer the leading cause of death in the developed world and the second leading in the developing world. Over half of cases occur in the developing world.

Deaths from cancer were 5.8 million in 1990. Deaths have been increasing primarily due to longer lifespans and lifestyle changes in the developing world. The most significant risk factor for developing cancer is age. Although it is possible for cancer to strike at any age, most patients with invasive cancer are over 65. According to cancer researcher Robert A. Weinberg, "If we lived long enough, sooner or later we all

would get cancer." Some of the association between aging and cancer is attributed to immunosenescence, errors accumulated in DNA over a lifetime and age-related changes in the endocrine system. Aging's effect on cancer is complicated by factors such as DNA damage and inflammation promoting it and factors such as vascular aging and endocrine changes inhibiting it.

Some slow-growing cancers are particularly common, but often are not fatal. Autopsy studies in Europe and Asia showed that up to 36% of people have undiagnosed and apparently harmless thyroid cancer at the time of their deaths and that 80% of men develop prostate cancer by age 80. As these cancers do not cause the patient's death, identifying them would have represented overdiagnosis rather than useful medical care.

The three most common childhood cancers are leukemia (34%), brain tumors (23%) and lymphomas (12%). In the United States cancer affects about 1 in 285 children. Rates of childhood cancer increased by 0.6% per year between 1975 and 2002 in the United States and by 1.1% per year between 1978 and 1997 in Europe. Death from childhood cancer decreased by half between 1975 and 2010 in the United States.

History



Engraving with two views of a Dutch woman who had a tumor removed from her neck in 1689

Cancer has existed for all of human history. The earliest written record regarding cancer is from c. 1600 BC in the Egyptian Edwin Smith Papyrus and describes breast cancer. Hippocrates (c. 460 BC – c. 370 BC) described several kinds of cancer, referring to them with the Greek word *καρκίνοϛ* *karkinos* (crab or crayfish). This name comes from the appearance of the cut surface of a solid malignant tumor, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name". Galen stated that "cancer of the breast is so called because of the fancied resemblance to a crab given by the lateral prolongations of the tumor and the adjacent distended veins". Celsus (c. 25 BC – 50 AD) translated *karkinos* into the Latin *cancer*, also meaning crab and recommended surgery

as treatment. Galen (2nd century AD) disagreed with the use of surgery and recommended purgatives instead. These recommendations largely stood for 1000 years.

In the 15th, 16th and 17th centuries, it became acceptable for doctors to dissect bodies to discover the cause of death. The German professor Wilhelm Fabry believed that breast cancer was caused by a milk clot in a mammary duct. The Dutch professor Francois de la Boe Sylvius, a follower of Descartes, believed that all disease was the outcome of chemical processes and that acidic lymph fluid was the cause of cancer. His contemporary Nicolaes Tulp believed that cancer was a poison that slowly spreads and concluded that it was contagious.

The physician John Hill described tobacco sniffing as the cause of nose cancer in 1761. This was followed by the report in 1775 by British surgeon Percivall Pott that chimney sweeps' carcinoma, a cancer of the scrotum, was a common disease among chimney sweeps. With the widespread use of the microscope in the 18th century, it was discovered that the 'cancer poison' spread from the primary tumor through the lymph nodes to other sites ("metastasis"). This view of the

disease was first formulated by the English surgeon Campbell De Morgan between 1871 and 1874.

Society and culture

Although many diseases (such as heart failure) may have a worse prognosis than most cases of cancer, cancer is the subject of widespread fear and taboos. The euphemism of "a long illness" to describe cancers leading to death is still commonly used in obituaries, rather than naming the disease explicitly, reflecting an apparent stigma. Cancer is also euphemised as "the C-word"; Macmillan Cancer Support uses the term to try to lessen the fear around the disease. In Nigeria, one local name for cancer translates into English as "the disease that cannot be cured". This deep belief that cancer is necessarily a difficult and usually deadly disease is reflected in the systems chosen by society to compile cancer statistics: the most common form of cancer—non-melanoma skin cancers, accounting for about one-third of cancer cases worldwide, but very few deaths are excluded from cancer statistics specifically because they are easily treated and almost always cured, often in a single, short, outpatient procedure.

Western conceptions of patients' rights for people with cancer include a duty to fully disclose the medical situation to the person, and the right to engage in shared decision-making in a way that respects the person's own values. In other cultures, other rights and values are preferred. For example, most African cultures value whole families rather than individualism. In parts of Africa, a diagnosis is commonly made so late that cure is not possible, and treatment, if available at all, would quickly bankrupt the family. As a result of these factors, African healthcare providers tend to let family members decide whether, when and how to disclose the diagnosis, and they tend to do so slowly and circuitously, as the person shows interest and an ability to cope with the grim news. People from Asian and South American countries also tend to prefer a slower, less candid approach to disclosure than is idealized in the United States and Western Europe, and they believe that sometimes it would be preferable not to be told about a cancer diagnosis. In general, disclosure of the diagnosis is more common than it was in the 20th century, but full disclosure of the prognosis is not offered to many patients around the world.

In the United States and some other cultures, cancer is regarded as a disease that must be "fought" to end the "civil insurrection"; a War on Cancer was declared in the US. Military metaphors are particularly common in descriptions of cancer's human effects, and they emphasize both the state of the patient's health and the need to take immediate, decisive actions himself rather than to delay, to ignore or to rely entirely on others. The military metaphors also help rationalize radical, destructive treatments. In the 1970s, a relatively popular alternative cancer treatment in the US was a specialized form of talk therapy, based on the idea that cancer was caused by a bad attitude. People with a "cancer personality"—depressed, repressed, self-loathing and afraid to express their emotions—were believed to have manifested cancer through subconscious desire. Some psychotherapists claimed that treatment to change the patient's outlook on life would cure the cancer. Among other effects, this belief allowed society to blame the victim for having caused the cancer (by "wanting" it) or having prevented its cure (by not becoming a sufficiently happy, fearless and loving person). It also increased patients' anxiety, as they incorrectly believed that natural emotions of sadness, anger or fear shorten their lives. The idea

was ridiculed by Susan Sontag, who published *Illness as Metaphor* while recovering from treatment for breast cancer in 1978. Although the original idea is now generally regarded as nonsense, the idea partly persists in a reduced form with a widespread, but incorrect, belief that deliberately cultivating a habit of positive thinking will increase survival. This notion is particularly strong in breast cancer culture.

One idea about why people with cancer are blamed or stigmatized, called the just-world fallacy, is that blaming cancer on the patient's actions or attitudes allows the blamers to regain a sense of control. This is based upon the blamers' belief that the world is fundamentally just and so any dangerous illness, like cancer, must be a type of punishment for bad choices, because in a just world, bad things would not happen to good people.

Economic effect

The total health care expenditure on cancer in the US was estimated to be \$80.2 billion in 2015. Even though cancer-related health care expenditure have increased in absolute terms during recent decades, the share of health expenditure devoted to cancer treatment has remained close to 5% between the 1960s and 2004. A similar pattern has been observed in Europe where

about 6% of all health care expenditure are spent on cancer treatment. In addition to health care expenditure and financial toxicity, cancer causes indirect costs in the form of productivity losses due to sick days, permanent incapacity and disability as well as premature death during working age. Cancer causes also costs for informal care. Indirect costs and informal care costs are typically estimated to exceed or equal the health care costs of cancer.^{[250][249]}

Workplace

In the United States, cancer is included as a protected condition by the Equal Employment Opportunity Commission (EEOC), mainly due to the potential for cancer having discriminating effects on workers. Discrimination in the workplace could occur if an employer holds a false belief that a person with cancer is not capable of doing a job properly, and may ask for more sick leave than other employees. Employers may also make hiring or firing decisions based on misconceptions about cancer disabilities, if present. The EEOC provides interview guidelines for employers, as well as lists of possible solutions for assessing and accommodating employees with cancer.

Effect on divorce

A study found women were around six times more likely to be divorced soon after a diagnosis of cancer compared to men. Rate of separation for cancer-survivors showed correlations with race, age, income, and comorbidities in a study. A review found a somewhat decreased divorce rate for most cancer types, and noted study heterogeneity and methodological weaknesses for many studies on effects of cancer on divorce.^[254]

Research



University of Florida Cancer Hospital

Because cancer is a class of diseases, it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases. Angiogenesis inhibitors were once incorrectly thought to have potential as a "silver bullet" treatment applicable to many types of

cancer. Angiogenesis inhibitors and other cancer therapeutics are used in combination to reduce cancer morbidity and mortality.

Experimental cancer treatments are studied in clinical trials to compare the proposed treatment to the best existing treatment. Treatments that succeeded in one cancer type can be tested against other types. Diagnostic tests are under development to better target the right therapies to the right patients, based on their individual biology.

Cancer research focuses on the following issues:

- Agents (e.g. viruses) and events (e.g. mutations) that cause or facilitate genetic changes in cells destined to become cancer.
- The precise nature of the genetic damage and the genes that are affected by it.
- The consequences of those genetic changes on the biology of the cell, both in generating the defining properties of a cancer cell and in facilitating additional genetic events that lead to further progression of the cancer.

The improved understanding of molecular biology and cellular biology due to cancer research has led to new treatments for

cancer since US President Richard Nixon declared the "War on Cancer" in 1971. Since then, the country has spent over \$200 billion on cancer research, including resources from public and private sectors. The cancer death rate (adjusting for size and age of the population) declined by five percent between 1950 and 2005.

Competition for financial resources appears to have suppressed the creativity, cooperation, risk-taking and original thinking required to make fundamental discoveries, unduly favoring low-risk research into small incremental advancements over riskier, more innovative research. Other consequences of competition appear to be many studies with dramatic claims whose results cannot be replicated and perverse incentives that encourage grantee institutions to grow without making sufficient investments in their own faculty and facilities.