LIVES IN MEDICINE

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Introduction[1]

Cultural history

We need to reform our educational systems, particularly the teaching of history. As it is taught today, history is a chronicle of power struggles and war, told from a biased national standpoint. We are taught that our own country is always heroic and in the right.

We urgently need to replace this indoctrination in chauvinism by a reformed view of history, where the slow development of human culture is described, giving credit to all who have contributed. When we teach history, it should not be about power struggles. It should be about how human culture was gradually built up over thousands of years by the patient work of millions of hands and minds. Our common global culture, the music, science, literature and art that all of us share, should be presented as a precious heritage - far too precious to be risked in a thermonuclear war.

Human nature has two sides: It has a dark side, to which nationalism and militarism appeal; but our species also has a genius for cooperation, which we can see in the growth of culture. Our modern civilization has been built up by means of a worldwide exchange of ideas and inventions. It is built on the achievements of many ancient cultures. China, Japan, India, Mesopotamia, Egypt, Greece, the Islamic world, Christian Europe, and the Jewish intellectual traditions all have contributed. Potatoes, corn, squash, vanilla, chocolate, chilli peppers, and quinine are gifts from the American Indians.

Culture is cooperative, not competitive!

Our modern civilization has been built on the achievements of all the peoples of the world throughout history. The true history of humanity is not the history of power struggles, conflicts, kings, dictators and empires. The true history of humanity is a history of ideas, inventions, progress, shared knowledge, shared culture and cooperation.

Our cultural heritage is not only immensely valuable; it is also so great that no individual comprehends all of it. We are all specialists, who un-

[1]This book makes use of articles and book chapters that I have previously written about the history of medicine, but most of the material is new

derstand only a tiny fragment of the enormous edifice. No scientist understands all of science. Perhaps Leonardo da Vinci could come close in his day, but today it is impossible. Nor do the vast majority people who use cell phones, personal computers and television sets every day understand in detail how they work. Our health is preserved by medicines, which are made by processes that most of us do not understand, and we travel to work in automobiles and buses that we would be completely unable to construct.

The sharing of scientific and technological knowledge is essential to modern civilization. The great power of science is derived from an enormous concentration of attention and resources on the understanding of a tiny fragment of nature. It would make no sense to proceed in this way if knowledge were not permanent, and if it were not shared by the entire world.

Science is not competitive. It is cooperative. It is a great monument built by many thousands of hands, each adding a stone to the cairn. This is true not only of scientific knowledge but also of every aspect of our culture, history, art and literature, as well as the skills that produce everyday objects upon which our lives depend. Civilization is not competitive. It is cooperative!

Lives in Medicine

With the aim of writing cultural history in mind, I have started to write a series of books about the lives of women and men who have contributed importantly to various fields. The completed books are:

- **Lives in Physics**
- **Lives in Economics**
- **Lives in Ecology**
- **Lives in the Peace Movement**

The present book, **Lives in Medicine**, is part of this series, and others are planned. I hope that they will make a small contribution to cultural history. The books may be freely downloaded and circulated from the following links:

http://eacpe.org/about-john-scales-avery/

https://wsimag.com/authors/716-john-scales-avery

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Chapter 1

HYPOCRATES AND GALEN

1.1 Medicine in ancient India

According to an article by Tejraj Aminabhavi in Asian Review[1], "Among India's many claims to fame is the ancient medical science known as Ayurveda (from the Sanskrit words ayur, or life, and veda, science). This is a healing method that relies on herbs as medicines for maintaining good health. The 5,000-year-old system of natural healing is originated in India's ancient Vedic culture. It was suppressed during the years of foreign occupation, but its medical practices have been enjoying a resurgence both in its native land and throughout the world.

"Early Greek medicine embraced many concepts originally described in classical Ayurvedic texts dating back thousands of years. Traditional Tibetan and Chinese medicine also have roots in Ayurveda. Over time, Ayurveda has become the science of life, encompassing body, mind and spirit. This body of knowledge is believed to have been originally delivered by God to sages and seers, who were yogis renowned in their insight, intuition and keen observation of human behavior. They handed down their knowledge to their disciples. An important goal of Ayurveda is to identify the ideal state of balance of a person and offer solutions using diet, herbs, music, massage treatments and meditation to restore the body's balance.

"The key concepts of Ayurvedic medicine are based on universal interconnections among people, their health, the universe, the body's constitution and life forces that are often compared to the "humors" of the ancient Greek system. Using these concepts, Ayurvedic physicians prescribe individualized treatments that include herbs, diet and exercise along with lifestyle recommendations. The majority of the Indian population today uses Ayurvedic medicine, combined with conventional Western medicine, a practice popular all over Southeast Asia as well."

[1] https://asia.nikkei.com/Business/Science/Ayurveda-the-ancient-Indian-medical-practice

1.2 Mesopotamian medicine

In medicine, the Mesopotamians believed that disease was a punishment inflicted by the gods on men, both for their crimes and for their errors and omissions in the performance of religious duties. They believed that the cure for disease involved magical and religious treatment, and the diseased person was thought to be morally tainted. However, in spite of this background of superstition, Mesopotamian medicine also contained some practical remedies. For example, the prescription for urinary retention was as follows: "Crush poppy seeds in beer and make the patient drink it. Grind some myrrh, mix it with oil and blow it into his urethra with a tube of bronze. Give the patient anemone crushed in alppanu-beer."

Until recently it was believed that the Mesopotamians had no idea of hygiene and preventive medicine. However, the following remarkable text was published recently. It is a letter, written by Zimri-Lim, King of Mari, who lived about 1780 B.C., to his wife Shibtu: "I have heard that Lady Nanname has been taken ill. She has many contacts with the people of the palace. She meets many ladies in her house. Now then, give severe orders that no one should drink in the cup where she drinks. No on should sit on the seat where she sits. No one should sleep in the bed where she sleeps. She should no longer meet many ladies in her house. This disease is contagious."

Mesopotamian treatments and prescriptions

The Encyclopedia of Ancient History states that: "Fees for services were on a sliding scale depending on one's social status. A doctor presiding over the birth of a noble was paid more than for a common birth. Prescriptions were on this same sliding scale and, whereas a doctor might be paid in gold for mixing a prescription for a prince, the payment for doing the same for a common person might be a bowl of soup or a clay cup. There is no evidence, however, that doctors refused to treat the poor and the same prescriptions were given, with the same ingredients, without regard for a patient's social status. Prescriptions were ground by the doctor, usually, in the presence of the patient, while some incantation was recited. A prescription from Babylon for an injury to the face reads: "If a man is sick with a blow on the cheek, pound together fir-turpentine, pine-turpentine, tamarisk, daisy, flour of Inninnu; mix in milk and beer in a small copper pan; spread on skin, bind on him, and he shall recover" (Te. Antiseptics were made from a mixture of alcohol, honey, and myrrh, and surgery was more advanced than in other regions of the time. Teall writes, "In the treatment of all wounds, there are three critical steps: washing, applying a plaster, and binding the wound". The Mesopotamians recognized that washing a wound with clean water, and making sure the doctor's hands were also clean, prevented infection and hastened healing. Hands and wounds were cleaned with a mixture of beer and hot water though, as Teall notes, "a liquid soap was already available". Teall continues: "While some aspects of ancient Mesopotamian wound dressing are completely lacking as seen through the lens of modern biomedical practices... others were surprisingly advanced, such as the washing and the preparation of poultices for wounds"

Figure 1.1: **An ancient Egyptian doctor and patient.**

1.3 Medicine in ancient Egypt

The Wikipedia article on Ancient Egyptian Medicine mentions several papyrus texts dealing with surgery and treatments for disease: "The Edwin Smith Papyrus is a textbook on surgery and details anatomical observations and the 'examination, diagnosis, treatment, and prognosis' of numerous ailments. It was probably written around 1600 BC, but is regarded as a copy of several earlier texts. Medical information in it dates from as early as 3000 BC. It is thus viewed as a learning manual. Treatments consisted of ointments made from animal, vegetable or fruit substances or minerals. There is evidence of oral surgery being performed as early as the 4th Dynasty (2900-2750 BC).

"The Ebers papyrus c. 1550 BC includes 877 prescriptions (as categorized by a modern editor) for a variety of ailments and illnesses, some of them involving magical remedies, for Egyptian beliefs regarding magic and medicine were often intertwined. It also contains documentation revealing awareness of tumors, along with instructions on tumor removal.

"The Kahun Gynecological Papyrus treats women's complaints, including problems with conception. Thirty four cases detailing diagnosis and treatment survive, some of them fragmentarily. Dating to 1800 BC, it is the oldest surviving medical text of any kind."

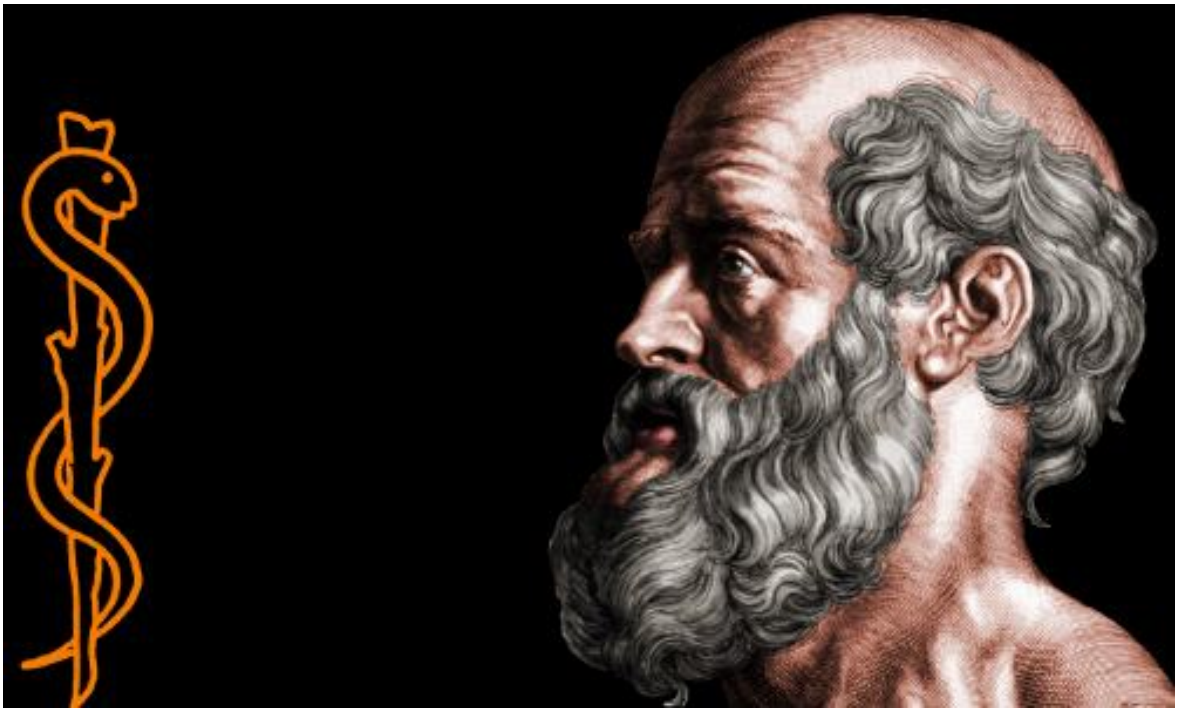

Figure 1.2: **Hipprocates (460 BC - c, 370 BC) has been called "the father of modern medicine".**

1.4 Hippocrates

The physician Hippocrates was born in about 460 B.C. on the island of Kos. His family belonged to the nobility, and for several generations they had been outstanding physicians. Hippocrates married a noblewoman, and they had two sons and a daughter. Both sons became physicians, and the daughter married a physician.

According to tradition, Hippocrates visited Egypt during the early part of his life. There he studied medicine, especially the medical works of Imhotep. He is also said to have studied under Democritus. Returning to the island of Kos, he founded the most rational school of medicine of the ancient world. He had many students, among whom were his sons and his sons-in law. During the later part of his life, he also taught and practiced in Thrace and Athens.

The medical school founded by Hippocrates was famous for its rationality and for its high ethical standard. The medical ethics of Hippocrates live on today in the oath taken by physicians. The rationality of Hippocrates is evident in all the writings of his school. For example, a book on epilepsy, called *The Sacred Disease*, contains the following passage:

"As for this disease called divine, surely it has its nature and causes, as have other diseases. It arises, like them, from things which enter and leave the body... Such things are divine or not - as you will, for the distinction matters not, and there is no need to make

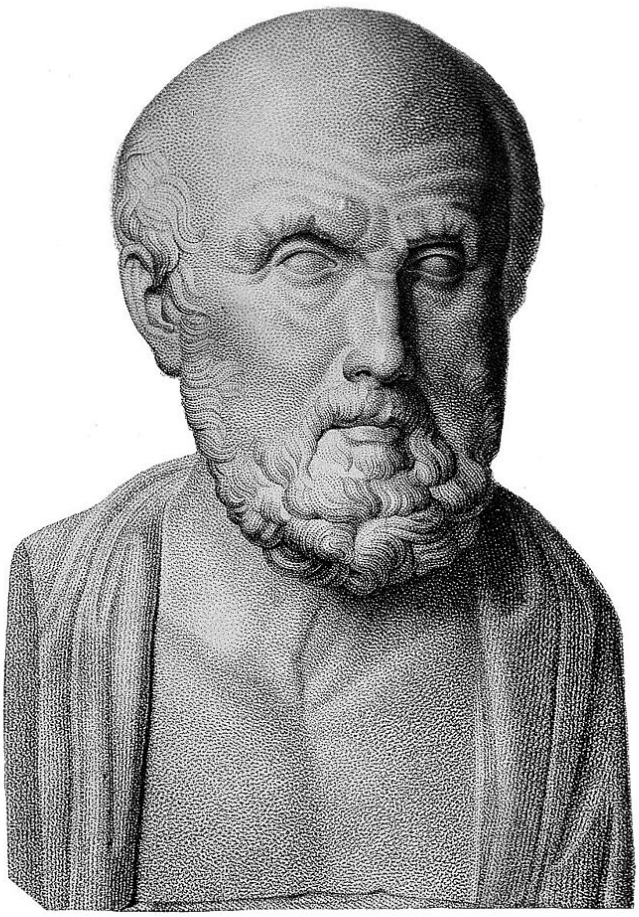

Figure 1.3: **A statue of Hippocrates.**

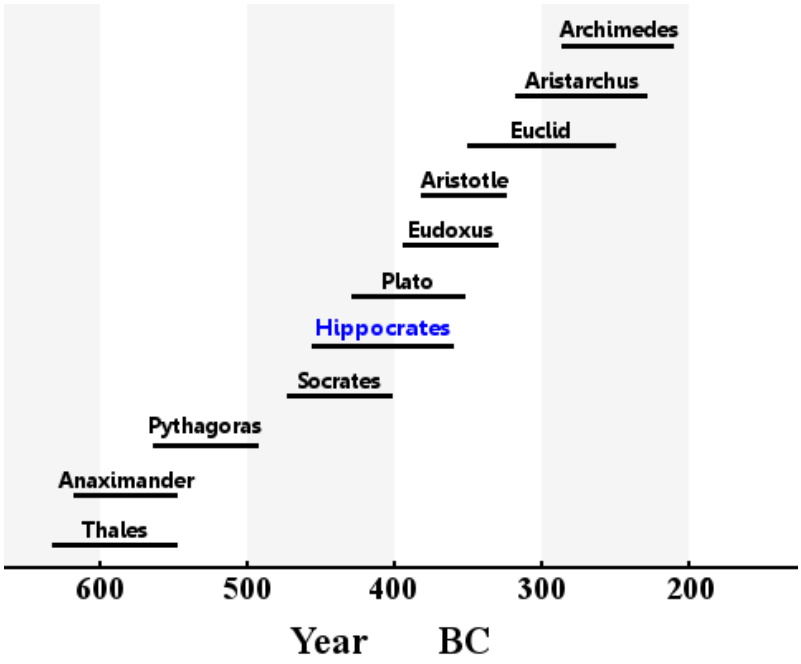

Figure 1.4: **Lifetimes of some ancient philosophers and scientists.**

such a division anywhere in nature; for all alike are divine, or all are natural. All have their antecedent causes, which can be found by those who seek them."

More than fifty books of Hippocrates' school were collected in Alexandria in the 3rd century B.C.. All of them were attributed by the Alexandrians to Hippocrates himself, but undoubtedly many of the books were written by his students. The physicians of the school of Hippocrates believed that cleanliness and rest are important for a sick or wounded patient, and that the physician should interfere as little as possible with the natural healing processes of the body. The books of the school contain much careful observation of disease. Hippocrates and his school resisted the temptation to theorize without a basis of carefully observed facts, just as they also resisted the temptation to introduce supernatural causes into medicine.

Hippocrates is said to have died in his hundredth year. According to tradition, he was humane, observant, learned, orderly and calm, with a grave and thoughtful attitude, a complete mastery of his own passions and a profound sympathy for the sufferings of his patients. We feel his influence today, both as one of the great founders of rational medicine, and as a pioneer of observation and inductive reasoning in science.

1.5 Galen

Aelius Galenus or Claudius Galenus (129 AD - c. 200 AD), whose name is commonly Anglicized as Galen, was born in Pergamon, in present-day Turkey. Pergamon was then a Greek city-state and a great cultural center, whose library rivaled the Great Library

of Alexandria. Because the Ptolemaic dynasty of pharaohs had forbidden the export of papyrus from Egypt, Pergamon developed a way of treating animal skins so that they could be used as written documents. The term of these skins *charta pergamena*, is the root of the English word "parchment".

Galen's father was a wealthy and highly cultured architect and builder. He originally gave his son an education in philosophy to prepare him for a career in politics. However, when Galen was 16, his father had a dream in which Asclepius, the Greek god of medicine, commanded him to make his son study medicine instead of philosophy. As the result of his father's dream, Galen continued to be given the best education available, but in a different field.

In AD 149. Galen's beloved father died, and he inherited a very large fortune. Now financially indepemdemt, Galen was able to follow the advice that Hippocrates had given to young physicians. Hippocrates advised them to travel widely and to study medical practice in many countries. Galen studied first in Smyrna under Pelops, then in Corinth, and finally in Alexandria, where he absorbed the knowledge of anatomy and physiology handed down by the 3rd century BC physicians, Herophilus and Eristratus, and the 1st century ad anatomist, Marinus.

In AD 157. after nine years of medical studies in foreign countries, Galen returned to Pergamon, where he spent three years as the surgeon of gladiators. He performed these duties so skillfully the the death rate of wounded gladiators dropped to a small fraction of what it had been.

The physician of Emperor Marcus Aurelius

In AD 161. Galen traveled to Rome, where he practiced medicine so successfully that he aroused the jealousy of local Roman physicians. Finally, fearing that he might be these jealous rivals, Galen returned to Pergamon. However, the Roman Emperor, Marcus Aurelius, had heard of his great skill as a physician, and commanded him to return to Rome. Galen then became the personal physician of Emperor Marcus Aurelius.

20 scribes, 400 books!

Since Galen was independently very wealthy, he could afford to employ scribes to take down his thoughts. He is said to have employed 20 of them! Whether or not this is an exaggeration, Galen certainly produces a huge volume of writing. an estimated 400 books, on philosophy and medicine, amounting to ten million words.

A few things that Galen said or wrote

My father taught me to despise the opinion and esteem of others and to seek only the truth . . . He insisted further that the primary end of personal possessions is to relieve hunger, thirst, and nakedness, and if more than sufficient remains it should be transmitted into good works.

Nature does nothing without a purpose. The physician is Nature's assistant.

A few more quotations from Galen

Employment is Nature's physician, and is essential to human happiness.

Confidence and hope do more good than physic.

It is impossible for anyone to find the correct function of a part unless he is perfectly acquainted with the action of the whole instrument

When I tell them this, and add that all voluntary movement is produced by muscles controlled by nerves coming from the brain, they call me a "teller of marvelous tales" . . . No one has ever been able to withstand me when I have demonstrated the muscles of respiration and voice. The muscles move certain organs, but they themselves require, in order to be moved, certain nerves from the brain, and if you intercept one of these with a ligature, immediately the muscle in which the nerve is inserted and the organ moved are rendered motionless.

After my twenty-eighth year from birth, having persuaded myself that there is a certain art of hygiene, I followed its precepts for my subsequent life, so I was no longer sick with any disease except an occasional fever.

To me it seems that those who through ambition or zeal have chosen some form of life so involved in affairs of business that they can have little leisure for the care of their bodies, are also willing slaves to hard masters. So that for these it is impossible to prescribe absolutely perfect care of the body. But whoever is completely free, both by fortune and by choice, for him it is possible to suggest how he may enjoy the most health, suffer the least sickness, and grow old most comfortably.

Figure 1.5: **A statue of the Greek god of healing, Asclepius. Although Galen believed Asclepius came to his aid, he also came to believe there was only one God. This made the later Christian and Muslim worlds much more receptive to his work.**

Figure 1.6: **Roman surgical instruments.**

Figure 1.7: **Galen dissecting a monkey, as imagined by Veloso Salgado in 1906**

Figure 1.8: **An 18th century portrait of Galen by Paul Busch**

Suggestions for further reading

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Chapter 2

THE BUKHT-YISHU FAMILY

2.1 The Hellenistic era

Alexandria

Nowhere was the cosmopolitan character of the Hellenistic Era more apparent than at Alexandria in Egypt. No city in history has ever boasted a greater variety of people. Ideally located at the crossroads of world trading routes, Alexandria became the capital of the world - not the political capital, but the cultural and intellectual capital.

Miletus in its prime had a population of 25,000; Athens in the age of Pericles had about 100,000 people; but Alexandria was the first city in history to reach a population of over a million!

Strangers arriving in Alexandria were impressed by the marvels of the city - machines which sprinkled holy water automatically when a five-drachma coin was inserted, water-driven organs, guns powered by compressed air, and even moving statues, powered by water or steam!

The Great Library

For scholars, the chief marvels of Alexandria were the great library and the Museum established by Ptolemy I. Credit for making Alexandria the intellectual capital of the world must go to Ptolemy I and his successors (all of whom were named Ptolemy except the last of the line, the famous queen, Cleopatra). Realizing the importance of the schools which had been founded by Pythagoras, Plato and Aristotle, Ptolemy I established a school at Alexandria. This school was called the Museum, because it was dedicated to the muses.

Near to the Museum, Ptolemy built a great library for the preservation of important manuscripts. The collection of manuscripts which Aristotle had built up at the Lyceum in Athens became the nucleus of this great library. The library at Alexandria was open to the general public, and at its height it was said to contain 750,000 volumes. Besides preserving important manuscripts, the library became a center for copying and distributing books.

Figure 2.1: **Burning of the Great Library at Alexandria.**

The material which the Alexandrian scribes used for making books was papyrus, which was relatively inexpensive. The Ptolemys were anxious that Egypt should keep its near-monopoly on book production, and they refused to permit the export of papyrus. Pergamum, a rival Hellenistic city in Asia Minor, also boasted a library, second in size only to the great library at Alexandria. The scribes at Pergamum, unable to obtain papyrus from Egypt, tried to improve the preparation of the skins traditionally used for writing in Asia. The resulting material was called *membranum pergamentum*, and in English, this name has become "parchment".

2.2 Burning of the Great Library of Alexandria

According to one account, the Great Library at Alexandria was partially destroyed in 48 BC, when Julius Caesar was pursuing his rival Ptolemy into Egypt. Caesar was suddenly cut off by the Egyptian fleet at Alexandria. Greatly outnumbered, Caesar ordered the ships in the harbor to be set on fire. The fire destroyed the Egyptian fleet, as Caesar had intended, but it also destroyed the part of the city where the Great Library stood.

2.3 The Nestorians and Islam

After the burning of the Great Library at Alexandria and the destruction of Hellenistic civilization, most of the books of the classical Greek and Hellenistic philosophers were lost. However, a few of these books survived and were translated from Greek, first into Syriac, then into Arabic and finally from Arabic into Latin. By this roundabout route, fragments from the wreck of the classical Greek and Hellenistic civilizations drifted back into the consciousness of the west.

We mentioned that the Roman empire was ended in the 5th century A.D. by attacks of barbaric Germanic tribes from northern Europe. However, by that time, the Roman empire had split into two halves. The eastern half, with its capital at Byzantium (Constantinople), survived until 1453, when the last emperor was killed vainly defending the walls of his city against the Turks.

The Byzantine empire included many Syriac-speaking subjects; and in fact, beginning in the 3rd century A.D., Syriac replaced Greek as the major language of western Asia. In the 5th century A.D., there was a split in the Christian church of Byzantium;and the Nestorian church, separated from the official Byzantine church. The Nestorians were bitterly persecuted by the Byzantines, and therefore they migrated, first to Mesopotamia, and later to south-west Persia. (Some Nestorians migrated as far as China.)

During the early part of the middle ages, the Nestorian capital at Gondisapur was a great center of intellectual activity. The works of Plato, Aristotle, Hippocrates, Euclid, Archimedes, Ptolemy, Hero and Galen were translated into Syriac by Nestorian scholars, who had brought these books with them from Byzantium.

Among the most distinguished of the Nestorian translators were the members of a family called Bukht-Yishu (meaning "Jesus hath delivered"), which produced seven generations of outstanding scholars. Members of this family were fluent not only in Greek and Syriac, but also in Arabic and Persian.

In the 7th century A.D., the Islamic religion suddenly emerged as a conquering and proselytizing force. Inspired by the teachings of Mohammad (570 A.D. - 632 A.D.), the Arabs and their converts rapidly conquered western Asia, northern Africa, and Spain. During the initial stages of the conquest, the Islamic religion inspired a fanaticism in its followers which was often hostile to learning. However, this initial fanaticism quickly changed to an appreciation of the ancient cultures of the conquered territories; and during the middle ages, the Islamic world reached a very high level of culture and civilization.

Thus, while the century from 750 to 850 was primarily a period of translation from Greek to Syriac, the century from 850 to 950 was a period of translation from Syriac to Arabic. It was during this latter century that Yuhanna Ibn Masawiah (a member of the Bukht-Yishu family, and medical advisor to Caliph Harun al-Rashid) produced many important translations into Arabic.

The skill of the physicians of the Bukht-Yishu family convinced the Caliphs of the value of Greek learning; and in this way the family played an extremely important role in the preservation of the western cultural heritage. Caliph al-Mamun, the son of Harun al-Rashid, established at Baghdad a library and a school for translation, and soon Baghdad

Figure 2.2: **Ibn Bakhtishu's Manafi' al-Hayawan, dated 12th century. Captions appear in Persian language.**

replaced Gondisapur as a center of learning.

The English word "chemistry" is derived from the Arabic words *"al-chimia"*, which mean "the changing". The earliest alchemical writer in Arabic was Jabir (760-815), a friend of Harun al-Rashid. Much of his writing deals with the occult, but mixed with this is a certain amount of real chemical knowledge. For example, in his *Book of Properties*, Jabir gives the following recipe for making what we now call lead hydroxycarbonate (white lead), which is used in painting and pottery glazes:

"Take a pound of litharge, powder it well and heat it gently with four pounds of vinegar until the latter is reduced to half its original volume. The take a pound of soda and heat it with four pounds of fresh water until the volume of the latter is halved. Filter the two solutions until they are quite clear, and then gradually add the solution of soda to that of the litharge. A white substance is formed, which settles to the bottom. Pour off the supernatant water, and leave the residue to dry. It will become a salt as white as snow."

Another important alchemical writer was Rahzes (c. 860 - c. 950). He was born in the ancient city of Ray, near Teheran, and his name means "the man from Ray". Rhazes studied medicine in Baghdad, and he became chief physician at the hospital there. He wrote the first accurate descriptions of smallpox and measles, and his medical writings include methods for setting broken bones with casts made from plaster of Paris. Rahzes was the first person to classify substances into vegetable, animal and mineral. The word *"al-kali"*, which appears in his writings, means "the calcined" in Arabic. It is the source of our word "alkali", as well as of the symbol K for potassium.

Figure 2.3: **A folio of the earliest manuscript of the Kitáb na't al-hayawán, attributed to ibn Bukhtishu, depicting Aristotle.**

Figure 2.4: **A painting of Caliph Harun al-Rashid.**

Suggestions for further reading

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Chapter 3

AVICENNA

3.1 The Persian Leonardo

The greatest physician of the middle ages, Avicenna, (Abu-Ali al Hussain Ibn Abdullah Ibn Sina, 980-1037), was a Persian. More than a hundred books are attributed to him. They were translated into Latin in the 12th century, and they were among the most important medical books used in Europe until the time of Harvey. Avicenna also wrote on alchemy, and he is important for having denied the possibility of transmutation of elements.

Avicenna was born in present-day Uzbekistan, which was then a part of the Samanian Empire, a Persian empire that ruled the region between 819 and 999 AD. His father was a respected scholar from Afghanistan. By the age of 10, Avicenna had memorized the entire Koran. With the help of a merchant from India, he taught himself the Indian form of mathematics. At the age of 16, he turned his attention to medicine and not only learned medical theory, but also attended the sick without payment and discovered many new methods and treatments. Avicina's later writings included an incredible range of topics - philosophy, medicine, astronomy, alchemy, geography. geology, psychology, Islamic theology, logic, mathematics, physics and works of poetry.

3.2 The Canon of Medicine

In 1025, Avicenna completed his masterpiece, *The Cannon of Medicine*, a five-volume encyclopedia of all the medical knowledge that the world possessed at that time. It included many of his own contributions. Translations reached the west, and it became the standard medical text both in Europe and in the Islamic world during the Middle Ages. Both *The Canon of Medicine* and Avicenna's other medical work, *The Art of Healing*, continued to be used as late as the 17th century. Because of his enormous influence, Avicena jas neen called "the father of modern medicine".

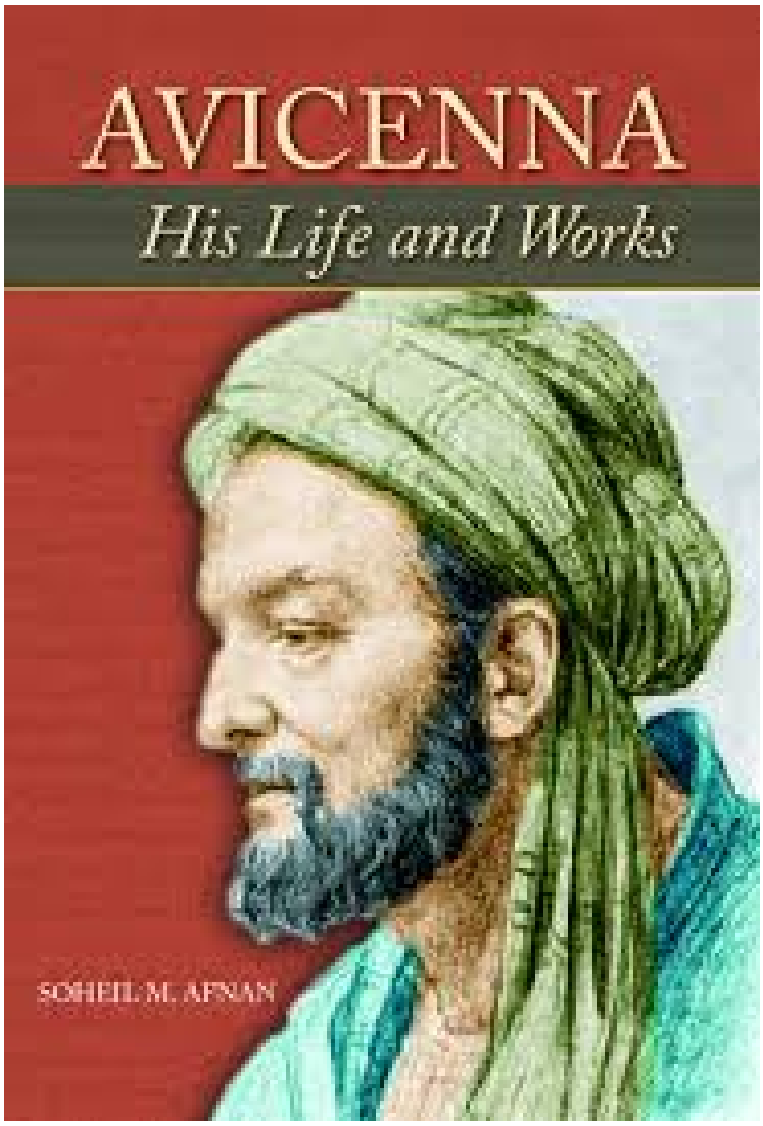

Figure 3.1: **Abu-Ali al Hussain Ibn Abdullah Ibn Sina, (980-1037), known in the west as Avicenna, was a universal genius. He is believed to have written 450 works, on philosophy, medicine, astronomy, alchemy, geography. geology, psychology, Islamic theology, logic, mathematics, physics and works of poetry. Of these, 150 have survived, including his books *The Book of Healing* and *The Cannon of Medicine*. These medical books were standard texts in Europe during the Middle Ages, and were even in use as late as the 17th century. Avicenna has been called "The father of modern medicine".**

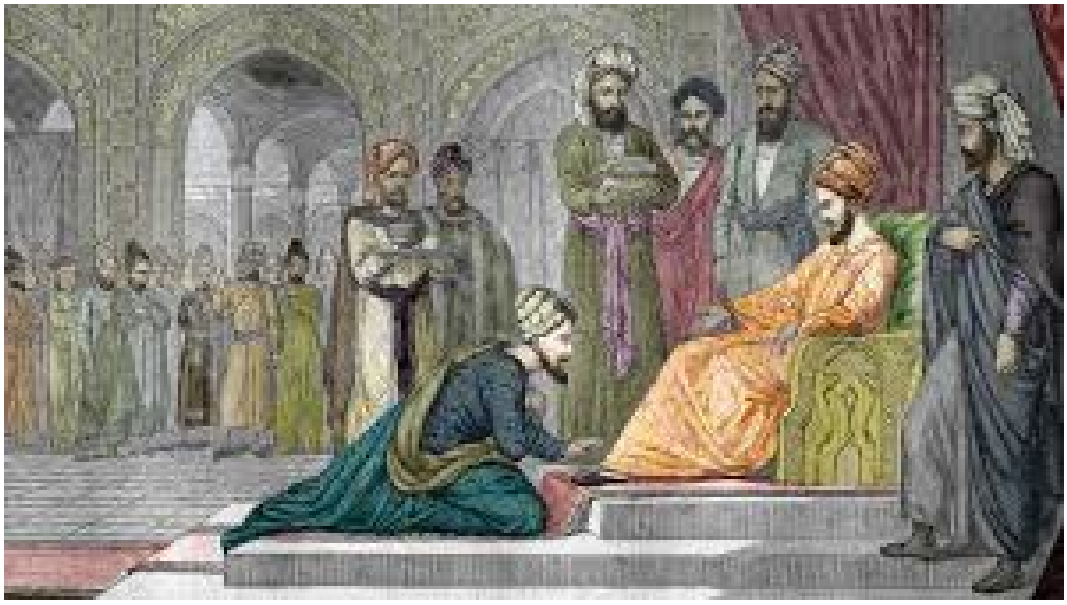

Figure 3.2: **Avicenna sought the patronage of rulers.**

Figure 3.3: **Avicenna writing one of his many books.**

Figure 3.4: **A monument to Avicenna in Qakh city, Azerbaijan.**

Figure 3.5: **Image of Avicenna on the Tajikistani somoni.**

Figure 3.6: **Avicenna statue in Milad Tower, Tehran, Iran.**

Figure 3.7: **The statue of Avicenna in United Nations Office in Vienna.**

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Chapter 4

LEONARDO'S ANATOMICAL STUDIES

4.1 East-west contacts during the Renaissance

Towards the end of the middle ages, Europe began to be influenced by the advanced Islamic civilization. European scholars were anxious to learn, but there was an "iron curtain" of religious intolerance which made travel in the Islamic countries difficult and dangerous for Christians. However, in the 12th century, parts of Spain, including the city of Toledo, were reconquered by the Christians. Toledo had been an Islamic cultural center, and many Moslem scholars, together with their manuscripts, remained in the city when it passed into the hands of the Christians. Thus Toledo became a center for the exchange of ideas between east and west; and it was in this city that many of the books of the classical Greek and Hellenistic philosophers were translated from Arabic into Latin.

In the 12th century, the translation was confined to books of science and philosophy. Classical Greek literature was forbidden by both the Christian and Moslem religions; and the beautiful poems and dramas of Homer, Sophocles and Euripides were not translated into Latin until the time of the Renaissance Humanists.

During the Mongol period (1279-1328), direct contact between Europe and China was possible because the Mongols controlled the entire route across central Asia; and during this period Europe received from China three revolutionary inventions: printing, gunpowder and the magnetic compass.

Another bridge between east and west was established by the crusades. In 1099, taking advantage of political divisions in the Moslem world, the Christians conquered Jerusalem and Palestine, which they held until 1187. This was the first of a series of crusades, the last of which took place in 1270. European armies, returning from the Middle East, brought with them a taste for the luxurious spices, textiles, jewelry, leatherwork and fine steel weapons of the orient; and their control of the Mediterranean sea routes made trade with the east both safe and profitable. Most of the profit from this trade went to a few cities, particularly to Venice and Florence.

At the height of its glory as a trading power, the Venetian Republic maintained six fleets of nationally owned ships, which could be chartered by private enterprise. All the ships of this fleet were of identical construction and rigged with identical components, so that parts could be replaced with ease at depots of the Venetian consular service abroad. The ships of these fleets could either serve as merchant ships, or be converted into warships by the addition of guns. Protected by a guard of such warships, large convoys of Venetian merchant ships could sail without fear of plunder by pirates.

In 1420, at the time of Venice's greatest commercial expansion, the doge, Tommaso Mocenigo, estimated the annual turnover of Venetian commerce to be ten million ducats, of which two million was profit. With this enormous income to spend, the Venetians built a city of splendid palaces, which rose like a shimmering vision above the waters of the lagoon.

The Venetians were passionately fond of pleasure, pageantry and art. The cross-shaped church of Saint Mark rang with the music of great composers, such as Gabrieli and Palestrina; and elegant triumphal music accompanied the doge as he went each year to throw a golden ring into the waters of the lagoon, an act which symbolized the marriage of Venice to the sea.

Like the Athenians after their victory in the Persian war, the Venetians were both rich and confident. Their enormous wealth allowed them to sponsor music, art, literature and science. The painters Titian, Veronese, Giorgione and Tintoretto, the sculptor Verrocchio and the architect Palladio all worked in Venice at the height of the city's prosperity.

The self-confidence of the Venetians produced a degree of intellectual freedom which was not found elsewhere in Europe at that time, except in Florence. At the University of Padua, which was supported by Venetian funds, students from all countries were allowed to study regardless of their religious beliefs. It was at Padua that Copernicus studied, and there Andreas Vesalius began the research which led to his great book on anatomy. At one point in his career, Galileo also worked at the University of Padua.

The prosperity of 15th century Florence, like that of Venice, was based on commerce. In the case of Florence, the trade was not by sea, but along the main north-south road of Italy, which crossed the Arno at Florence. In addition to this trade, Florence also had an important textile industry. The Florentines imported wool from France, Flanders, Holland and England. They wove the wool into cloth and dyed it, using superior techniques, many of which had come to them from India by way of the Islamic civilization. Later, silk weaving (again using eastern techniques) became important. Florentine banking was also highly developed, and our present banking system is derived from Florentine commercial practices.

Humanism

In the 15th and 16th centuries, Florence was ruled by a syndicate of wealthy merchant families, the greatest of whom were the Medicis. Cosimo de' Medici, the unofficial ruler of Florence from 1429 to 1464, was a banker whose personal wealth exceeded that of most

contemporary kings. In spite of his great fortune, Cosimo lived in a relatively modest style, not wishing to attract attention or envy; and in general, the Medici influence tended to make life in Florence more modest than life in Venice.

Cosimo de' Medici is important in the history of ideas as one of the greatest patrons of the revival of Greek learning. In 1439, the Greek Patriarch and the Emperor John Palaeologus attended in Florence a council for the reunification of the Greek and Latin churches. The Greek-speaking Byzantine scholars who accompanied the Patriarch brought with them a number of books by Plato which excited the intense interest and admiration of Cosimo de' Medici.

Cosimo immediately set up a Platonic Academy in Florence, and chose a young man named Marsilio Ficino as its director. In one of his letters to Ficino, Cosimo says:

"Yesterday I came to the villa of Careggi, not to cultivate my fields, but my soul. Come to us, Marsilio, as soon as possible. Bring with you our Plato's book *De Summo Bono*. This, I suppose, you have already translated from the Greek language into Latin, as you promised. I desire nothing so much as to know the road to happiness. Farewell, and do not come without the Orphian lyre!"

Cosimo's grandson, Lorenzo the Magnificent, continued his grandfather's policy of reviving classical Greek learning, and he became to the golden age of Florence what Pericles had been too the golden age of Athens. Among the artists whom Lorenzo sponsored were Michelangelo, Botticelli and Donatello. Lorenzo established a system of bursaries and prizes for the support of students. He also gave heavy financial support to the University of Pisa, which became a famous university under Lorenzo's patronage. (It was later to be the university of Galileo and Fermi.)

At Florence, Greek was taught by scholars from Byzantium; and Poliziano, who translated Homer into Latin could say with justice: "Greek learning, long extinct in Greece itself, has come to life and lives again in Florence. There Greek literature is taught and studied, so that Athens, root and branch, has been transported to make her abode - not in Athens in ruins and in the hands of barbarians, but in Athens as she was, with her breathing spirit and her very soul."

4.2 Leonardo da Vinci

Against this background, it may seem strange that Lorenzo the Magnificent did not form a closer relationship with Leonardo da Vinci, the most talented student of Verrocchio's school in Florence. One might have expected a close friendship between the two men, since Lorenzo, only four years older than Leonardo, was always quick to recognize exceptional ability.

The explanation probably lies in Leonardo's pride and sensitivity, and in the fact that, while both men were dedicated to knowledge, they represented different points of view. Lorenzo was full of enthusiasm for the revival of classical learning, while Leonardo had already taken the next step: Rejecting all blind obedience to authority, including the authority of the ancients, he relied on his own observations. Lorenzo was fluent in Latin

and Greek, and was widely educated in Greek philosophy, while Leonardo was ignorant of both languages and was largely self-taught in philosophy and science (although he had studied mathematics at the school of Benedetto d'Abacco).

While he did not form a close friendship with Lorenzo the Magnificent, Leonardo was lucky in becoming the friend and protegé of the distinguished Florentine mathematician, physician, geographer and astronomer, Paolo Toscanelli, who was also the friend and advisor of Columbus. (Toscanelli furnished Columbus with maps of the world and encouraged him in his project of trying to reach India and China by sailing westward. Toscanelli's maps mistakenly showed the Atlantic Ocean with Europe on one side, and Asia on the other!)

Gradually, under Toscanelli's influence, young Leonardo's powerful and original mind was drawn away from the purely representational aspects of art, and he became more and more involved in trying to understand the underlying structure and mechanism of the things which he observed in nature - the bodies of men and animals, the flight of birds, the flow of fluids and the features of the earth.

Both in painting and in science, Leonardo looked directly to nature for guidance, rather than to previous masters. He wrote:

"The painter will produce pictures of small merit if he takes as his standard the pictures of others; but if he will study from natural objects, he will produce good fruits... And I would say about these mathematical studies, that those who study the authorities and not the works of nature are descendents but not sons of nature."

In another place, Leonardo wrote:

"But first I will test with experiment before I proceed further, because my intention is to consult experience first, and then with reasoning to show why such experience is bound to operate in such a way. And that is the true rule by which those who analyze the effects of nature must proceed; and although nature begins with the cause and ends with the experience, we must follow the opposite course, namely (as I said before) begin with the experience and by means of it investigate the cause."

Lorenzo the Magnificent finally did help Leonardo in a backhanded way: In 1481, when Leonardo was 29 years old, Lorenzo sent him as an emissary with a gift to the Duke of Milan, Ludovico Sforza. Although Milan was far less culturally developed than Florence, Leonardo stayed there for eighteen years under the patronage of Sforza. He seemed to work better in isolation, without the competition and criticism of the Florentine intellectuals.

In Milan, Leonardo began a series of anatomical studies which he developed into a book, intended for publication. Leonardo's anatomical drawings make previous work in this field seem like the work of children, and in many respects his studies were not surpassed for hundreds of years. Some of his anatomical drawings were published in a book by Fra Pacioli, and they were very influential; but most of the thousands of pages of notes which Leonardo wrote have only been published in recent years.

The notebooks of Leonardo da Vinci cover an astonishing range of topics: mathematics, physics, astronomy, optics, engineering, architecture, city planing, geology, hydrodynamics and aerodynamics, anatomy, painting and perspective, in addition to purely literary works. He was particularly interested in the problem of flight, and he made many studies of the

flight of birds and bats in order to design a flying machine. Among his notes are designs for a helicopter and a parachute, as well as for a propeller-driven flying machine.

In astronomy, Leonardo knew that the earth rotates about its axis once every day, and he understood the physical law of inertia which makes this motion imperceptible to us except through the apparent motion of the stars. In one of his notebooks, Leonardo wrote: "The sun does not move." However, he did not publish his ideas concerning astronomy. Leonardo was always planning to organize and publish his notes, but he was so busy with his many projects that he never finished the task. At one point, he wrote what sounds like a cry of despair: "Tell me, tell me if anything ever was finished!"

Leonardo ended his life in the court of the king of France, Francis I. The king gave him a charming chateau in which to live, and treated him with great respect. Francis I visited Leonardo frequently in order to discuss philosophy, science and art; and when Leonardo died, the king is said to have wept openly.

Leonardo's anatomical drawings

Leonardo's interest in anatomy began when he was a student in Verrocio's workshop. It is not known exactly when he began dissecting the human body, but a likely guess is that it was shortly after his move to Milan. He began this research in order to aid his artistic representation of the human body, but as the work progressed, his main interest shifted to determining the mechanisms by which the human body performed its functions.

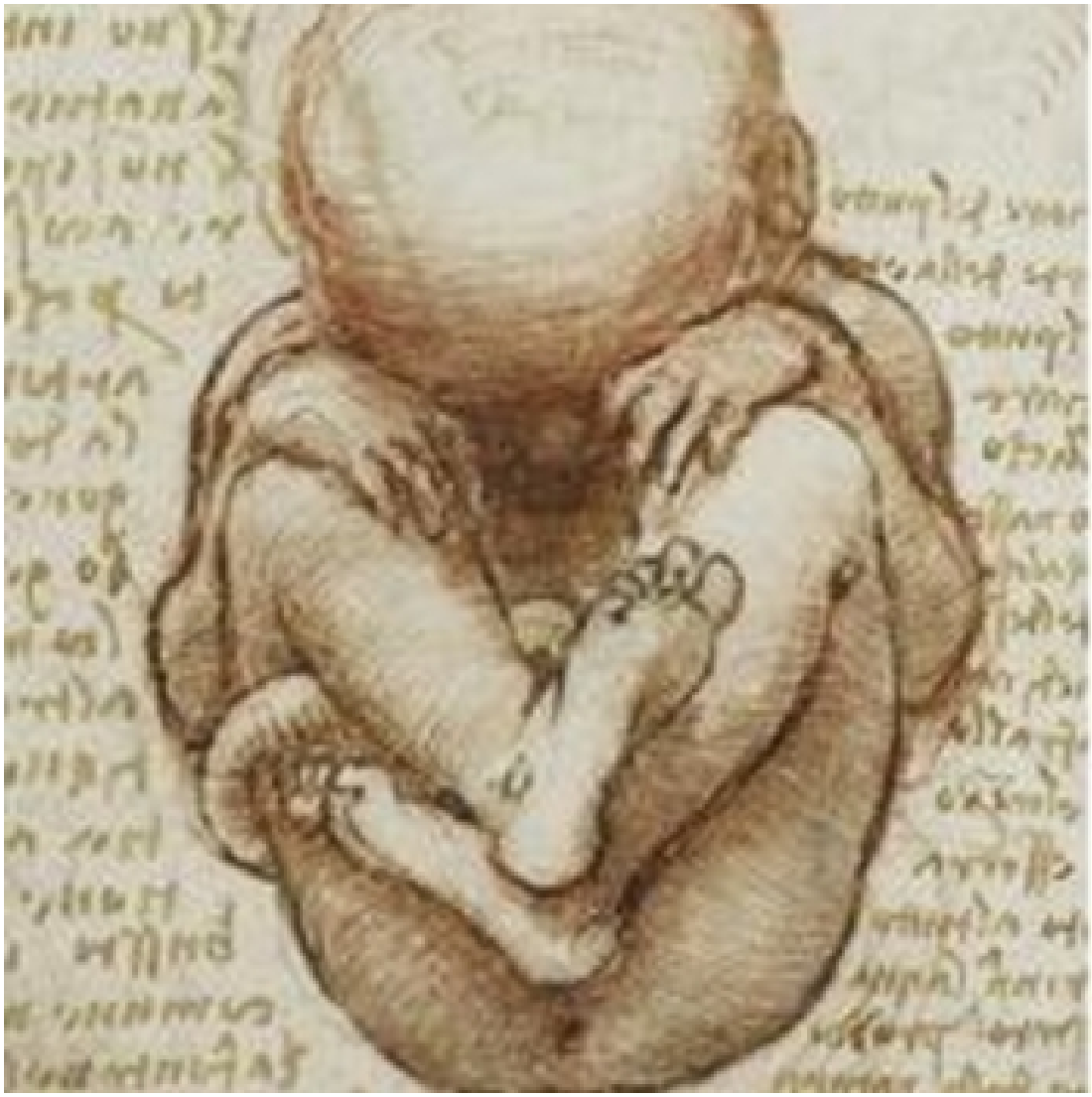
During the next twenty years, he did practical work in anatomy on the dissection table in Milan, then at hospitals in Florence and Rome, and in Pavia, where he collaborated with the physician-anatomist Marcantonio della Torre. By his own count Leonardo dissected 30 corpses in his lifetime.

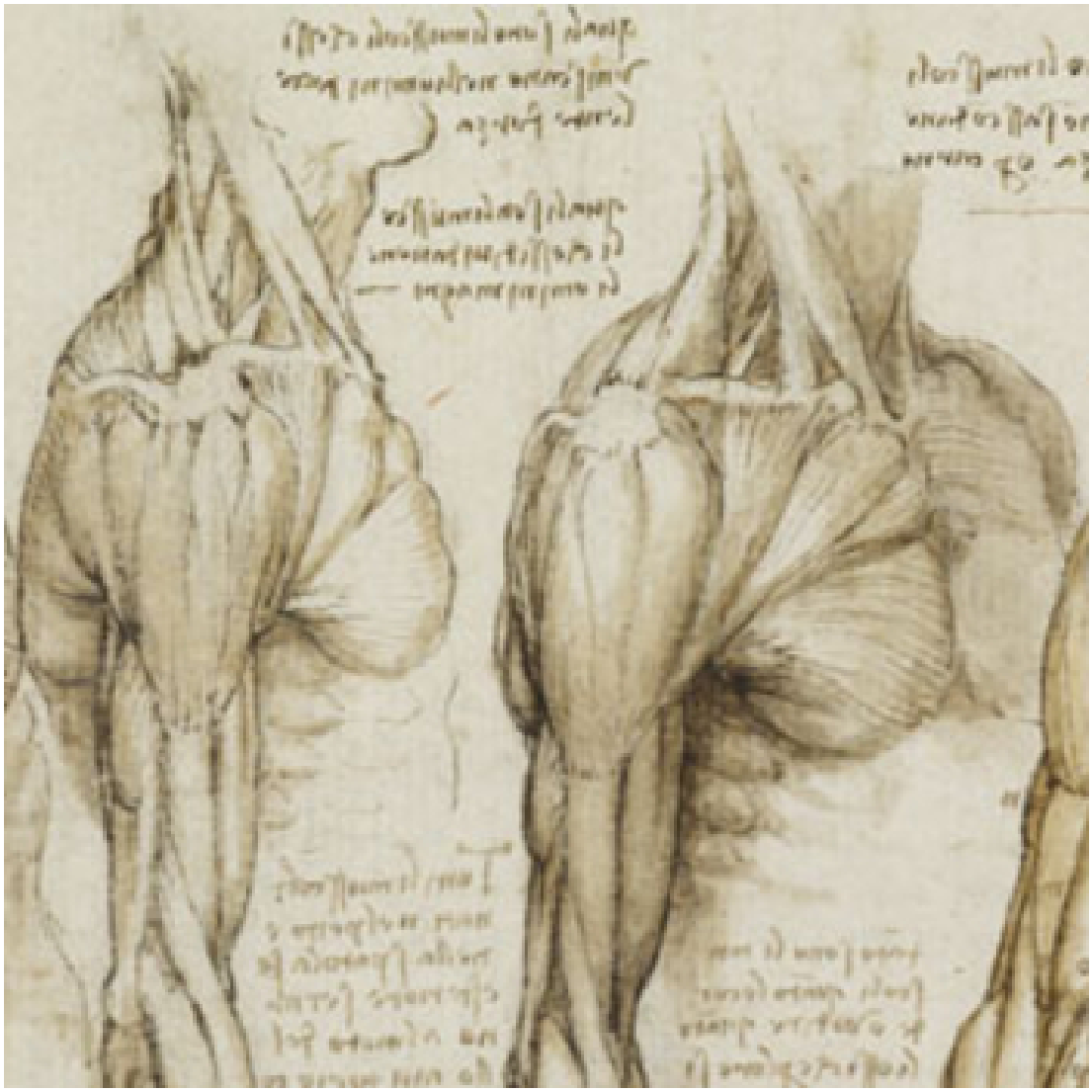
Leonardo intended to publish his drawings in a treatise on anatomy- If he had done so his discoveries would have transformed European knowledge of the subject. But when he died in 1519, the drawings remained a mass of undigested material among his private papers and their significance was lost to the world for almost 400 years.

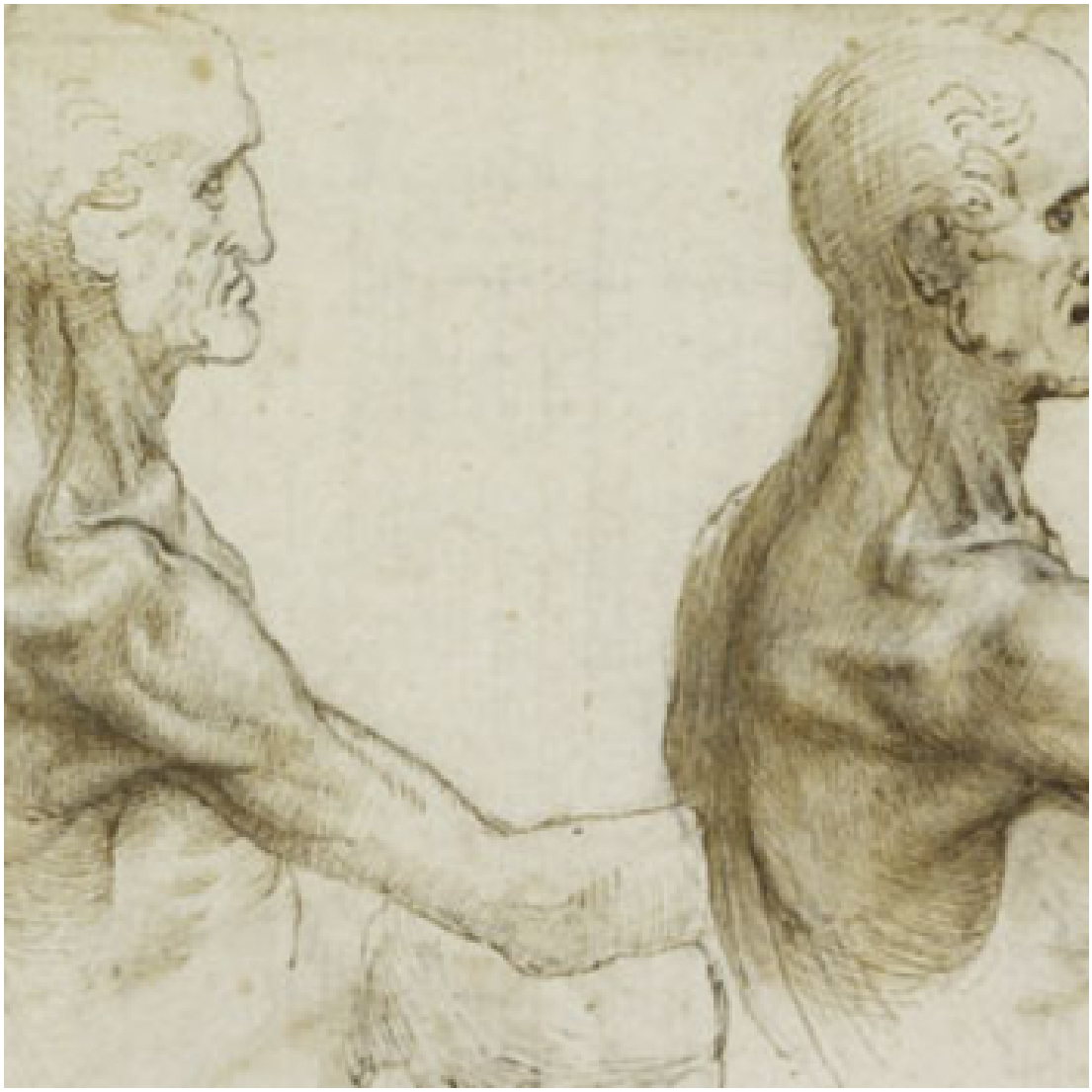
Paper and printing in the Renaissance

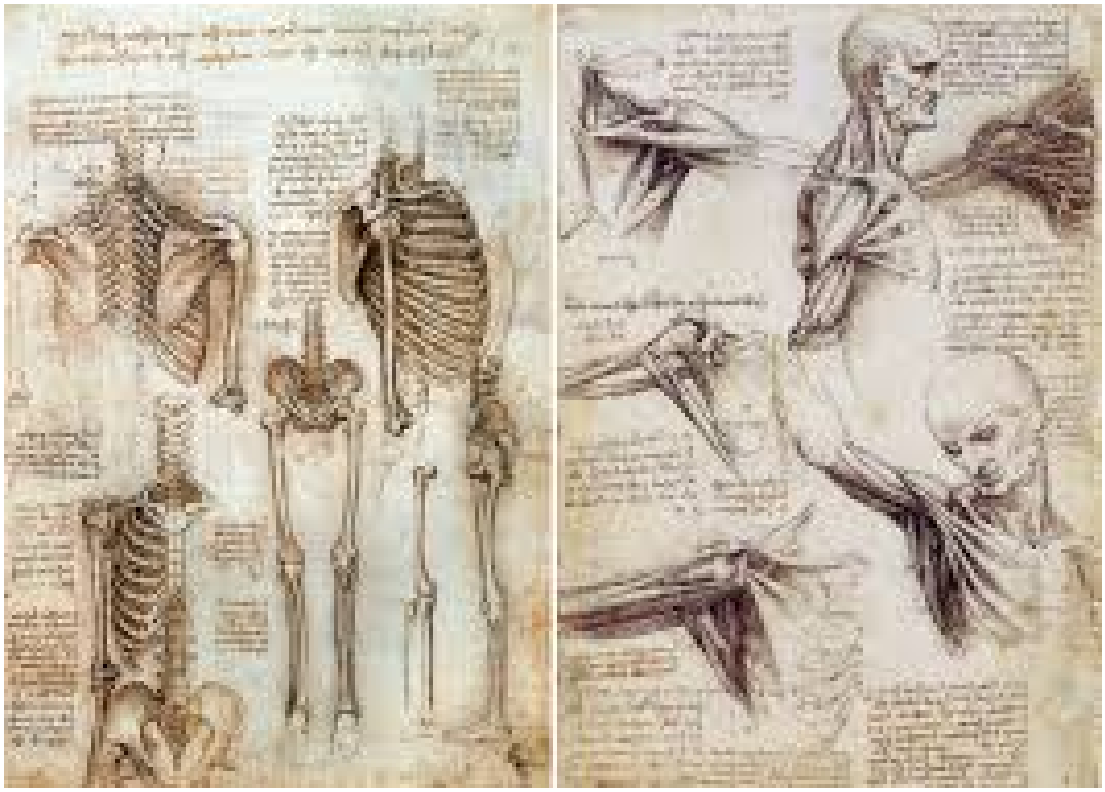
The career of Leonardo da Vinci illustrates the first phase of the "information explosion" which has produced the modern world: Inexpensive paper was being manufactured in Europe, and it formed the medium for Leonardo's thousands of pages of notes. His notes and sketches would never have been possible if he had been forced to use expensive parchment as a medium. On the other hand, the full force of Leonardo's genius and diligence was never felt because his notes were not printed.

Copernicus, who was a younger contemporary of Leonardo, had a much greater effect on the history of ideas, because his work was published. Thus, while paper alone made a large contribution to the information explosion, it was printing combined with paper which had an absolutely decisive and revolutionary impact: The modern scientific era began with the introduction of printing.









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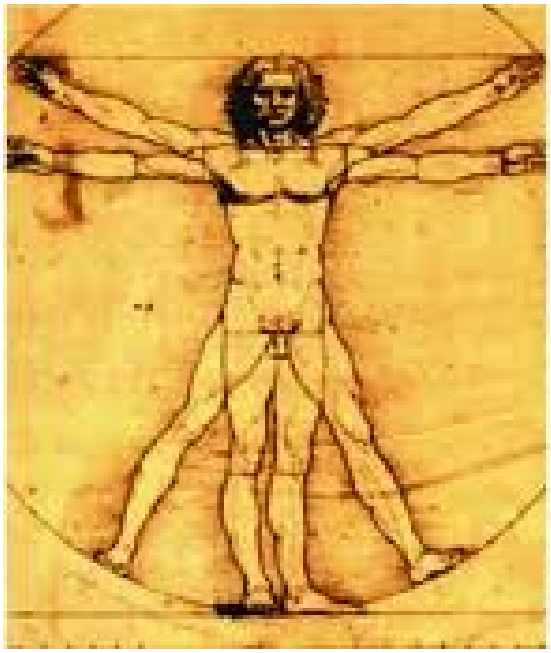

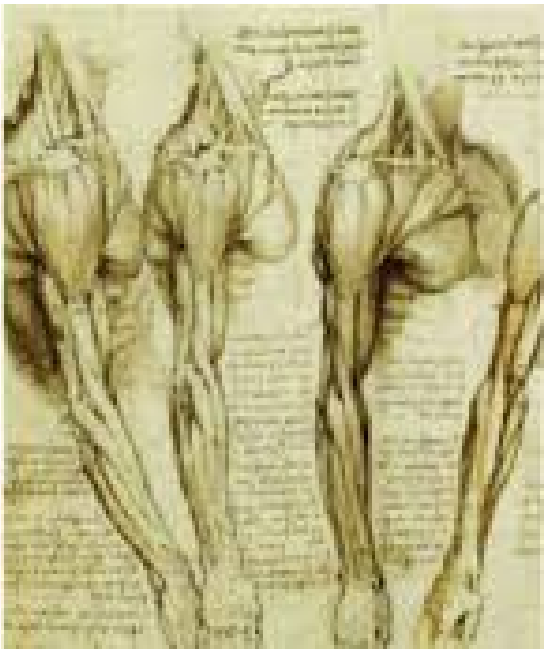

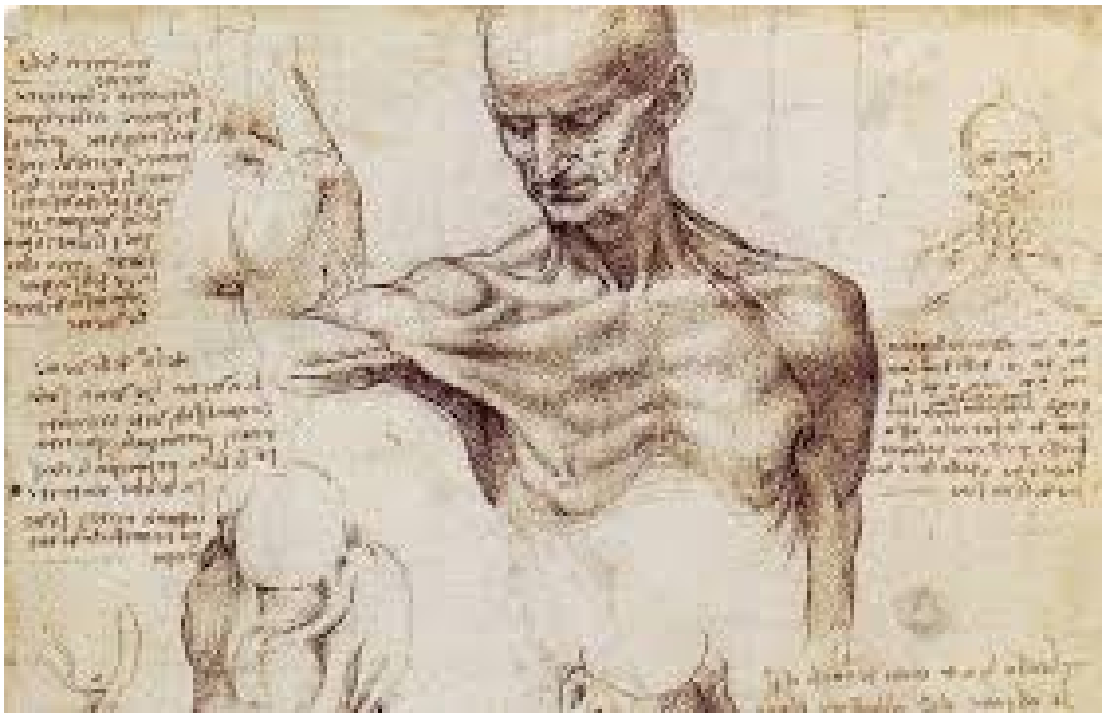

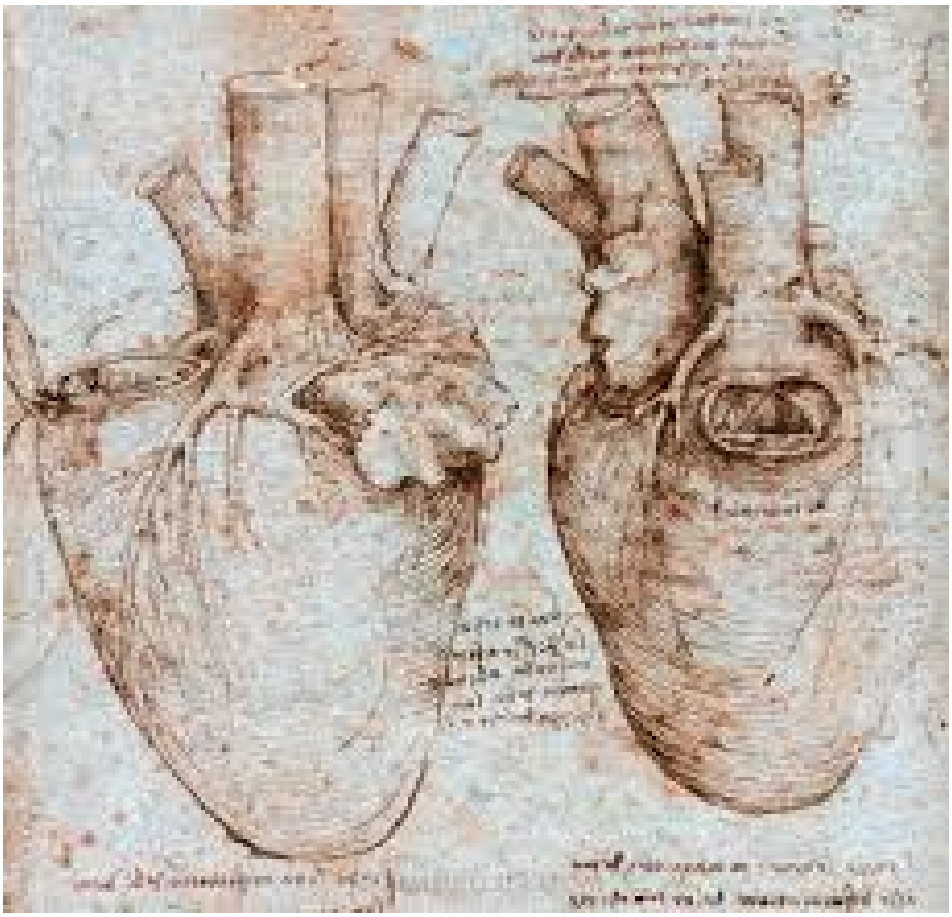

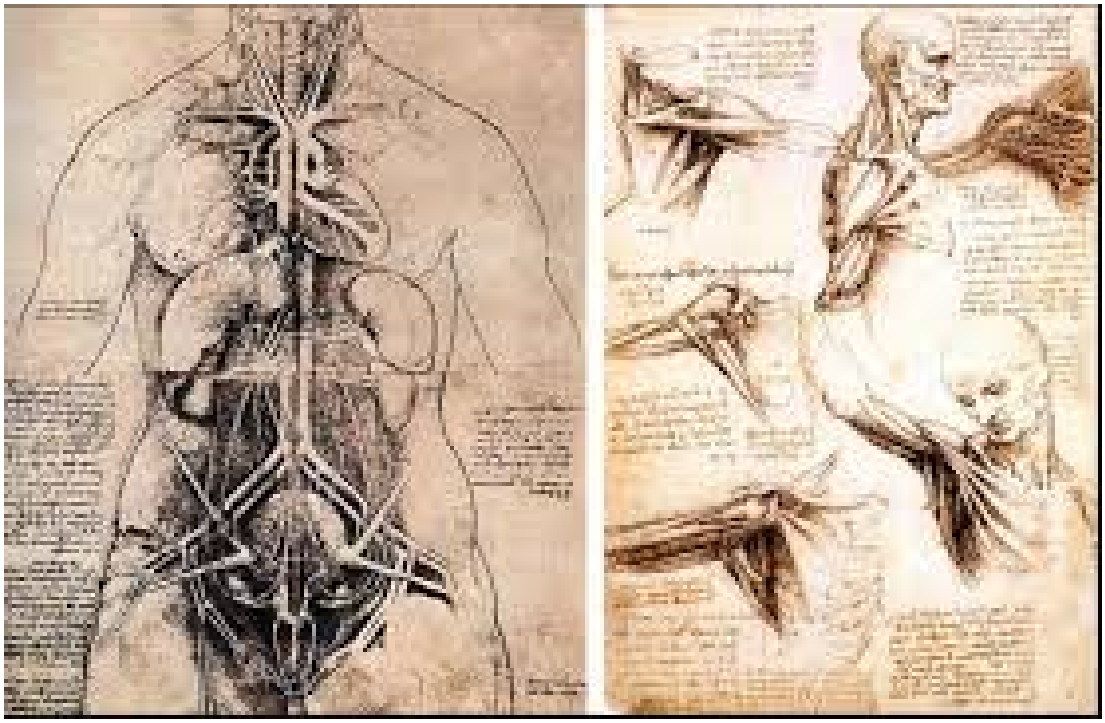

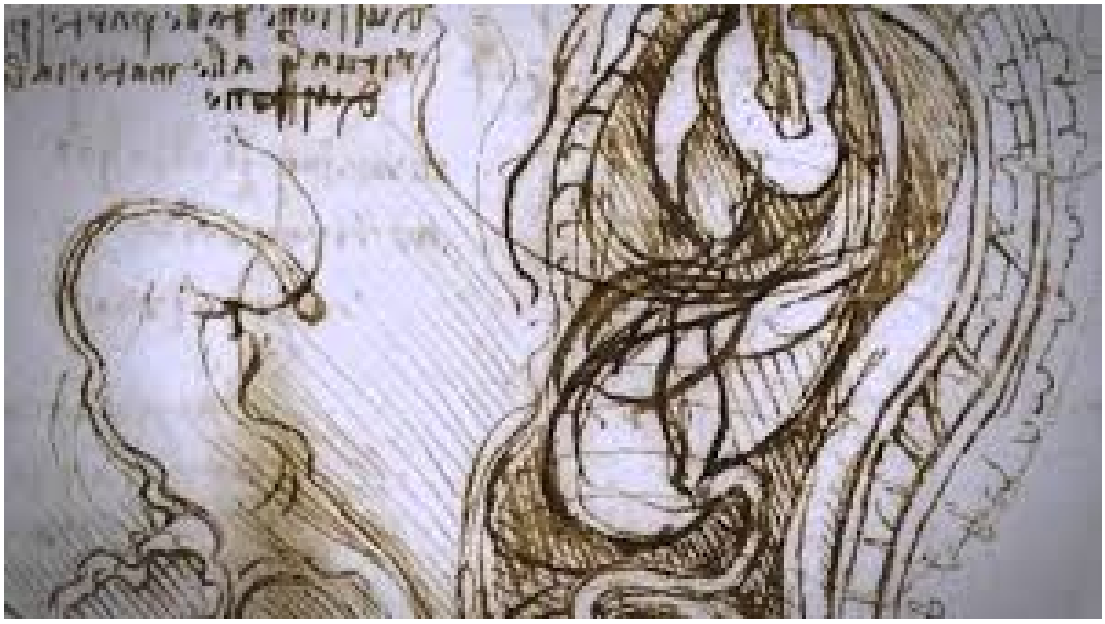

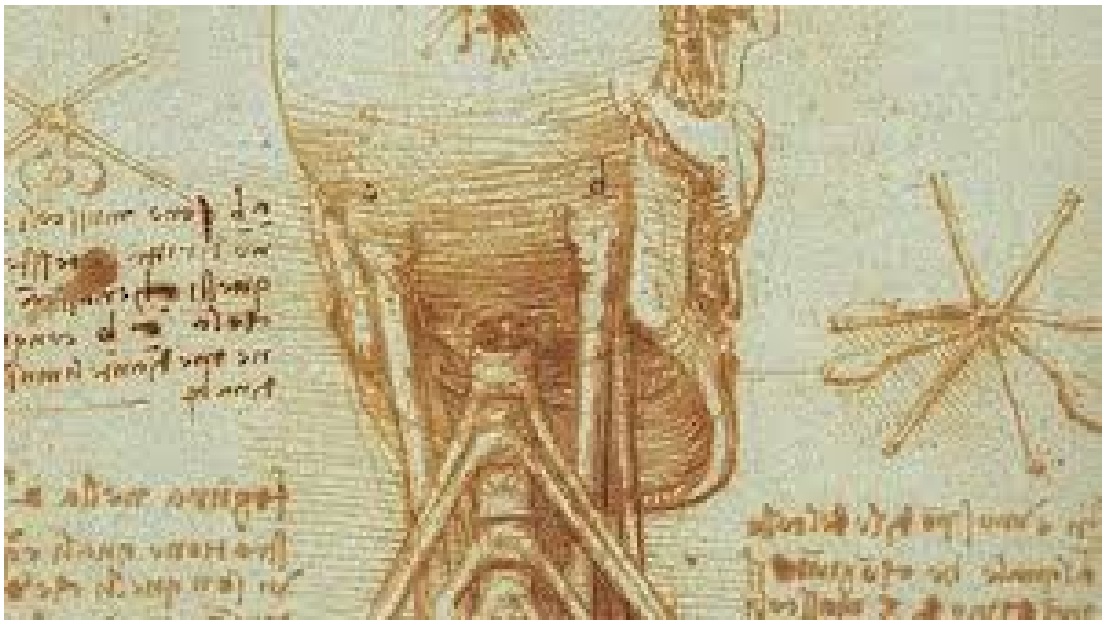

Chapter 5

EPIDEMICS OF PLAGUE AND CHOLERA

5.1 The "Black Death"

The great plague epidemic that peaked in Europe during the years 1347-1351 is thought to have originated in the grasslands of Kyrgyzstan or China, where the disease is commonly present in ground rodents such as marmots. These rodents carry fleas capable of transmitting the disease to humans. The epidemic of plague was one of the most devastating events in human history. It reduced the global population from an estimated 450 million to approximately 350 million. On Europe, between 30% and 60% of the population perished.

By the end of 1346, reports of plague had reached the seaports of Europe: "India was depopulated, Tartary, Mesopotamia, Syria, Armenia were covered with dead bodies".

There are several routes by which the plague may have come from Asia to Europe. It may have come along the Silk Road with armies or traders. Another report of the transmission of the plague to Europe is an early example of the dangers of biological warfare: During a long siege of the port city of Kaffa in the Crimea, the Mongol army under the command of Jani Beg was suffering from the plague. As a weapon of war, they catapulted the bodies of men who had died from the disease into the city, thus infecting the defenders, many of whom were Genoese merchants. The merchants later escaped and returned to Genoa by shiom thus transmitting the plague epidemic to Europe.

Figure 5.3 shows the chronology of the spread of plague in Europe. Because of the great speed with which the epidemic spread northward, some scientists have have speculated that the epidemic which killed such a large fraction of the population of Europe was a variant of the disease caused by the bacterium Yersinia pestis which was transmitted, not by rats and fleas, but by coughing.

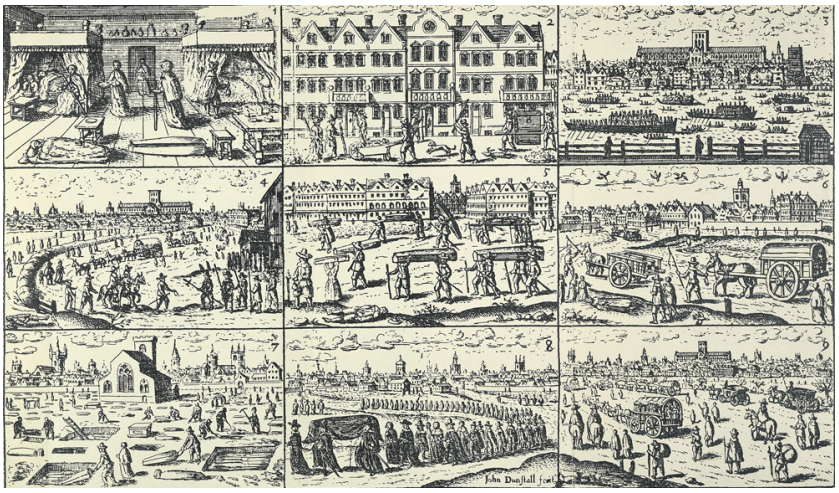

Figure 5.1: **Carrying coffins of the dead during the great plague epidemic.**

Figure 5.2: **Danse Macabre, a drawing inspired by the plague epidemic.**

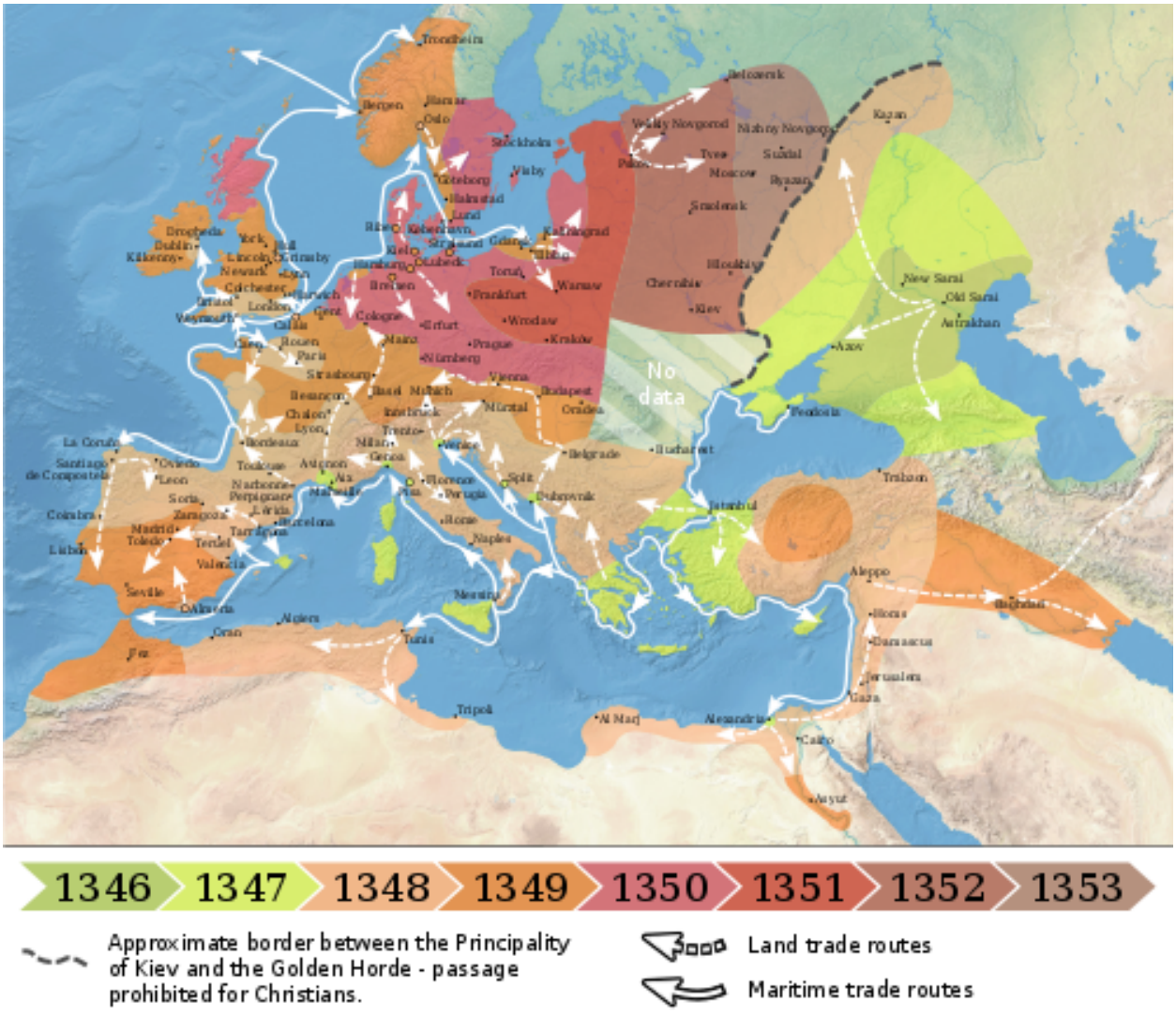

Figure 5.3: **Spread of the Black Death in Europe.**

5.2 Control of plague by quarantine

Here are some quotations from an article by Eugenia Tognotti entitled *Lessons from the History of Quarantine, from Plague to Influenza A*. The article was published in 2013 by the Center for Disease Control and Prevention.

"Organized institutional responses to disease control began during the plague epidemic of 1347-1352. The plague was initially spread by sailors, rats, and cargo arriving in Sicily from the eastern Mediterranean; it quickly spread throughout Italy, decimating the populations of powerful city-states like Florence, Venice, and Genoa. The pestilence then moved from ports in Italy to ports in France and Spain. From northeastern Italy, the plague crossed the Alps and affected populations in Austria and central Europe. Toward the end of the fourteenth century, the epidemic had abated but not disappeared; outbreaks of pneumonic and septicemic plague occurred in different cities during the next 350 years

"Quarantine was first introduced in 1377 in Dubrovnik on Croatia's Dalmatian Coast, and the first permanent plague hospital (lazaretto) was opened by the Republic of Venice in 1423 on the small island of Santa Maria di Nazareth. The lazaretto was commonly referred to as Nazarethum or Lazarethum because of the resemblance of the word lazaretto to the biblical name Lazarus. In 1467, Genoa adopted the Venetian system, and in 1476 in Marseille, France, a hospital for persons with leprosy was converted into a lazaretto. Lazarettos were located far enough away from centers of habitation to restrict the spread of disease but close enough to transport the sick. Where possible, lazarettos were located so that a natural barrier, such as the sea or a river, separated them from the city; when natural barriers were not available, separation was achieved by encircling the lazaretto with a moat or ditch. In ports, lazarettos consisted of buildings used to isolate ship passengers and crew who had or were suspected of having plague. Merchandise from ships was unloaded to designated buildings. Procedures for so-called "purgation" of the various products were prescribed minutely; wool, yarn, cloth, leather, wigs, and blankets were considered the products most likely to transmit disease. Treatment of the goods consisted of continuous ventilation; wax and sponge were immersed in running water for 48 hours."

Later outbreaks of plague

In Europe, smaller outbreaks of the plague continued for another 350 years. The disease is not present in Europe today, but is still found in other parts of the world.

5.3 Discovering the connection between cholera and sanitation

Cholera is a serious gastrointestinal disease caused by drinking water contaminated with cholera bacilli. If untreated, the death rate can be as high as 60%. Although in most developed countries, cholera is no longer a threat, it remains prevelant in many less-developed

regions.

Wikipedia states that "Since it became widespread in the 19th century, cholera has killed tens of millions of people. In Russia alone, between 1847 and 1851, more than one million people perished of the disease. It killed 150,000 Americans during the second pandemic. Between 1900 and 1920, perhaps eight million people died of cholera in India.[Cholera became the first reportable disease in the United States due to the significant effects it had on health. John Snow, in England, was the first to identify the importance of contaminated water as its cause in 1854. Cholera is now no longer considered a pressing health threat in Europe and North America due to filtering and chlorination of water supplies, but still heavily affects populations in developing countries..."

Dr. John Snow and the Soho pumphandle

Dr. John Snow (1813-1858) made several important contributions to medicine. He was a leader in the development of anesthesia, and medical hygene, and because if his work on the Soho cholera epidemic, he is considered to be one of the fathers of modern epidemiology. His work led to changes in public health practices throughout the world.

Born into a poor family in York, Snow exhibited an early aptitude for mathematics and science. At the age of only 14, he was apprenticed to a physician, and had the opportunity to observe the victims of a cholera epidemic. Snow later graduated from the University of London, and was admitted to the Royal College of Physicians in 1850.

Snow was a pioneer of anesthesiology, calculating the optimal dosages for the use of both chloroform and ether. He used these anesthetics in his practice of obstetrics. This created opposition from the Church of England, since it was believed the women were "meant to suffer in childbirth". However, in 1853 Queen Victoria asked Snow to administer chloroform during the birth of her eighth child. She repeated the request for her next birth, three years later.

John Snow lived before the establishment of the germ theory of disease by Louis Pasteur, Robert Koch and others. At the time when Snow lived, it was believed that diseases were caused by "miasma" or "bad air". Even before the Soho cholera epidemic, Snow's observations on the relationship between disease and sanitation had caused him to be skeptical of the "bad air" theory. In 1849 he published an essay entitled *On the Mode of Communication of Cholera*. However, it was the Soho cholera epidemic of 1854 that provided him with the hard evidence that he needed.

Describing his actions in the Soho epidemic, Snow later wrote in a letter to the editor of the Medical Times and Gazette, "On proceeding to the spot, I found that nearly all the deaths had taken place within a short distance of the [Broad Street] pump. There were only ten deaths in houses situated decidedly nearer to another street-pump. In five of these cases the families of the deceased persons informed me that they always sent to the pump in Broad Street, as they preferred the water to that of the pumps which were nearer. In three other cases, the deceased were children who went to school near the pump in Broad Street...

"The result of the inquiry, then, is, that there has been no particular outbreak or

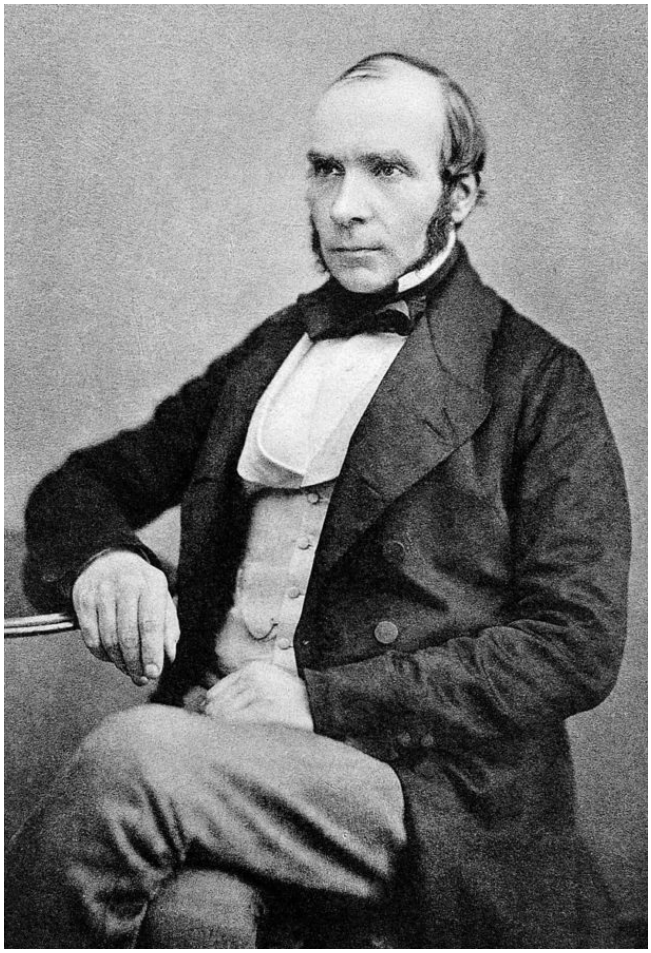

Figure 5.4: **John Snow in 1852.**

prevalence of cholera in this part of London except among the persons who were in the habit of drinking the water of the above-mentioned pump well.

"I had an interview with the Board of Guardians of St James's parish, on the evening of the 7th inst [7 September], and represented the above circumstances to them. In consequence of what I said, the handle of the pump was removed on the following day."

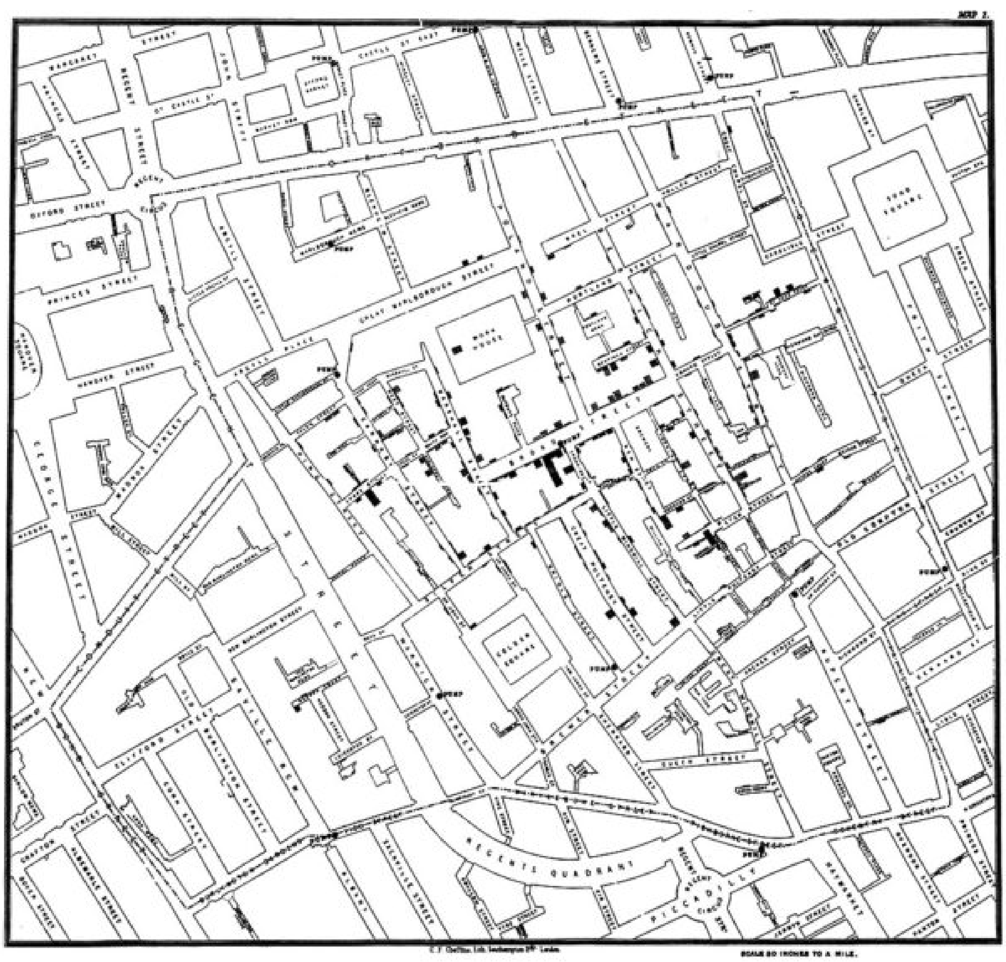

Figure 5.5: **John Snow's map, showing that all of the cases of cholera in the Soho outbreak occurred near to a particular pump.**

Figure 5.6: **The John Snow memorial and public house.**

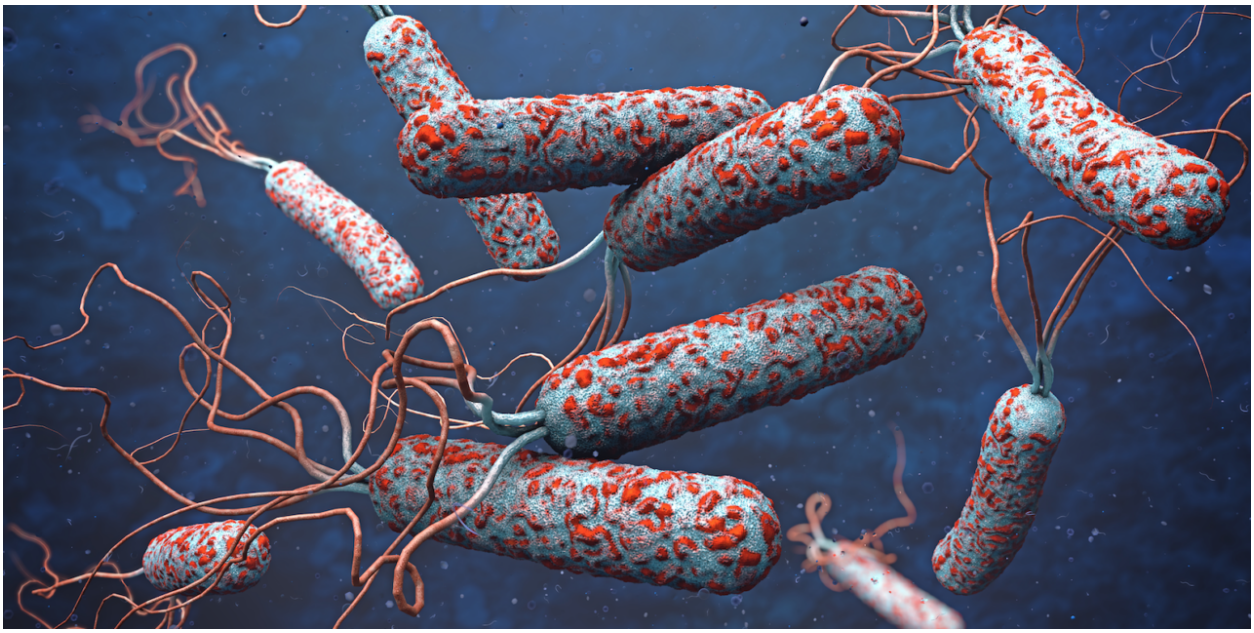

Figure 5.7: **Cholera bacteria. In 1883, the German physician, Robert Koch, isolated the bacterium Vibrio cholerae, finally discovering the cause of the disease. He determined that cholera is spread through unsanitary water or food supply sources, supporting Snow's theory from 20 years earlier. However, John Snow did not live to see his theories vindicated. At the time of Koch's discoveries, he had died of a stroke in 1858, at the age of only 45.**

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Chapter 6

JENNER

6.1 Smallpox

Smallpox a frightful disease from which 300 million people died during the 20th century alone. Approximately one third of the people infected with smallpox die from it, and those who survive are often severely disfigured.

Smallpox inadvertently became a biological weapon aiding the Spanish conquistadors in Central and South America. Much of their military success was due to the fact that they brought smallpox and measles with them, diseases to which the Indians had never been exposed. Since they had no immunity, the majority of the Indians died of these diseases whenever they contracted them in epidemics brought by the Spanish.

In North America, smallpox was deliberately used as a weapon by the British. In 1763, during the Pontiac Rebellion, Sir Jeffrey Amherst, the Commander in Chief of British forces in North America, wrote to Col. Henry Bouquet, "Could it not be contrived to send smallpox among these disaffected tribes of Indians? We must use every stratagem within our power to reduce them." Bouquet replied: "I will try to inoculate [them] with some blankets that may fall into their hands, and take care not to get the disease myself." As in South America, the disease was horrifyingly effective as a weapon, since the Indians had no immunity.

Thus smallpox played an early role in the history of biological warfare, a dark chapter in human history. But as we shall see, it also played a role in the some of the greatest successes of modern medicine. Smallpox is the first disease against which vaccination proved to be possible.

In 18th century Europe, smallpox was so common that people scarcely hoped to avoid it entirely, they hoped instead to have a mild case. It had been noticed that anyone who survived an attack of smallpox could never be attacked again. In Turkey and China, people sometimes inoculated themselves with pus taken from the blisters of patients sick with smallpox in a mild form. The Turkish and Chinese custom of inoculation was introduced into Europe in the 18th century by Lady Mary Montague, the widow of a diplomat who had spent some time in Turkey. Diderot, the editor of the Encyclopedia, did much to make

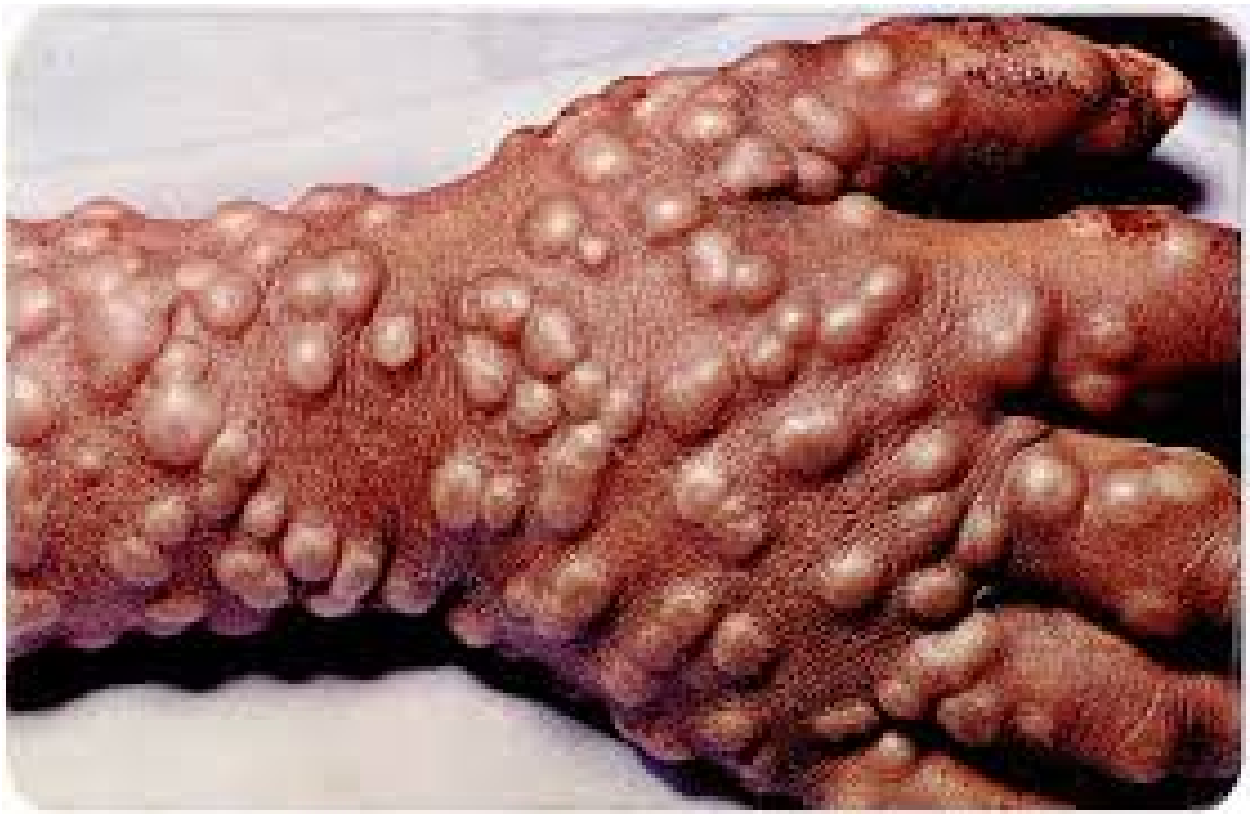

Figure 6.1: **Blisters produced by smallpox. Even when victems of the disease survived, they were often disfigured for life.**

Figure 6.2: **Lady Mary Wortley Montague (1689-1762), was the wife of the British Ambassador to Turkey. She witnessed the practice of inoculation in Turkey, brought back to England and popularized it. Despite the dangers of inoculation, the fear of smallpox was so great that it was widely adopted.**

this practice popular. However, this type of inoculation was dangerous: It gave protection against future attacks, but often the inoculated person became severely ill or died. In addition, the person inoculated was an active source of contagion for some time.

6.2 Edward Jenner and the milkmaid

The story of safe immunization against smallpox began when an English physician named Edward Jenner (1749-1823) treated a dairymaid. He suspected that she might have smallpox; but when he told her this, she replied: "I cannot take the smallpox sir, because I have had the cowpox". She told him that it was common knowledge among the people of her district that anyone who had been ill with cowpox (a mild disease of cattle which sometimes affected farmers and dairymaids), would never be attacked by smallpox.

Jenner realized that if her story were true, it might offer humanity a safe method of immunization against one of its most feared diseases. On May 14, 1796, he found a dairymaid with active cowpox, and taking a little fluid from a blister on her hand, he scratched it into the skin of a boy. The boy became ill with cowpox but he recovered quickly, because the disease is always mild.

Jenner then took the dangerous step of inoculating the boy with smallpox. If the boy had died, Jenner would have been a criminal - but he was immune! It took Jenner two years to find the courage and the opportunity to try the experiment again; but when he repeated it in 1798 with the same result, he decided to publish his findings.

So great was the terror of smallpox, that Jenner was immediately besieged with requests for immunization by inoculation with cowpox (which he called "vaccination" after *vacca*, the Latin word for "cow"). The practice quickly became accepted: The English Royal Family was vaccinated, and Parliament voted Jenner rewards totaling thirty thousand pounds - in those days an enormous sum.

In 1807 Bavaria made vaccination compulsory[1], and celebrated Jenner's birthday as a holiday. Russia also enthusiastically adopted vaccination. The first child in Russia to be vaccinated was given the name "Vaccinov", and was educated at the expense of the state.

In France, the great chemist and bacteriologist Louis Pasteur (1822-1895) and his coworkers were able to apply Jenner's discovery to other diseases. Working first with chicken cholera, and later with anthrax[2], they discovered methods for producing a safe vaccines by weakening cultures of bacteria, so that they no longer produced the disease but still conferred immunity. Pasteur and his coworkers even discovered how to make a vaccine against rabies, which is a virus disease. Thus smallpox played a special role in the history of modern medicine.

[1]This was the initiative of Benjamin Thompson, Count Rumford, the American physicist-soldier-politician.

[2]Anthrax is an often-fatal disease of animals and humans. The anthrax bacilli can form spores that which are able to live in the ground for years. These bacilli have been cultured and stockpiled as a biological weapon by several countries, including the United States and the former Soviet Union.

Figure 6.3: **Edward Jenner (1749-1823). Although he was not the first person to propose vaccination with cowpox as a method for preventing smallpox, it was Jenner's scientific studies of the method that first made it widely accepted.**

Figure 6.4: **Jenner performing his first vaccination on 8-year-old James Phipps, his gardener's son.**

Figure 6.5: **A painting showing Jenner advising a farmer to inoculate his family.**

6.3 Some other discoveries of Edward Jenner

Edward Jenner grew up with a strong interest in science. As the son of a clergyman, he was given a good education. In 1770, at the age of 21, he became apprenticed in surgery and anatomy under John Hunter at St. George's Hospital in London. In 1773 he returned to his native Glostershire, where he became a successful medical practitioner.

Strongly interested in scientific problems of all kinds, Jenner published a study of the previously misunderstood habits of the cuckoo. In this careful study, which was based on observations, experiment and dissection, he showed that the offspring of the cuckoo pushed its host's eggs and fledgling chicks out of the nest (contrary to existing belief that the adult cuckoo did it). In an article published in Philosophical Transactions of the Royal Society in 1788, he wrote: "The singularity of its shape is well adapted to these purposes; for, different from other newly hatched birds, its back from the scapula downwards is very broad, with a considerable depression in the middle. This depression seems formed by nature for the design of giving a more secure lodgement to the egg of the Hedge-sparrow, or its young one, when the young Cuckoo is employed in removing either of them from the nest. When it is about twelve days old, this cavity is quite filled up, and then the back assumes the shape of nestling birds in general." This study won Jenner election to the Royal Society.

In addition, Jenner made a number of medical discoveries and observations, for example in relation to angina.

6.4 The complete eradication of smallpox

After his discovery of safe vaccination against smallpox, Edward Jenner had written, "It now becomes too manifest to admit of controversy, that the annihilation of the Small Pox, the most dreadful scourge of the human species, must be the final result of this practice." In 1959, Jenner's prophecy began to move towards fulfillment when the World Health Assembly passed a resolution initiating a program for the global eradication of smallpox.

A World Health Organization team led by D.A. Henderson devised a strategy in which cases of smallpox were isolated and all their contacts vaccinated, so that the disease had no way of reaching new victims. Descriptions of the disease were circulated, and rewards offered for reporting cases. The strategy proved to be successful, and finally, in 1977, the last natural case of smallpox was isolated in Somalia. After a two-year waiting period, during which no new cases were reported, WHO announced in 1979 that smallpox, one of the most frightful diseases of humankind, had been totally eliminated from the world. This was the first instance of the complete eradication of a disease, and it was a demonstration of what could be achieved by the enlightened use of science combined with international cooperation. The eradication of smallpox was a milestone in human history.

But our species is not really completely wise and rational; we do not really deserve to be called "Homo sapiens". Stone-age emotions and stone-age politics are alas still with us. Samples of smallpox virus were taken to "carefully controlled" laboratories in the United

States and the Soviet Union. Why? Probably because these two Cold War opponents did not trust each other, although both had signed the Biological Weapons Convention. Each feared that the other side might intend to use smallpox as a biological weapon. There were also rumors that unofficial samples of the virus had been saved by a number of other countries, including North Korea, Iraq, China, Cuba, India, Iran, Israel, Pakistan and Yugoslavia.

In 1989 Vladimir Pasechnik, a senior Soviet scientist, defected to the UK. According to Pasechnik, the civilian pharmaceutical firm Biopreparat was in fact a front for a massive Soviet bio-weapons program. His testimony was echoed by Dr. Kanatjan Alibekov, who had been Chief Scientist at Biopreparat between 1987 and 1992, but who defected to the United States in 1992. Alibekov said that a particularly virulent strain of smallpox virus was being cultivated at Biopreparat and that it was being developed as an offensive weapon. In November, 2001, the United States announced that it will not destroy its stocks of smallpox virus, and that it intends to keep them.

To make the discouraging story of smallpox complete, the financial section of a European newspaper ("Metroxpress") recently published a photograph of two very satisfied-looking businessmen. The accompanying article explained the reason for their satisfaction: It was considered likely that smallpox might be used by terrorists. Hence a massive vaccination program would undoubtedly soon take place in Europe, and the company of these two businessmen would make large profits by manufacturing the vaccine. One despairs for the human race!

There is another idea of the biological weapons community that equally repellent, if not more so - racially selective bio-weapons. Basically the idea is this: The Human Genome Project has revealed the sequences of junk DNA (i.e., sequences that do not code for useful proteins) are racially specific. Thus the various races of humankind can be identified by looking at their junk DNA sequences. This being so, it should in principle be possible to construct a virus or toxin that will selectively attack people of a particular race. This idea is particularly abhorrent because it simultaneously violates two important principles of human solidarity. The first principle is that, since disease is the common enemy of mankind, all humans must to work together for its eradication. The second is that all humans must regard each other as members of a single large family. This is absolutely necessary if we are to survive on our small planet.

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Chapter 7

PASTEUR

7.1 Semmelweis

In 1800, when vaccination began to be used against smallpox, no one understood why it worked. No one, in fact, understood what caused infectious diseases. It had been more than a century since Anton van Leeuwenhoek had studied bacteria with his home-made microscopes and described them in long letters to the Royal Society. However, the great Swedish naturalist, Carolus Linnaeus, left microscopic organisms out of his classification of all living things on the grounds that they were too insignificant and chaotic to be mentioned.

Etiology, Concept and Prophylaxis of Childbed Fever

Puerperal fever, or "childbed fever", was common in mid-19th-century hospitals and often fatal. The Hungarian physician Ignaz Semmelweis, working in the maternity division of the Vienna General Hospital began to require that the doctors working under him should wash their hands in chlorinated lime solution between visits to patients. He found that this practice reduced mortality from childbed fever to less than 1%. In 1861, he published a book describing his results entitled *Etiology, Concept and Prophylaxis of Childbed Fever*. However, despite this and other publications, his results were not only rejected by the medical establishment of the time, but Semmelweis was also vilified, ridiculed and treacherously assigned to an insane asylum, where he was beaten to death by guard. He was before his time. His discoveries came before the germ theory of disease. Today, Semmelweis is recognized as a great pioneer of antiseptic practice in medicine.

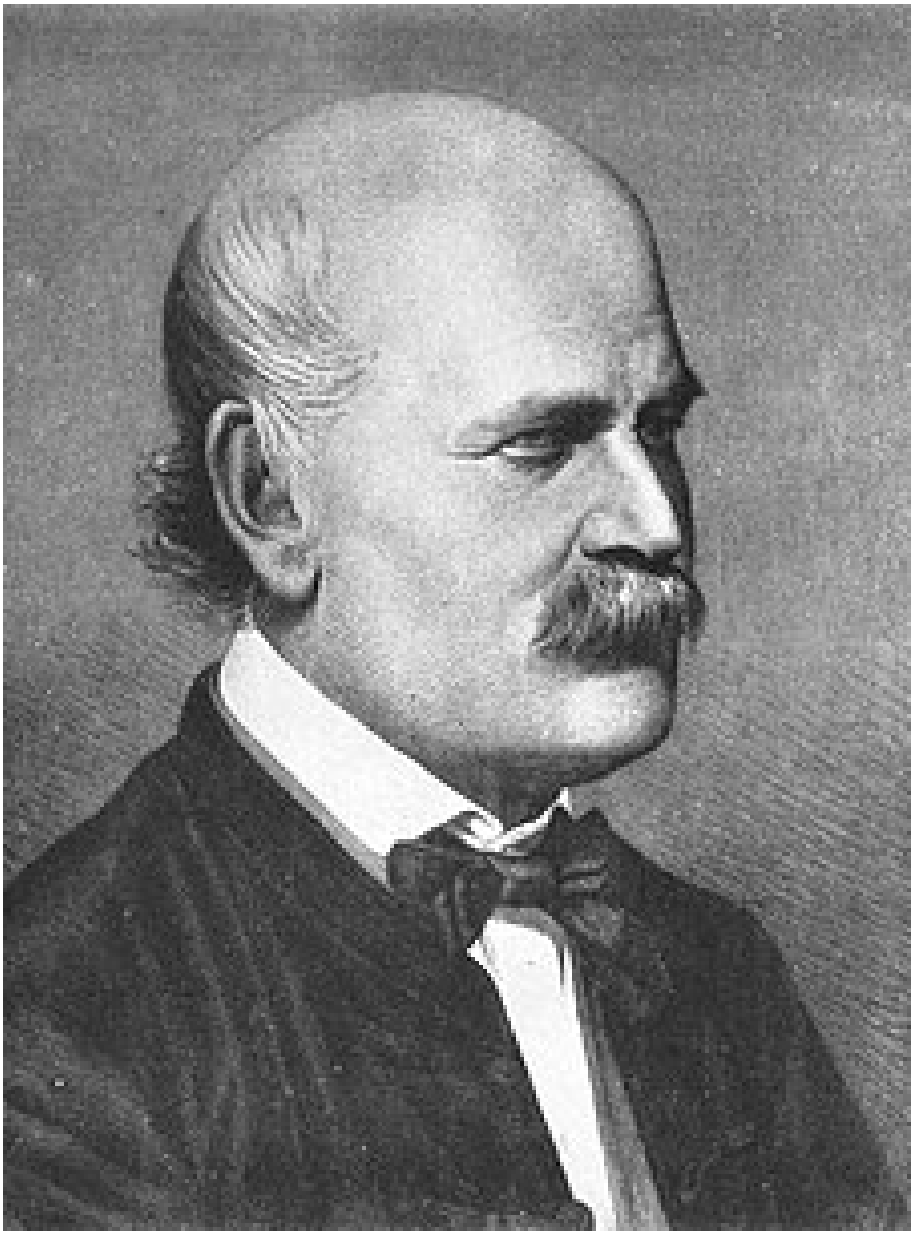

Figure 7.1: **Dr. Ignaz Semmelweis, (1818-1865). He discovered that if physicians at the Vienna General Hospital's Obstetrical Clinic washed their hands in chlorinated lime solution between visits to patients, the mortality was drastically reduced. Tragically, since this discovery was made prior to the germ theory of disease, it was rejected by the medical establishment of his time. Semmelweis was sent to an insane asylum, where he died after being beaten by guards.**

Figure 7.2: **Semmelweis statue at the University of Tehran.**

Figure 7.3: **2008 Austrian commemorative coin picturing Semmelweis.**

7.2 Pasteur: artist or chemist?

This was the situation when Louis Pasteur was born in 1822, in the Jura region of France, near the Swiss border. His father was a tanner in the small town of Arbois. Pasteur's parents were not at all rich, but they were very sincere and idealistic, and they hoped that their son would one day become a teacher.

As a boy, Louis Pasteur was considered to be a rather slow student, but he was artistically gifted. Between the ages of 13 and 19, he made many realistic and forceful portraits of the people of his town. His ambition was to become a professor of the fine arts; and with this idea he studied to qualify for the entrance examination of the famous École Normale of Paris, supporting himself with a part-time teaching job, and sometimes enduring semi-starvation when the money sent by his father ran out.

The earnest, industrious and artistically gifted boy would certainly have succeeded in becoming an excellent professor of the fine arts if he had not suddenly changed his mind and started on another path. This new path was destined to win Louis Pasteur a place among the greatest benefactors of humanity.

The change came when Pasteur attended some lectures by the famous chemist Jean Baptiste Dumas. Professor Dumas was not only a distinguished researcher; he was also a spellbinding speaker, whose lectures were always attended by six or seven hundred excited students. "I have to go early to get a place", Pasteur wrote to his parents, "just as in the theatre". Inspired by these lectures, Pasteur decided to become a chemist. He put away his brushes, and never painted again.

While he was still a student, Pasteur attracted the attention of Antoine Jerome Balard, the discoverer of the element bromine. Instead of being sent to teach at a high-school in the provinces after his graduation, Pasteur became an assistant in the laboratory of Balard, where he had a chance to work on a doctor's degree, and where he could talk with the best chemists in Paris. Almost every Thursday, he was invited to the home of Professor Dumas, where the conversation was always about science.

Pasteur's first important discovery came when he was 25. He had been studying the tartarates - a group of salts derived from tartaric acid. There was a mystery connected with these salts because, when polarized light was passed through them, they rotated the direction of polarization. On the other hand, paratartaric acid (now called racemic acid), did not exhibit this effect at all, nor did its salts. This was a mystery, because there seemed to be no chemical difference between tartaric acid and racemic acid.

Studying tiny crystals of paratartaric acid under his microscope, Pasteur noticed that there were two kinds, which seemed to be mirror images of one another. His vivid imagination leaped to the conclusion that the two types of crystals were composed of different forms of tartaric acid, the molecules of one form being mirror images of the other. Therefore the crystals too were mirror images, since, as Pasteur guessed, the shapes of the crystals resulted from the shapes of the molecules.

By painstakingly separating the tiny right-handed crystals from the left-handed ones, Pasteur obtained a pure solution of right-handed molecules, and this solution rotated polarized light. The left-handed crystals, when dissolved, produced the opposite rotation!

Pasteur ran from the laboratory, embraced the first person that he met in the hall, and exclaimed: "I have just made a great discovery! I am so happy that I am shaking all over, and I am unable to set my eyes again to the polarimeter."

Jean Baptiste Biot, the founder of the field of polarimetry, was sceptical when he heard of Pasteur's results; and he asked the young man to repeat the experiments so that he could see the results with his own eyes. Under Biot's careful supervision, Pasteur separated the two types of crystals of racemic acid, and put a solution of the left-handed crystals into the polarimeter.

"At the first sight of the color tints presented by the two halves of the field", Pasteur wrote, "and without having to make a reading, Biot recognized that there was a strong rotation to the left. Then the illustrious old man, who was visibly moved, seized me by the hand and said: 'My dear son, all my life I have loved science so deeply that this stirs my heart!'"

As he continued his work with right- and left-handed molecules, Pasteur felt that he was coming close to an understanding of the mysteries of life itself, since, as Biot had shown, the molecules which rotate polarized light are almost exclusively molecules produced by living organisms. He soon discovered that he could make an optically active solution of tartaric acid in another way: When he let the mould *penicillium glaucum* grow in a solution of racemic acid, the left-handed form disappeared, and only the right-handed form remained. In this way, Pasteur became interested in the metabolism of microscopic organisms.

Pasteur's work on crystallography and optical activity had made him famous among chemists, and he was appointed Professor of Chemistry at the University of Strasbourg. He soon fell in love with and married the daughter of the Rector of the university, Marie Laurent. This marriage was very fortunate for Pasteur. In the words of Pasteur's assistant, Emil Roux, "Madame Pasteur loved her husband to the extent of understanding his studies... She was more than an incomparable companion for her husband: She was his best collaborator". She helped him in every way that she could - protecting him from everyday worries, taking dictation, copying his scientific papers in her beautiful handwriting, discussing his experiments and asking intelligent questions which helped him to clarify his thoughts.

7.3 Saving the French wine industry

After a few years at Strasbourg, Pasteur was appointed Dean of the Faculty of Sciences at the University of Lille. In appointing him, the French government explained to Pasteur that they expected him to place the Faculty of Sciences of the university at the service of the industry and agriculture of the district.

Pasteur took this commission seriously, and he soon put his studies of microorganisms to good use in the service of a local industry which produced alcohol from beet juice. He was able to show that whenever the vats of juice contained bacteria, they spoiled; and he showed the local manufacturers how eliminate harmful bacteria from their vats. As a result of this work, the industry was saved.

PASTEUR en 1857

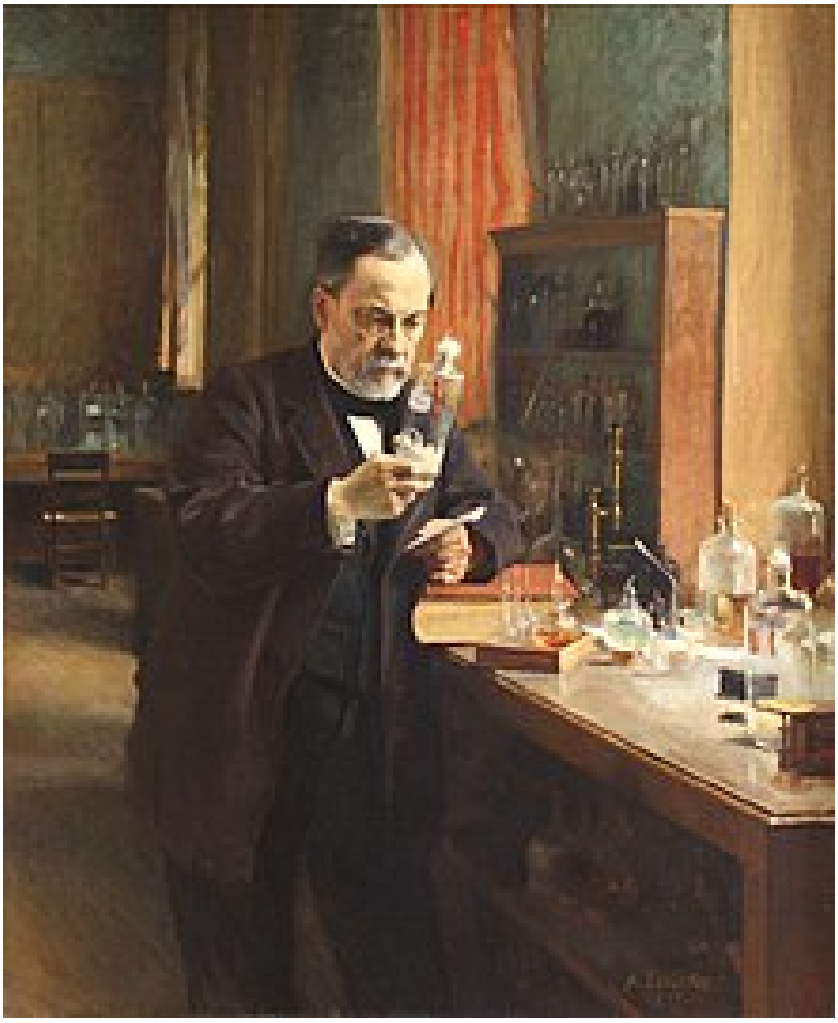

Figure 7.4: **Louis Pasteur in his laboratory, as painted by A. Edelfeldt.**

His work on fermentation put Pasteur into conflict with the opinions of the most famous chemists of his time. He believed that it was the action of the living yeast cells which turned sugar into alcohol, since he had observed that the yeasts were alive and that the amount of alcohol produced was directly proportional to the number of yeasts present. On the other hand, the Swedish chemist, Jöns Jakob Berzelius (1779-1848), had considered fermentation to be an example of catalysis, while Justus von Liebig (1805-1875) thought that the yeasts were decaying during fermentation, and that the breakdown of the yeast cells somehow assisted the conversion of sugar to alcohol. (Both Pasteur and Berzelius were right! Although the fermentation observed by Pasteur was an example of the action of living yeasts, it is possible to extract an enzyme from the yeasts which can convert sugar to alcohol without the presence of living cells.)

Pasteur studied other fermentation processes, such as the conversion of sugar into lactic acid by the bacilli which are found in sour milk, and the fermentation which produces

butyric acid in rancid butter. He discovered that each species of microorganism produces its own specific type of fermentation; and he learned to grow pure cultures of each species.

At the suggestion of Napoleon III, Pasteur turned his attention to the French wine industry, which was in serious difficulties. He began to look for ways to get rid of the harmful bacteria which were causing spoilage of the wine. After trying antiseptics, and finding them unsatisfactory, Pasteur finally found a method for killing the bacteria, without affecting the taste of the wine, by heating it for several minutes to a temperature between 50 and 60 degrees centigrade. This process ("Pasteurization") came to be applied, not only to wine, but also to milk, cheese, butter, beer and many other kinds of food.

Pasteur developed special machines for heat-treating large volumes of liquids. He patented these, to keep anyone else from patenting them, but he made all his patents available to the general public, and refused to make any money from his invention of the Pasteurization process. He followed the same procedure in patenting an improved process for making vinegar, but refusing to accept money for it.

Pasteur was now famous, not only in the world of chemists and biologists, but also in the larger world. He was elected to membership by the French Academy of Sciences, and he was awarded a prize by the Academy for his research refuting the doctrine of spontaneous generation.

7.4 The germ theory of disease

In 1873, Louis Pasteur was elected to membership by the French Academy of Medicine. Many conservative physicians felt that he had no right to be there, since he was really a chemist, and had no medical "union card". However, some of the younger doctors recognized Pasteur as the leader of the most important revolution in medical history; and a young physician, Emil Roux, became one of Pasteur's devoted assistants.

When he entered the Academy of Medicine, Pasteur found himself in the middle of a heated debate over the germ theory of disease. According to Pasteur, every contagious disease is caused by a specific type of microorganism. To each specific disease there corresponds a specific germ.

Pasteur was not alone in advocating the germ theory, nor was he the first person to propose it. For example, Varro (117 B.C. - 26 B.C.), believed that diseases are caused by tiny animals, too small to be seen, which are carried by the air, and which enter the body through the mouth and nose.

In 1840, Jacob Henle, a distinguished Bavarian anatomist, had pointed out in an especially clear way what one has to do in order to prove that a particular kind of germ causes a particular disease: The microorganism must be found consistently in the diseased tissue; it must be isolated from the tissue and cultured; and it must then be able to induce the disease consistently. Finally, the newly-diseased animal or human must yield microorganisms of the same type as those found originally.

Henle's student, Robert Koch (1843-1910), brilliantly carried out his teacher's suggestion. In 1872, Koch used Henle's method to prove that anthrax is due to rodlike bacilli

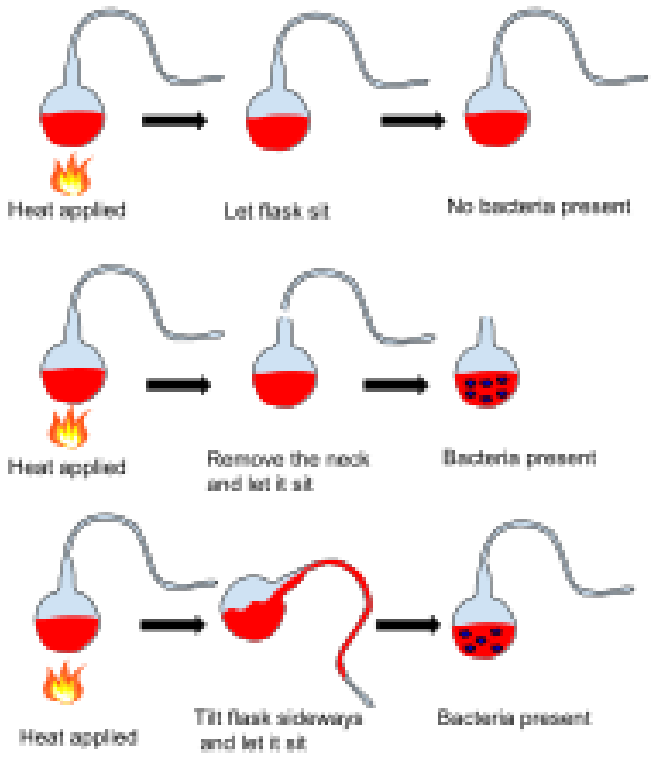

Figure 7.5: **One of Louis Pasteur's experiment illustrates the fact that the spoilage of liquid is caused by particles in the air rather than the air itself. These experiments were important evidence supporting the germ theory of disease..**

in the blood of the infected animal. Koch's pioneering contributions to microbiology and medicine were almost as great as those of Pasteur. Besides being the first person to prove beyond doubt that a specific disease was caused by a specific microorganism, Koch introduced a number of brilliant technical improvements which paved the way for rapid progress in bacteriology and medicine.

Instead of using liquids as culture media,Koch and his assistant, Petri, pioneered the use of solid media. Koch developed a type of gel made from agar-agar (a substance derived from seaweed). On the surface of this gel, bacteria grew in tiny spots. Since the bacteria could not move about on the solid surface, each spot represented a pure colony of a single species, derived from a single parent. Koch also pioneered techniques for staining bacteria, and he introduced the use of photography in bacteriology. He was later to isolate the bacillus which causes tuberculosis, and also the germ which causes cholera.

When Koch's work was attacked in the French Academy of Medicine, Pasteur rushed to his defense. In order to demonstrate that it was living bacilli in the blood of a sheep with anthrax which transmitted the disease, and not something else in the blood, Pasteur took a drop of infected blood and added it to a large flask full of culture medium. He let this stand until the bacteria had multiplied; and then he took a tiny drop from the flask and transferred it to a second flask of nutrient broth. He did this a hundred times, so that there was no possibility that anything whatever remained from the original drop of sheep's blood. Nevertheless, a tiny amount of liquid from the hundredth flask was just as lethal as fresh blood drawn from a sheep with anthrax.

Figure 7.6: **Robert Koch (1843-1910) was one of the important founders of modern bacteriology. He received the Nobel Prize for Physiology or Medicine in 1905.**

7.5 Vaccines

Pasteur read and reread the papers of Jenner on immunization against smallpox. He searched continually for something analogous to smallpox vaccination which could be applied to other diseases. Finally, the answer came by chance.

Pasteur and his assistants had been studying chicken cholera, an invariably fatal disease of chickens. Roux and Chamberland were carrying out a series of experiments where they made a fresh culture of chicken cholera bacteria every day. When they injected a bit of liquid from any of these cultures into a chicken, the chicken always died.

It was summer, and the young men went off for two weeks of vacation. When they came back, they took their two-week-old culture of chicken cholera out of the cupboard and injected it into a hen; but the hen didn't die. They decided that while they had been on vacation, the culture must have lost its strength; and after some effort, they obtained a new specimen of active chicken cholera bacteria, which they injected into their hens. All the hens died except one. The hen which had previously been inoculated with two-week-old culture didn't even get sick!

When Pasteur returned to his laboratory, the two young men hesitated to tell him about this strange result because they were afraid that he might be angry with them for going off on a holiday and breaking off the series of experiments. However, they finally confessed what had happened, and added the strange detail about the chicken which had not died. In the middle of their apologies, Pasteur raised his hand. "Please be quiet for a moment", he said, "I want to think". After a few moments of silence, Pasteur looked at Roux and Chamberland and said, "That's it! The hen that didn't die was *vaccinated* by the old culture!"

This was the big breakthrough - a turning point in medical history. Pasteur, Roux and Chamberland had discovered by chance a method of weakening a culture of bacteria so that it would not produce the fatal disease with which it was usually associated; but on the other hand, it was still able to alert the body's defense mechanisms, so that the inoculated animal became immune. This great discovery was made by chance, but, as Pasteur was fond of saying, "In research, chance favors the prepared mind".

Pasteur, Roux and Chamberland dropped everything else and began a series of experiments to find the best way of weakening their cultures of chicken cholera. They found that the critical factor was the proper amount of exposure to air. (Probably the culture contained a few mutant bacteria, able to grow well in air, but not able to produce chicken cholera; and during the exposure of a culture, these mutants multiplied rapidly, until the entire population was composed of mutants.)

Pasteur now began research on a vaccine against anthrax - a disease which was causing serious economic loss to farmers, and which could affect humans as well as animals. With anthrax, the problem was to keep the bacilli from forming spores. After much experimentation, the group found that if they held their anthrax cultures at a temperature between 42 C and 43 C, the bacilli would still grow, but they did not form spores.

Pasteur and his coworkers allowed their cultures to grow at 42 C in shallow dishes, where there was good contact with the air. They found that after two weeks, the cultures

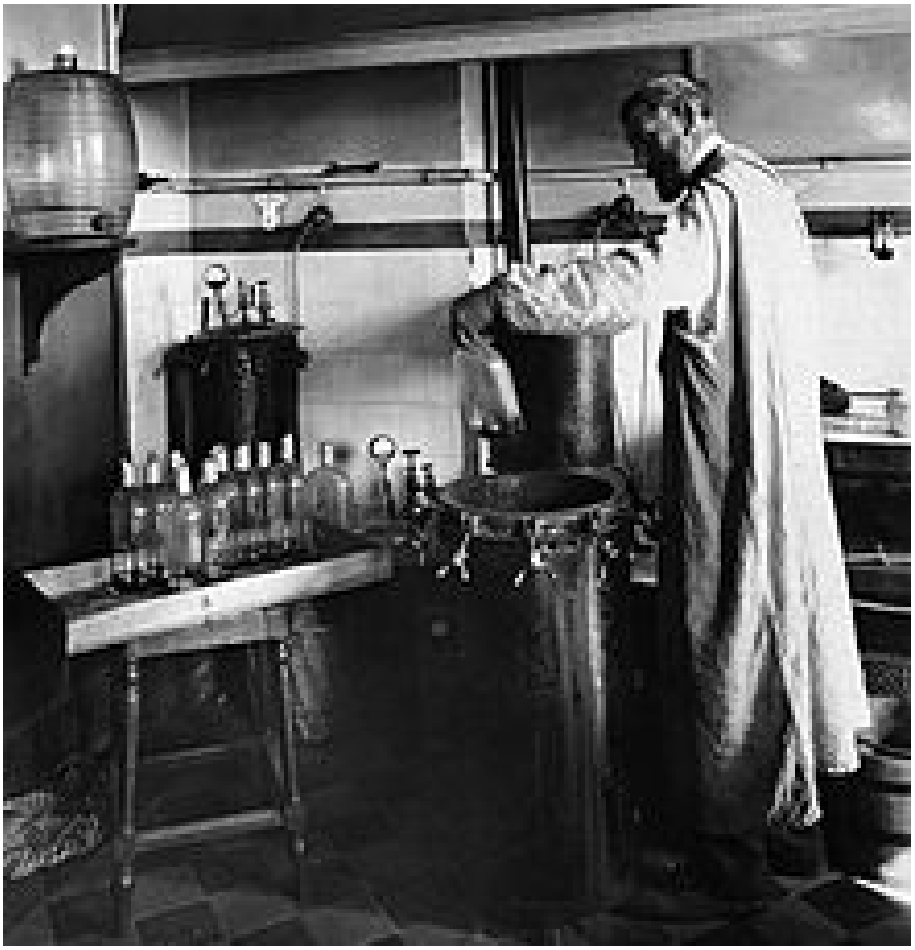

Figure 7.7: **Louis Pasteur at work in his laboratory.**

were weakened to the point where they would make a sheep sick, but not kill it. They developed a method for inoculating animals in two stages - first with a very much weakened culture, and later with a stronger one. After the second inoculation, the animals could stand an injection of even the most virulent anthrax bacilli without becoming ill.

When Pasteur published these results, there was much sarcasm among veterinarians. The editor of the *Veterinary Press*, a surgeon named Rossignol, wrote: "Monsieur Pasteur's discovery, *if it were genuine*, should not be kept in the laboratory". Rossignol proposed a public trial of the anthrax vaccine, and he started a campaign to collect money for the purchase of experimental animals.

Pasteur's friends warned him against accepting the risk of a public trial at such an early stage. He had not tested his vaccine sufficiently, and a failure would make him the laughing stock of Europe. However, Pasteur saw the trial as a chance to focus public attention on microorganisms and vaccines. Like Galileo, Pasteur had a flair for dramatic gestures and public debate; and the impact of his career was greatly enhanced by his ability to attract widespread attention.

A farm near Melun called Poully le Fort was chosen as the site for the experiment; and sixty sheep, together with several cows, were put at Pasteur's disposal. Thousands of people made the journey from Paris to Melun to watch the first injections, which were made on May 5, 1881. Twelve days later, the same sheep were inoculated with a stronger vaccine. Then, on May 31, the big test was made - both the vaccinated and unvaccinated animals were inoculated with a highly lethal culture of anthrax. Pasteur went back to Paris. There was nothing to do but wait.

The next afternoon, a telegram from Rossignol shattered Pasteur's confidence: It said that one of the vaccinated sheep was dying. Pasteur spent a sleepless night. The following morning, however, at nine o'clock, another telegram arrived from Rossignol: All the vaccinated sheep were well, even the one which had seemed to be dying; and all the unvaccinated sheep were either dying or already dead! Rossignol, who had been Pasteur's enemy, was completely converted; and his telegram ended with the words, "Stunning success!" When the aging Pasteur limped onto the field at Poully le Fort that afternoon, a great cheer went up from the thousands of people present.

Rabies

The next disease which Pasteur attempted to conquer was rabies, the terrifying and invariably fatal disease which often follows the bite of a mad dog. The rabies virus travels slowly through the body from the wounds to the spinal cord, where, after one or two months, it attacks the nervous system. If a victim is offered water and attempts to swallow, his head jerks back in terrible spasms, which make rabies extremely frightening, both for the victim and for the onlooker. For this reason, the disease is sometimes called hydrophobia - fear of water.

Pasteur and his coworkers soon discovered that even with their best microscopes, they were unable to see the organism which causes rabies. In fact, the disease is caused by a

Figure 7.8: **A French stamp commemorating Pasteur's fight against rabes.**

virus, much too small to be seen with an optical microscope. Thus the aging Pasteur was confronted with an entirely new technical problem, never before encountered in microbiology.

He soon found that it was impossible to culture the rabies virus in a flask or dish, as he was in the habit of doing with bacteria. Absorbed in his research, he forgot his wedding anniversary. Marie Pasteur, however, remembered; and she wrote in a letter to her daughter:

"Your father is absorbed in his thoughts. He talks little, sleeps little, rises at dawn, and in a word, continues the life which which I began with him this day thirty-five years ago."

Besides being technically difficult, the work on rabies was also dangerous. When Pasteur, Roux and Chamberland took samples of saliva from the foaming jaws of mad dogs, they risked being bitten by accident and condemned to an agonizing death from the convulsions of rabies. Since they could not culture the rabies virus in a dish or a flask of nutrient fluid, they were forced to grow it inside the nervous systems of experimental animals. After four years of difficult and hazardous work, they finally succeeded in developing a vaccine against rabies.

In the method which finally proved successful, they took a section of spinal cord from a rabbit with rabies and exposed it to air inside a germproof bottle. If the section of spinal cord remained in the bottle for a long time, the culture was very much weakened or "attenuated", while when it was exposed to air for a shorter time, it was less attenuated. As in the case of anthrax, Pasteur built up immunity by a series of injections, beginning with a very much attenuated culture, and progressing to more and more virulent cultures.

At last, Pasteur had a method which he believed could be used to save the lives of the victims of mad dogs and wolves; and he found himself faced with a moral dilemma: Everyone who developed rabies died of it; but not everyone who was bitten by a mad dog developed rabies. Therefore if Pasteur gave his vaccine to a human victim of a mad dog, he might harm someone who would have recovered without treatment.

He had published the results of his research, and he was inundated with requests for treatment, but still he hesitated. If he treated someone, and the person afterward died, he might be accused of murder; and all the work which he had done to build up public support for the new movement in medicine might be ruined.

Finally, on July 6, 1885, Pasteur's indecision was ended by the sight of a man and woman who had come to him with their frightened nine-year-old son. The boy, whose name was Joseph Meitner, had been severely bitten by a mad dog. It was one thing to write letters refusing requests for treatment, and another thing to look at a doomed and frightened child and turn him away.

Pasteur felt that he had to help the boy. He consulted Alfred Vulpian, a specialist in rabies, and Vulpian assured him that Joseph Meitner had been bitten so severely that without treatment, he would certainly develop rabies and die. Pasteur also consulted Dr. Granchier, a young physician who had joined his staff, and together the three men agreed that there was no time to lose - they would have to begin inoculations immediately if they were to save the boy's life. They decided to go ahead. To Pasteur's great joy, Joseph Meitner remained completely well.

The second rabies victim to be treated by Pasteur was a fourteen-year-old shepard named Jupille. He had seen a mad dog about to attack a group of small children, and he had bravely fought with the maddened animal so that the children could escape. Finally he had managed to tie its jaws together, but his hands were so badly bitten that without treatment, he was certain to die. Like Joseph Meitner, Jupille was saved by the Pasteur treatment. A statue of Jupille in front of the Pasteur Institute commemorates his bravery.

Pasteur had now grown so old, and was so worn out by his labors that he could do no more. The task of winning a final victory over infectious diseases was not finished - it was barely begun; but at least the feet of researchers had been placed on the right road; and there were younger men and women enthusiastically taking up the task which Pasteur laid down.

On December 27, 1892, physicians and scientists from many countries assembled in Paris to celebrate Pasteur's seventieth birthday. The old man was so weak that he was unable to reply in his own words to the address of Sir Joseph Lister and to the cheers of the crowd; but his words were read by his son. Pasteur spoke to the young men and women who would take his place in the fight against disease:

"Do not let yourselves be discouraged by the sadness of certain hours which pass over nations. Live in the serene peace of your laboratories and libraries. Say to yourselves first, 'What have I done for my instruction?', and as you gradually advance, 'What have I done for my country?', until the time comes when you may have the intense happiness of thinking that you have contributed in some way to the progress and good of humanity."

Figure 7.9: **Institut Pasteur de Lille.**

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Chapter 8

EHRLICH, MECHNIKOV AND JERNE

8.1 The language of molecular complementarity

In living (and even non-living) systems, signals can be written and read at the molecular level. The language of molecular signals is a language of complementarity. The first scientist to call attention to complementarity and pattern recognition at the molecular level was Paul Ehrlich, who was born in 1854 in Upper Silesia (now a part of Poland). Ehrlich was not an especially good student, but his originality attracted the attention of his teacher, Professor Waldeyer, under whom he studied chemistry at the University of Strasbourg. Waldeyer encouraged him to do independent experiments with the newly-discovered aniline dyes; and on his own initiative, Ehrlich began to use these dyes to stain bacteria. He was still staining cells with aniline dyes a few years later (by this time he had become a medical student at the University of Breslau) when the great bacteriologist Robert Koch visited the laboratory. "This is young Ehrlich, who is very good at staining, but will never pass his examinations", Koch was told. Nevertheless, Ehrlich did pass his examinations, and he went on to become a doctor of medicine at the University of Leipzig at the age of 24. His doctoral thesis dealt with the specificity of the aniline dyes: Each dye stained a special class of cell and left all other cells unstained.

Paul Ehrlich had discovered what might be called "the language of molecular complementarity": He had noticed that each of his aniline dyes stained only a particular type of tissue or a particular species of bacteria. For example, when he injected one of his blue dyes into the ear of a rabbit, he found to his astonishment that the dye molecules attached themselves selectively to the nerve endings. Similarly, each of the three types of phagocytes could be stained with its own particular dye, which left the other two kinds unstained[1].

[1] The specificity which Ehrlich observed in his staining studies made him hope that it might be possible to find chemicals which would attach themselves selectively to pathogenic bacteria in the blood stream and kill the bacteria without harming normal body cells. He later discovered safe cures for both sleeping sickness and syphilis, thus becoming the father of chemotherapy in medicine. He had already received the Nobel Prize for his studies of the mechanism of immunity, but after his discovery of a cure for syphilis, a

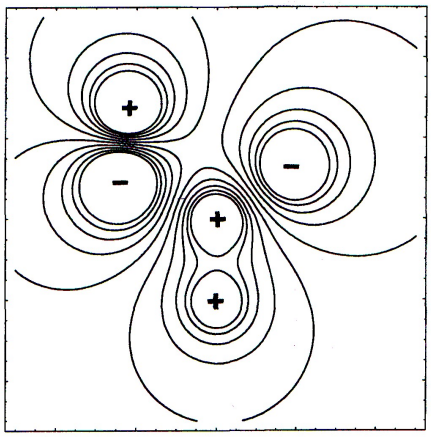

Figure 8.1: **This figure shows the excess charges and the resulting electrostatic potential on a molecule of formic acid, HCOOH. The two oxygens in the carboxyl group are negatively charged, while the carbon and the two hydrogens have positive excess charges. Molecular recognition involves not only steric complementarity, but also complementarity of charge patterns.**

Ehrlich believed that this specificity came about because the side chains on his dye molecules contained groupings of atoms which were complementary to groups of atoms on the surfaces of the cells or bacteria which they selectively stained. In other words, he believed that biological specificity results from a sort of lock and key mechanism: He visualized a dye molecule as moving about in solution until it finds a binding site which exactly fits the pattern of atoms in one of its side chains. Modern research has completely confirmed this picture, with the added insight that we now know that the complementarity of the "lock" and "key" is electrostatic as well as spatial.

Two molecules in a biological system may fit together because the contours of one are complementary to the contours of the other. This is how Paul Ehrlich visualized the fit - a spatial (steric) complementarity, like that of a lock and key. However, we now know that for maximum affinity, the patterns of excess charges on the surfaces of the two molecules must also be complementary. Regions of positive excess charge on the surface of one molecule must fit closely with regions of negative excess charge on the other if the two are to bind maximally. Thus the language of molecules is not only a language of contours, but also a language of charge distributions.

8.2 Paul Ehrlich, the father of chemotherapy

The first real understanding of the mechanism of the immune system was due to the work of Paul Ehrlich and Ilya Mechnikov, and in 1908 they shared a Nobel Prize for this work. Paul

street in Frankfurt was named after him!

Ehrlich can be said to be the discoverer of biological specificity. As a young medical student at the University of Strasbourg, he was fortunate to work under the distinguished chemist Heinrich von Waldeyer, who took a great interest in Ehrlich. Stimulated by Waldeyer, Ehrlich began to do experiments in which he prepared thin slices of various tissues for microscopic examination by staining them with the newly discovered aniline dyes. During the last half of the 19th century, there was a great deal of interest in histological staining. It was during this period that Walther Flemming in Germany discovered chromosomes by staining them with special dyes, and Christian Gram in Denmark showed that bacteria can be classified into two types by staining methods. (We now call these two types "gram positive" and "gram negative"). During this same period, and while he was still a student, Paul Ehrlich made the important discovery that mammalian blood contains three different types of white cells which can be distinguished by staining.

Ehrlich's early work on staining made him famous, and it also gave him a set of theories which led him to his great discoveries in immunology and chemotherapy. According to Ehrlich's ideas, the color of the aniline dyes is due to the aniline ring. However, dyes used commercially must also adhere to fabrics, and this adherence, according to Ehrlich, is due to the specific structure of the side chains. If the pattern of atoms on a side chain is complementary to the pattern of atoms on the binding site, the dye will adhere, but otherwise not. Thus there is a "lock and key" mechanism, and for this reason dyes with specific side chains stain specific types of tissue.

In one of his experiments, Paul Ehrlich injected methylene blue into the ear of a living rabbit, and found that it stained only the nerve endings of the rabbit. Since the rabbit seemed to be unharmed by the treatment, the experiment suggested to Ehrlich that it might be possible to find antibacterial substances which could be safely injected into the bloodstream of a patient suffering from an infectious disease. Ehrlich hoped to find substances which would adhere selectively to the bacteria, while leaving the tissues of the patient untouched.

With the help of a large laboratory especially constructed for him in Frankfurt, the center of the German dye industry, Ehrlich began to screen thousands of modified dyes and other compounds. In this way he discovered trypan red, a chemical treatment for sleeping sickness, and arsphenamine, a drug which would cure syphilis. Ehrlich thus became the father of modern chemotherapy. His success pointed the way to Gerhard Domagk, who discovered the sulphonamide drugs in the 1930s, and to Fleming, Waksman, Dubos and others, who discovered the antibiotics.

Ehrlich believed that in the operation of the immune system, the body produces molecules which have a pattern of atoms complementary to patterns (antigens) on invading bacteria, and that these molecules (antibodies) in the blood stream kill the bacteria by adhering to them.

Figure 8.2: **Paul Ehrlich (1854-1915). By the time that he developed a drug that could cure syphilis, he had already received the Nobel Prize for Physiology or Medicine, but to further honor Ehrlich, a street in Frankfurt was named after him**

Figure 8.3: **Dr. Paul Ehrlich and his assistant Dr. Sahachiro Hata. They worked together to find cures for many diseases.**

Figure 8.4: **A West German postage stamp (1954) commemorating Paul Ehrlich and Emil von Behring, who worked together at Robert Koch's suggestion, producing a drug that could cure diptheria.**

8.3 Mechnikov

Meanwhile, the Russian naturalist Ilya Mechnikov discovered another mechanism by which the immune system operates. While on vacation in Sicily, Mechnikov was studying the digestive process in starfish larvae. In order to do this, he introduced some particles of carmine into the larvae. The starfish larvae were completely transparent, and thus Mechnikov could look through his microscope and see what happened to the particles. He saw that they were enveloped and apparently digested by wandering amoebalike cells inside the starfish larvae. As he watched this process, it suddenly occurred to Mechnikov that our white cells might similarly envelop and digest bacteria, thus protecting us from infection. Describing this discovery, Mechnikov wrote in his diary: "I suddenly became a pathologist! Feeling that there was in this idea something of surpassing interest, I became so excited that I began striding up and down the room, and even went to the seashore to collect my thoughts."

Mechnikov later named the white cells "phagocytes" (which means "eating cells"). He was able to show experimentally that phagocytosis (i.e., the envelop- ment and digestion of bacteria by phagocytes) is an important mechanism in immunity.

Metchnikov's ideas were not immediately accepted. Wikipedia states that "His theory, that certain white blood cells could engulf and destroy harmful bodies such as bacteria, met with scepticism from leading specialists including Louis Pasteur, Behring and others. At the time, most bacteriologists believed that white blood cells ingested pathogens and then spread them further through the body. His major supporter was Rudolf Virchow, who published his research in his *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin* (now called the Virchows Archiv). His discovery of these phagocytes ultimately won him the Nobel Prize in 1908."

For a number of years, there were bitter arguments between those who thought that the immune system operates through phagocytosis, and those who thought that it operates through antibodies. Finally it was found that both mechanisms play a role. In phagocytosis, the bacterium will not be ingested by the phagocyte unless it is first studded with antibodies. Thus both Mechnikov and Ehrlich were proved to be right.

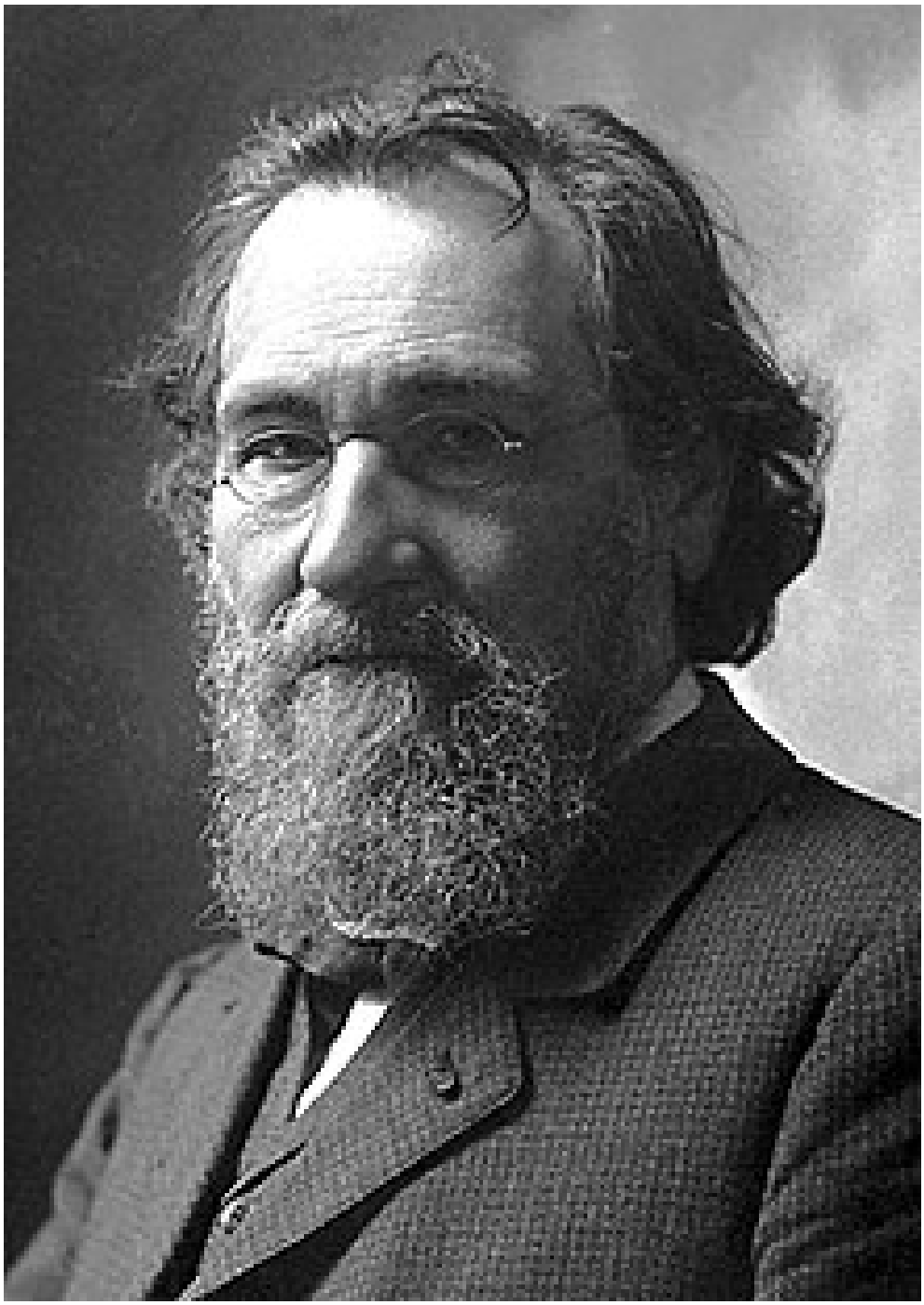

Figure 8.5: **Ilya Mechnikov (1845-1916), sometimes spelled Élie Metchnikoff. He shared the 1908 Nobel Prize in Physiology or Medicine with Paul Ehrlich. Mechnikov has been called "the father of immunology" because of his discovery of phagocytosis.**

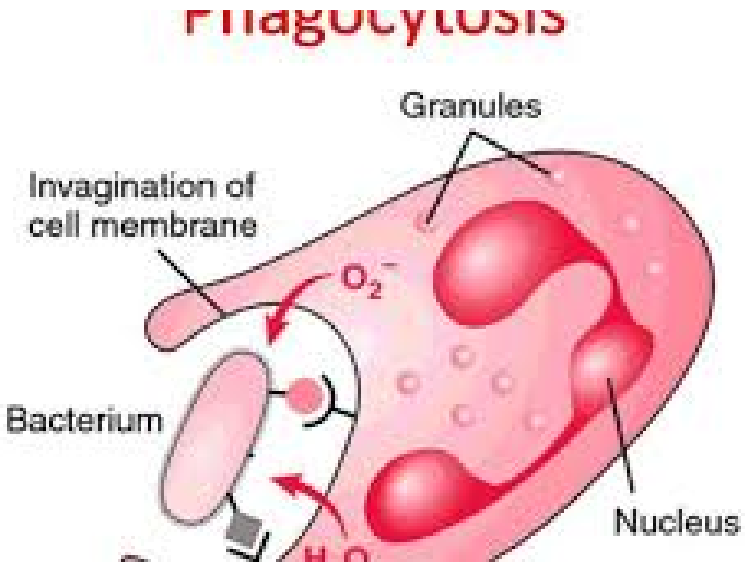

Figure 8.6: **Phagocytosis: A lymphocyte "eats" a bacterium, but only if it is coated with the right antigens.**

8.4 Burnet, Jerne and the clonal theory of immunity

As everyone knows, recovery from an infectious disease involves a response of our immune systems. Recovery occurs after the immune system had had some time to respond, and a recovered patient generally has some immunity to the disease.

During the 20th century, there were conflicting ideas about how and why this process occurs. One of these theories was proposed by Linus Pauling, who thought that an antigen on the surface of a bacteria or virus provides a template, and that the immune system uses this template to produce the specific antibodies needed to combat the disease. However, experimental evidence accumulated showing Pauling's template theory to be wrong and supporting the clonal theory of immunity proposed by Sir Frank Macfarlane Burnet and Niels Kai Jerne.

According to the clonal theory of immunity, there are extremely many strains of lymphocytes, each of which produces a specific single antibody. Populations of all these many strains are always present in small numbers. When a patient becomes ill with an infection, the antigens of the ingesting bacteria or virus stimulate one specific strain of lymphocyte to reproduce itself in large numbers, i.e. to become a clone. This large population produces exactly the right antibodies needed to combat the disease, and the large population remains after recovery, conferring continued immunity.

In order for the immune system not to attack the cells of our own bodies, a learning process must take place, early in our lives, in which the difference between self and non-self is established, and the lymphocyte strains that attack self are suppressed. Jerne postulated (correctly) that this learnubg process takes place in the thymus gland, which is very large in infants, and much smaller in adults.

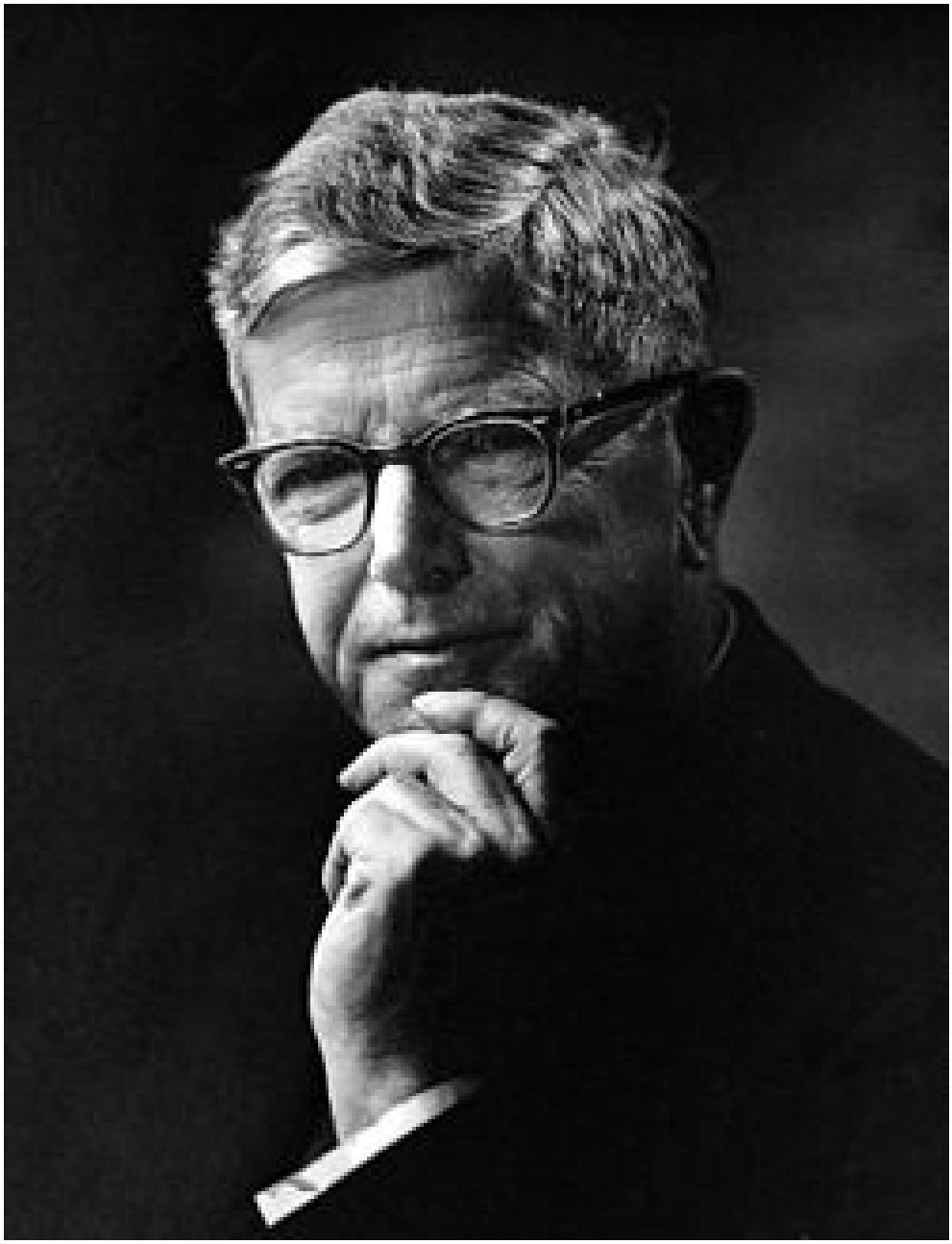

Figure 8.7: **Sir Frank Macfarlane Burnet (1899-1995). Both he and Niels Kai Jerne proposed the clonal theory of immunity.**

Figure 8.8: **The Danish immunologist Niels Kai Jerne (1911-1994). He shared the 1984 Nobel Prize for Physiology or Medicine with Georges Köhler and César Milstein "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies".**

Figure 8.9: **Georges Köhler (1046-1995).**

Figure 8.10: **César Milstein (1927-2002).**

8.5 Köhler, Milstein and monoclonal antibodies

Once the clonal theory of immunity became established, the way seemed open to clone in vitro B lymphocytes of a predetermined specificity. However, such clone cannot be made to live forever because like all other cells, except cancer cells, they are subject to "programed cell death". To overcome this difficulty, Georges Köhler and César Milstein found a way to give the desired lymphocytes immortality by fusing them with myoloma cells, thus producing clones that could be cultured indefinitely.

The Wikipedia article on Monoclonal Antibodies states that "In the 1970s, the B-cell cancer multiple myoloma was known. It was understood that these cancerous B-cells all produce a single type of antibody (a paraprotein). This was used to study the structure of antibodies, but it was not yet possible to produce identical antibodies specific to a given antigen.

"In 1975, Georges Köhler and César Milstein succeeded in making fusions of myeloma cell lines with B cells to create hybridomas that could produce antibodies, specific to known antigens and that were immortalized. They and Niels Kaj Jerne shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery.

"In 1988, Greg Winter and his team pioneered the techniques to humanize monoclonal antibodies, eliminating the reactions that many monoclonal antibodies caused in some patients.

"In 2018, James P. Allison and Tasuku Honjo received the Nobel Prize in Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation, using monoclonal antibodies that prevent inhibitory linkages."

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Chapter 9

FLEMING, FLOREY AND CHAIN

9.1 Fleming

Education

Alexander Fleming was born in Ayrshire, Scotland in 1881, where his parents had a farm. Following in his elder brother's footsteps, he studied medicine, enrolling at St. Mary's Hospital Medical School in London. After serving in the Royal Army Medical Corps during World War I, he returned to St. Mary's, and was elected Professor of Bacteriology in 1928.

Treating the wounds of soldiers

While treating wounded soldiers during the First World War, Fleming had noticed that the antiseptics commonly applied to wounds did more harm than good. These antiseptics killed bacteria on the surface of wounds, but below, untouched by the antiseptics, anaerobic bacteria continued the infection, and the body's natural defenses were damaged by the antiseptics. Fleming published these observations, but the practice of treating wounds with strong antiseptics nevertheless continued.

The discovery of lysozyme

After the war, continuing his work at St. Mary's Hospital, Fleming searched for effective antibacterial substances. The first that he discovered was the enzyme lysozyme, which he found in the nasal secretions of a patient with a heavy cold. Working with lysozyme, he was disappointed to find that it was effective only against relatively harmless bacteria. In fact the reason those bacteria are harmless is that our bodies are already heavily armed with lysozyme. It occurs in tears, saliva, skin, hair and nails as well as mucus. In nature, egg whites contain large amounts of lysozyme.

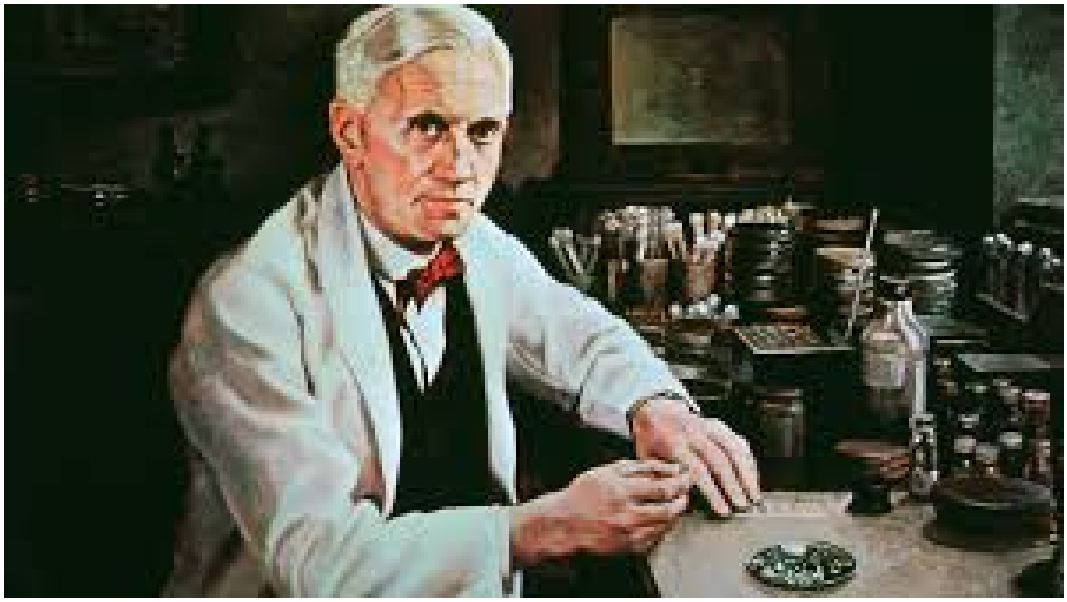

Figure 9.1: **Sir Alexander Fleming (1881-1955).**

The discovery of penicillin

"One sometimes finds, what one is not looking for. When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did." Alexander Fleming

Fleming was a brilliant researcher, but his laboratory was often messy. When he left with his family for a vacation in August, 1928, a jumble of petri dishes with staphylococci cultures were piled in a corner of the laboratory. Returning, a month later, Fleming noticed a mold growing in one of the culture dishes. Around the mold, the staphylococci were dead. He showed the dish to his former assistant, Merlyn Pryce. who said: "That's how you discovered lysozyme".

The Wikipedia article on the history of penicillin states that "The Scottish physician Alexander Fleming was the first to suggest that a Penicillium mold must secrete an antibacterial substance, and the first to concentrate the active substance involved, which he named penicillin, in 1928. Penicillin was the first modern antibiotic. During the next twelve years Fleming grew, distributed, and studied the original mold, which was determined to be a rare variant of Penicillium notatum (now Penicillium chrysogenum)."

Fleming was not the first person to suggest that molds could be used to treat infections. In fact the use of molds for this purpose has been known since ancient times. But it was Fleming's work that initiated the modern mass production and use of antibiotics.

Figure 9.2: **Fleming (center) receiving the Nobel prize from King Gustav V of Sweden (right) in 1945.**

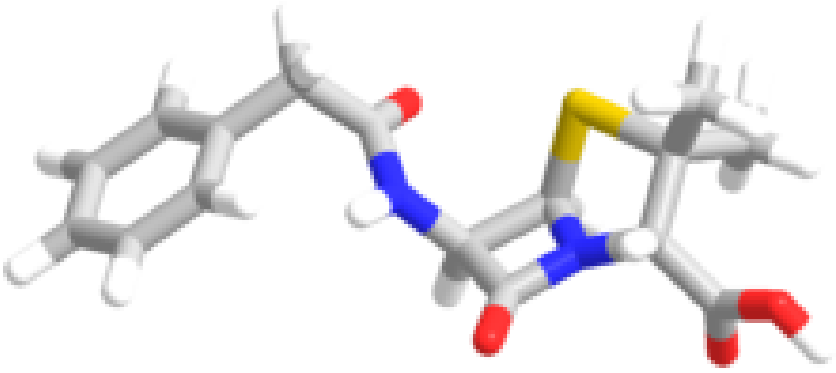

Figure 9.3: **3D-model of benzylpenicillin.**

Figure 9.4: **Faroe Islands postage stamp commemorating Fleming.**

Honors and awards

- Fleming, Florey and Chain jointly received the Nobel Prize in Medicine in 1945. According to the rules of the Nobel committee a maximum of three people may share the prize. Fleming's Nobel Prize medal was acquired by the National Museums of Scotland in 1989 and is on display after the museum re-opened in 2011.
- Fleming was a member of the Pontifical Academy of Sciences.
- Fleming was elected a Fellow of the Royal Society (FRS) in 1943.
- Fleming was awarded the Hunterian Professorship by the Royal College of Surgeons of England.
- Fleming was knighted, as a Knight Bachelor, by king George VI in 1944.
- He was made a Knight Grand Cross of the Order of Alfonso X the Wise in 1948.
- In 1999, Time magazine named Fleming one of the 100 Most Important People of the 20th century, statiimg: "It was a discovery that would change the course of history. The active ingredient in that mould, which Fleming named penicillin, turned out to be an infection-fighting agent of enormous potency. When it was finally recognized for what it was, the most efficacious life-saving drug in the world, penicillin would alter forever the treatment of bacterial infections. By the middle of the century, Fleming's discovery had spawned a huge pharmaceutical industry, churning out synthetic penicillin that would conquer some of mankind's most ancient scourges, including syphilis, gangrene and tuberculosis."

- The importance of his work was recognized by the placement of an International Historic Chemical Landmark plaque at the Alexander Fleming Laboratory Museum in London on November 19, 1999.
- When 2000 was approaching, at least three large Swedish magazines ranked penicillin as the most important discovery of the millennium.
- In 2002, Fleming was named in the BBC's list of the 100 Greatest Britons following a nationwide vote.
- A statue of Alexander Fleming stands outside the main bullring in Madrid, Plaza de Toros de Las Ventas. It was erected by subscription from grateful matadors, as penicillin greatly reduced the number of deaths in the bullring.
- Flemingovo námestÃ is a square named after Fleming in the university area of the Dejvice community in Prague.
- A secondary school is named after him in Sofia, Bulgaria.
- In Athens, a small square in the downtown district of Votanikos is named after Fleming and bears his bust. There are also a number of Streets in greater Athens and other towns in Greece named after either Fleming or his Greek second wife Amalia.
- In mid-2009, Fleming was commemorated on a new series of banknotes issued by the Clydesdale Bank; his image appears on the new issue of Â£5 notes.
- In 2009, Fleming was voted third greatest Scot in an opinion poll conducted by STV, behind only Scotland's national poet Robert Burns and national hero William Wallace.
- 91006 Fleming, an asteroid in the Asteroid Belt, is named after Fleming.
- Fleming station, on the Thessaloniki Metro system, takes its name from Fleming Street on which it is located, which in term is named after him.
- Sir Alexander Fleming College, a British school in Trujillo, northern Peru

9.2 Florey and Chain

Oxford University takes up the challenge

Alexander Fleming had been unable to produce large quantities of penicillin and to make it stable, so he became discouraged about the practical possibilities of using on a large scale as an antibacterial agent. However, a group of researchers at Oxford University in the department of the Professor of Pathology, Howard Florey, took up the challenge. Many researchers were involved in the effort to produce penicillin on a large scale and to make it in a stable form. At times the whole department was involved in the work, but the contributions of Ernst Boris Chain, Norman Heatley and Edward Abraham were especially important, especially those of Chain. In 1945 Chain shared the Nobel Prize in Physiology or Medicine with Fleming and Florey.

Figure 9.5: **Sir Howard Florey (1898-1968), later Lord Florey.**

Figure 9.6: **An Australian banknote with Florey's image.**

Figure 9.7: **Sir Ernst Boris Chain in 1945.**

Figure 9.8: **Ernst Chain in his laboratory.**

Figure 9.9: **Dr Ernst Chain undertakes an experiment in his office at the School of Pathology at Oxford University in 1944.**

9.3 War between micro-organisms

Antibiotics are the chemical weapons of microorganisms

Bacteria, viruses and molds do not live peacefully together. They are constantly at war. They kill each other with chemical weapons. Alexander Fleming was lucky enough to discover one of the chemical weapons with which molds fight against bacteria, but there are many many others. There are also extremely many chemical weapons used by bacteria to fight against each other. Finally some viruses, known as bacteriophages, attack and kill bacteria. Each of these cases offers humans new weapons in their fight against infectious disease.

The weapons of bacteria against other bacteria

If we grow cultures of two different species of bacteria on the same culture medium in a single petri dish, then often, after a few days, we will notice that one species of bacteria has died when it came in contact with the other. Such an event offers us the possibility of developing a new antibiotic. We merely need to culture, on a large scale, the bacterial strain that has successfully killed the other. Then we can isolate the active chemical agent. Research using the method just described is in progress to discover new antibiotics.

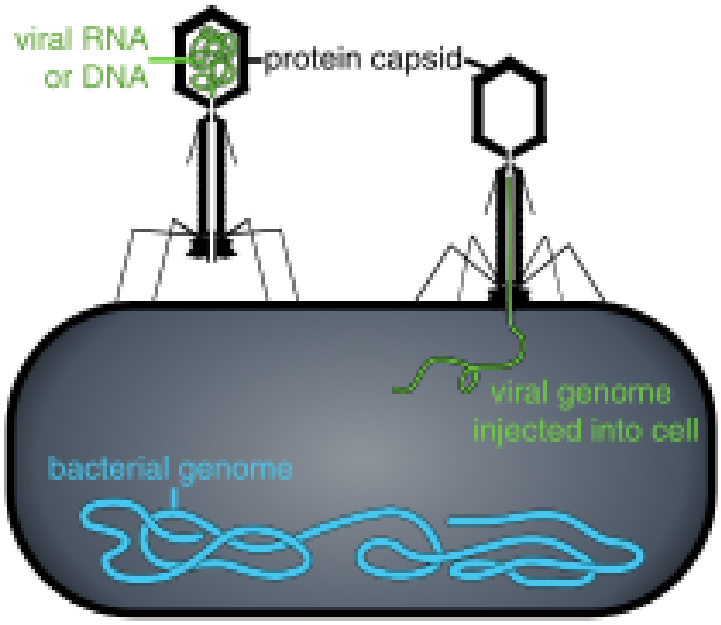

Figure 9.10: **Phage injecting its genome into bacterial cell.**

The use of bacteriophages in medicine

The Wikipedia article on Phage Therapy states that "The discovery of bacteriophages was reported by the Englishman Frederick Twort in 1915, and the French-Canadian Felix d'Hérelle in 1917. D'Hérelle said that the phages always appeared in the stools of *Shigella* dysentery patients shortly before they began to recover. He "quickly learned that bacteriophages are found wherever bacteria thrive: in sewers, in rivers that catch waste runoff from pipes, and in the stools of convalescent patients". Phage therapy was immediately recognized by many to be a key way forward for the eradication of pathogenic bacterial infections. A Georgian, George Eliava, was making similar discoveries. He travelled to the Pasteur Institute in Paris where he met d'Hérelle, and in 1923 he founded the Eliava Institute in Tbilisi, Georgia, devoted to the development of phage therapy. Phage therapy is used in Russia, Georgia and Poland...

"Isolated from Western advances in antibiotic production in the 1940s, Russian scientists continued to develop already successful phage therapy to treat the wounds of soldiers in field hospitals. During World War II, the Soviet Union used bacteriophages to treat many soldiers infected with various bacterial diseases e.g. dysentery and gangrene. Russian researchers continued to develop and to refine their treatments and to publish their research and results. However, due to the scientific barriers of the Cold War, this knowledge was not translated and did not proliferate across the world. A summary of these publications was published in English in 2009 in *A Literature Review of the Practical Application of Bacteriophage Research*."

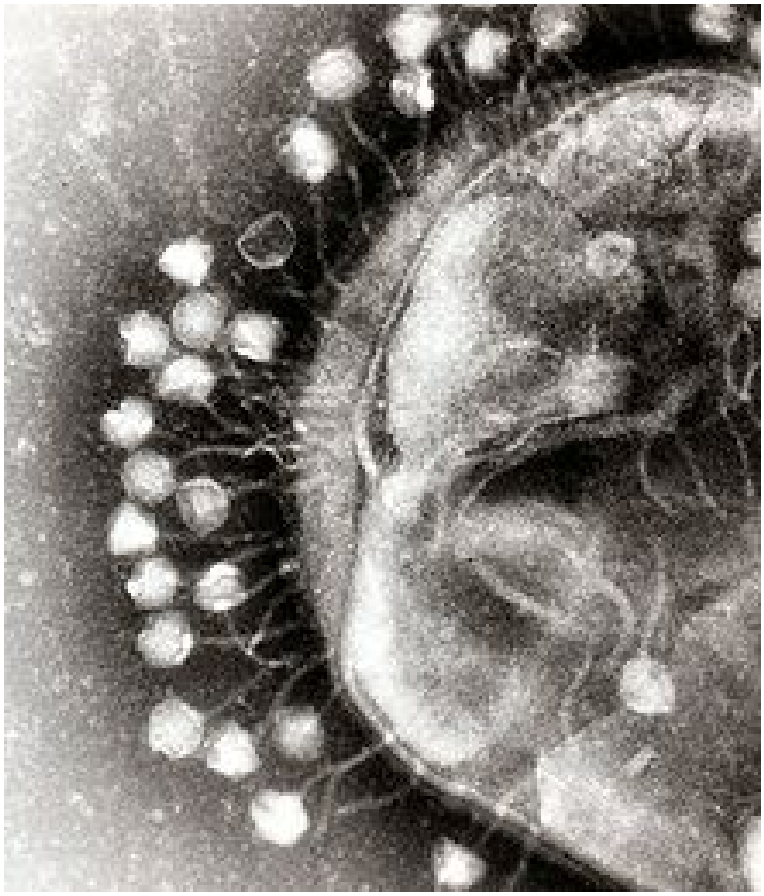

Figure 9.11: **An electron micrograph of bacteriophages attached to a bacterial cell. These viruses are the size and shape of coliphage T1.**

Figure 9.12: **Frederick Twort (1877-1950) discovered in 1915 that phages infect bacteria.**

Figure 9.13: **Félix d'Hérelle (1873-1949), co-discoverer of phages and pioneer of phage therapy.**

9.4 Overuse of antibiotics in agriculture

Pharming

A major global public health crisis may soon be produced by the wholesale use of antibiotics in the food of healthy farm animals. The resistance factors produced by shoveling antibiotics into animal food produces resistance factors (plasmids) which can easily be transferred to human pathogens. Pharming (instead of farming) is not a joke. It is a serious threat.[1]

Plasmids

Bacteria belong to a class of organisms (prokaryotes) whose cells do not have a nucleus. Instead, the DNA of the bacterial chromosome is arranged in a large loop

In the early 1950's, Joshua Lederberg discovered that bacteria can exchange genetic information. He found that a frequently-exchanged gene, the F-factor (which conferred fertility), was not linked to other bacterial genes; and he deduced that the DNA of the F-factor was not physically a part of the main bacterial chromosome. In 1952, Lederberg coined the word "plasmid" to denote any extrachromosomal genetic system.

In 1959, it was discovered in Japan that genes for resistance to antibiotics can be exchanged between bacteria; and the name "R-factors" was given to these genes. Like the F-factors, the R-factors did not seem to be part of the main loop of bacterial DNA. Because of the medical implications of this discovery, much attention was focused on the R-factors. It was found that they were plasmids, small loops of DNA existing inside the bacterial cell, but not attached to the bacterial chromosome. Further study showed that, in general, between one percent and three percent of bacterial genetic information is carried by plasmids, which can be exchanged freely even between different species of bacteria.

In the words of the microbiologist, Richard Novick, "Appreciation of the role of plasmids has produced a rather dramatic shift in biologists' thinking about genetics. The traditional view was that the genetic makeup of a species was about the same from one cell to another, and was constant over long periods of time. Now a significant proportion of genetic traits are known to be variable (present in some individual cells or strains, absent in others), labile (subject to frequent loss or gain) and mobile, all because those traits are associated with plasmids or other atypical genetic systems."

According to Ecowatch, "Roughly 80 percent of antibiotics purchased in the U.S. are fed to livestock to accelerate growth and prevent disease in healthy animals. Yet this

[1] http://ecowatch.com/2014/03/06/misuse-antibiotics-fatal-superbug-crisis/
http://ecowatch.com/2013/12/06/8-scary-facts-about-antibiotic-resistance/
http://ecowatch.com/2015/03/27/obama-fight-superbug-crisis/
http://ecowatch.com/2014/03/12/fda-regulation-antibiotics-factory-farms/
http://www.bbc.com/news/health-35153795
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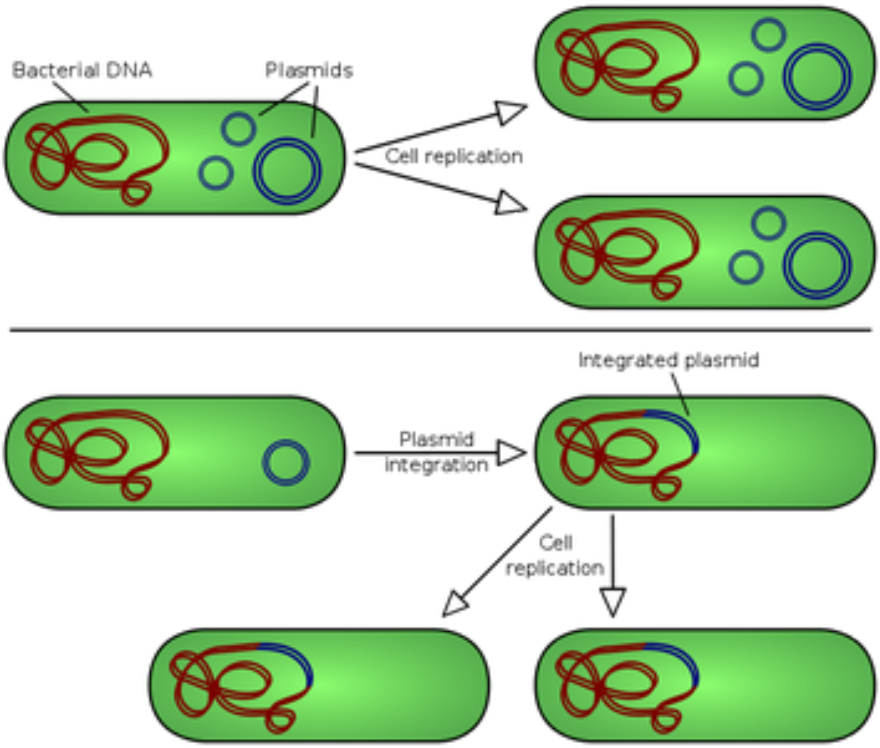

Figure 9.14: **There are two types of plasmid integration into a host bacteria: Non-integrating plasmids replicate as with the top instance, whereas episomes, the lower example, can integrate into the host.**

seemingly harmless practice also breeds superbugs, which can spread in the environment, contaminate food supplies and undermine the effectiveness of antibiotics.

"Antibiotic-resistant infections, like staphylococcus aureus, sicken at least 2 million Americans per year and kill more than 23,000, according to a 2013 CDC report. Those infections can happen anywhere, but they're especially deadly when they're spread in hospitals, nursing homes or other health care centers.

"Now the crisis is slowly worsening as drugmakers spend less time and money creating new antibiotics, even as more bacteria are becoming resistant to older drugs."

Suggestions for further reading

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Chapter 10

SZENT-GYÖRGYI

10.1 Summer work at Szent-Györgyi's laboratory

During the summers of 1960 and 1961, while I was still a postgraduate student in theoretical physics at the University of Chicago, I had the privilege of spending two summers working in the laboratory of the great Hungarian-American physiologist and biochemist, Albert Szent-Györgyi. He was famous for isolating vitamin C and for discovering the molecular mechanism of muscle contraction. But more importantly, he founded a new field of study: Bioenergetics.

Szent-Györgyi wondered how the chemical energy from food is harnessed to do mechanical work or to drive our metabolisms. He reasoned that there must be structures in living organisms which are analogous to the structures of engines. If you pour gasoline onto the street and set fire to it, no useful work results, only heat But if you burn it inside an engine, the chemical energy of the gasoline can be converted into useful mechanical work.

Following this line of thought, Szent-Györgyi looked for energy-transducing structures in the tissues of living organisms. Among the structures that caught Szent-Györgyi's attention were mitochondria, which power the metabolism of all animals, and he also studied the microscopic photosynthetic unit (thylakoids) in plants. After some years of work, he became convinced that quantum theory was needed in order to gain a complete understanding of how these microscopic engines work. Therefore he spent a year at the Institute for Advanced Study in Princeton, where he learned quite a lot of quantum theory.

Although he knew enough quantum theory to understand what physicists were talking about, he nevertheless thought that for the research which he wanted to undertake, he needed to collaborate with people whose whole education was in that field, and he brought some theoretical physicists (including me) to his laboratory. During the time that I was there, we worked to obtain a quantum theoretical understanding of the mechanism of the primary process in photosynthesis, where the energy of a photon is stabilized and trapped, ready to drive the synthesis of sugars.

I had heard about Albert Szent-Györgyi before the opportunity to work in his labora-

Figure 10.1: **Albert Szent-Györgyi in Italy in 1917.**

Figure 10.2: **Albert Szent-Györgyi in 1937, when he won the Nobel Prize in Physiology or Medicine. The prize was awarded partly for his work on the biochemistry of respiration, and partly for his isolation of vitamin C.**

Figure 10.3: **Szent-Györgyi working in his laboratory.**

tory presented itself. My brother Gordon had worked at the Woods Hole Marine Biological Laboratory during a previous summer and had told me that he considered Szent-Györgyi to be a great genius. Also, a University of Chicago classmate, David Freifelder, had said to me "You absolutely must read Szent-Györgyi's book, 'Bioenergetics'!"

10.2 Muscle contraction

Here are some excerpts from an article by Jack A. Roll, entitled *Generation of life in a test tube: Albert Szent-Györgyi, Bruno Straub, and the discovery of actin.* The article was published on 20 April, 2918 in Advances in Physiology Education[1]- Bruno Straub was Szent-Györgyi's student, with whom he collaborated on the work.

"Albert Szent-Györgyi, at 44 years of age, won the Nobel Prize in 1937 for his work on vitamin C and the establishment of the groundwork of the citric acid cycle. He now wanted to investigate one of the fundamental aspects of life and settled on the study of muscle contraction. The Szent-Györgyi laboratory in Hungary during World War II demonstrated that contraction could be reproduced in vitro by threads consisting of just two proteins, myosin and the newly discovered protein by Bruno Straub that they called actin. Szent-Györgyi called seeing the contraction of these threads, which occurred in the presence of ATP and ions, "the most thrilling moment" of his scientific life.

This major discovery of the generation of "life" in a test tube was totally unknown for years by the rest of the world because of the war. When the discovery was finally communicated to the world, it was not immediately accepted by all as being relevant to the physiology of muscle contraction.

10.3 Mitochondria

Mitochondria are believed to be descended from free-living bacteria. According to one theory for their evolution, they were engulfed and eaten by an ancient eukariotic cell, i.e. a large amoeba-like cell containing a nucleus and many organelles. The free-living bacteria thus eaten somehow escaped complete digestion and an endosymbiotic relationship was formed. This event may have occurred when the atmosphere of the earth changed from being reducing to oxidizing, because of the oxygen produced by plants. The benefit conferred by the symbiosis was the ability to perform oxidative phosphorolation, i.e. the synthesize ATP in an oxidizing atmosphere. Since that time, eukaryotes have contained mitochondria.

[1] https://www.physiology.org/doi/full/10.1152/advan.00189.2017

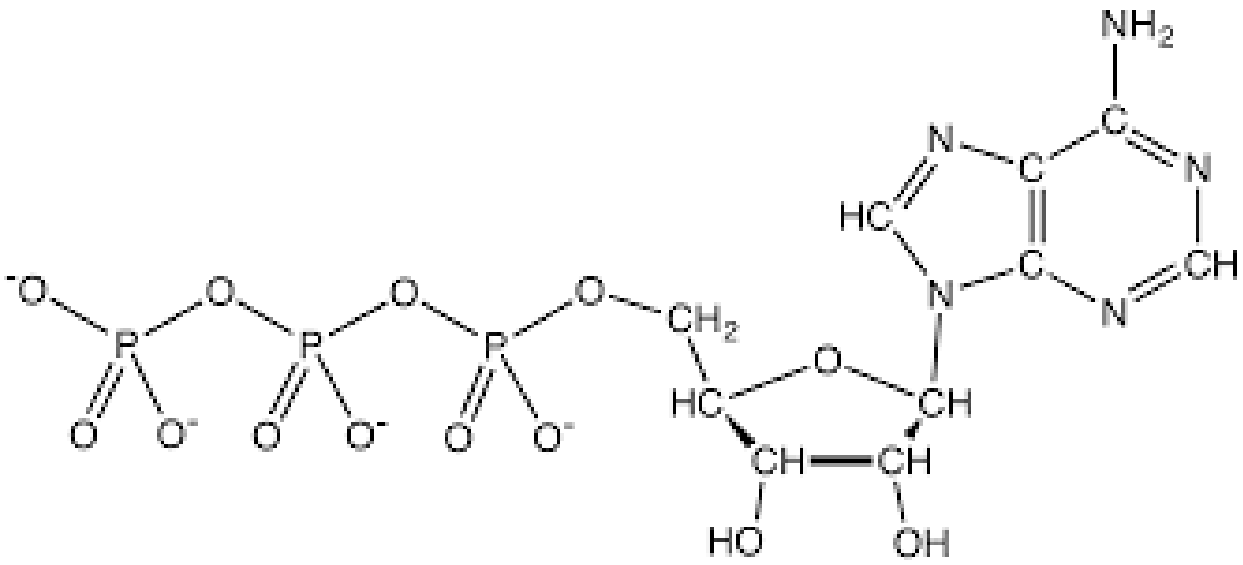

Figure 10.4: **The adinosine triphosphate (ATP) molecule acts as a universal fuel for both muscle contraction and metabolic processes within our bodies. Mitochondria use the stored chemical energy of sugars to synthesize ATP.**

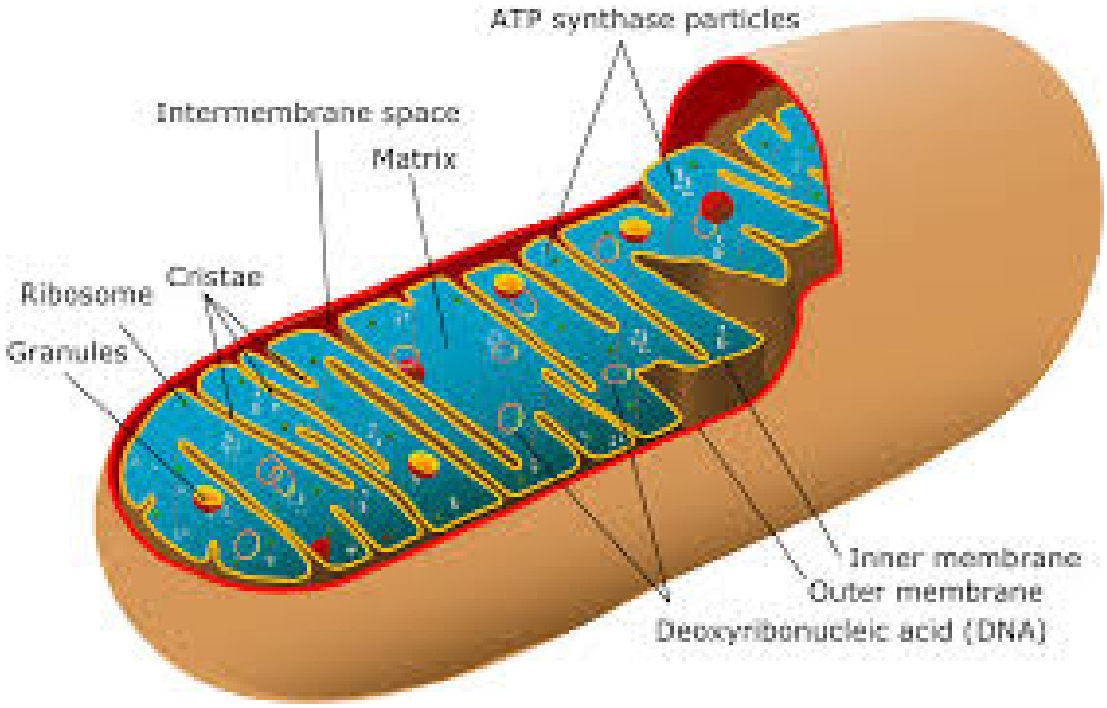

Figure 10.5: **Mitochondria contain membrane-bound enzymes that use the chemical energy of sugars to produce the high-energy phosphate bonds of adinosine triphosphate (ATP).**

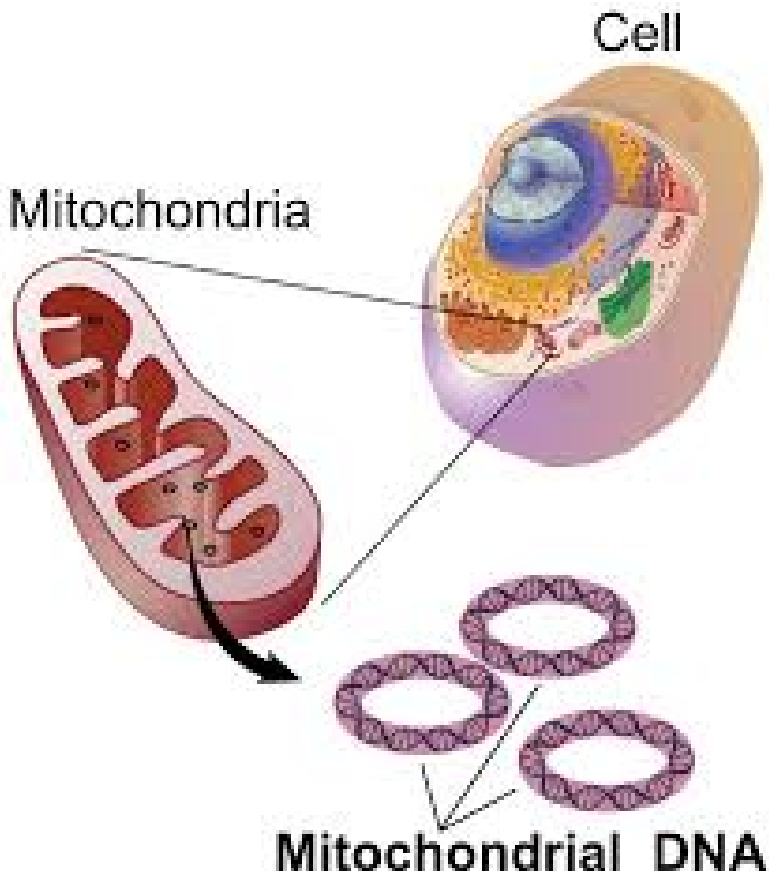

Figure 10.6: **Mitochondria are thought to be descended from free-living organisms, as is shown in Figure 12.6, and they have their own DNA.**

10.4 The photosynthetic unit

Like mitochondria, the chloroplasts that contain the photosynthetic unit of plants are thought to he the descendents of free-living cyanaobacteria, as is shown in Figure 12.6. Inside the chloroplasts are pocket-like structures called *thylakoids*. The membrane of thylakoids is like a sandwich. The middle part of this sandwich consists of pigment molecules, for example chloraphyl, which absorb the light, and produce an electron-hole pair. The outer layer of the thylakoid membrane sandwich consists of charge donor molecules, i.e. molecules whose highest filled molecular orbital is relatively high in energy, while the innermost layer consists of charge acceptor molecules, that is, molecules whose lowest empty orbital is quite low in energy. After a ohoton has been absorbed, the electron migrates to the charge.acceptors, while the hole migrates to the electron-donor molecules on the outside. Thus the electron and hole are rapidly separated, and the back-reaction is prevented. The mechanism is similar to the separation of the charge and hole in a silicon solar cell.

The Calvin cycle (the dark reaction)

After the primary process of photon absorption and charge-hole separation has taken place in the thylakoid, the available energy is stabilized in a dark reaction studies by Melvin Calvin (1911-1997) and his co.workers at the University of California, Berkeley. In the dark reaction, which is known as the *Calvin cycle*, the energy originally derived from absorption of a photon is further stabilized by being converted into the chemical energy of sugars. Calvin also contributed importantly to theories of the origin of life, and he is the author of a book entitles *Chemical Evolution Towards the Origin of Life On Earth and Elsewhere*. He was awarded the Nobel Prize for Chemistry in 1961.

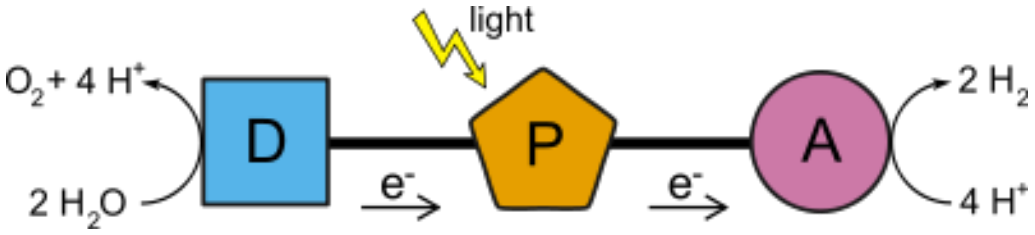

Figure 10.7: **The donor-pigment-acceptor triad needed for charge-hole separation.**

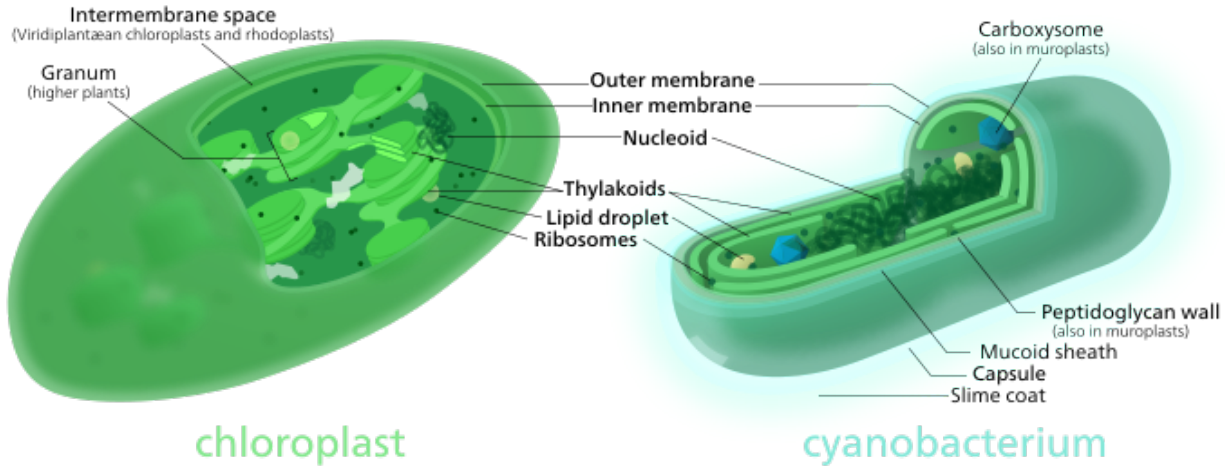

Figure 10.8: **Like mitochondria, chloroplasts were once free-living organisms, as is shown in Figure 12.6. Both chloroplasts and cyanobacteria have a double membrane, DNA, ribosomes, and thylakoids. Both the chloroplast and cyanobacterium depicted are idealized versions (the chloroplast is that of a higher plant) - a lot of diversity exists among chloroplasts and cyanobacteria.**

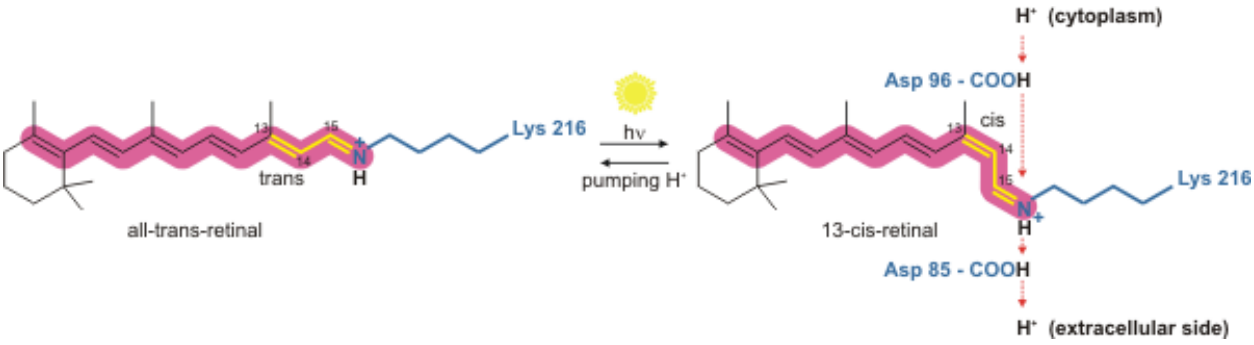

Figure 10.9: **Bacterial rhodopsin is interesting because it is a single molecule which is embedded in the membrane of the salt-loving bacterium *halobacterium halobium*, and which is capable of using the energy of sunlight to pump H^+ ions across the membrane against the electrochemical gradient. The molecule is almost identical to rhodopsin that occurs in our eyes. Perhaps, when our remote ancestors lived in the sea, they had a symbiotic relationship with halobacteria which led to the evolution of the vertebrate eye.**

10.5 Some of Albert Szent-Györgyi's personal reflections

On my Mother's side, I am the fourth generation of scientists. My Father was interested only in farming and so my Mother's influence prevailed. Music filled the house and the conversation at the table roamed about the intellectual achievements of the entire world. Politics and finance had no place in our thoughts. I am a scientist, myself, because at an early age I learned that only intellectual values were worth striving for, artistic or scientific creation being the highest aim. I strongly believe that we establish the coordinates of our evaluation at a very early age. What we do later depends on this scale of values which mostly cannot be changed later.

I wanted to understand life but found the complexity of physiology overÂ whelming. So I shifted to pharmacology where, at least, one of the partners, the drug, was simple. This, I found, did not relieve the difficulty. So, I went into bacteriology, but found bacteria too complex, too. I shifted on, to physicochemistry and then to chemistry, that is, to molecules, the smallest units in those days. Ten years ago I found molecules too complex and shifted to electrons, hoping to have reached bottom. But Nature has no bottom: its most basic principle is "organization." If Nature puts two things together she produces something new with new qualities, which cannot be expr essed in terms of qualities of the components. When going from electrons and protons to atoms, from here to molecules, molecular aggregates, etc., up to the cell or the whole animal, at every level we find something new, a new breathtaking vista. Whenever we separate two things, we lose something, something which may have been the most essential feature. So now, at 68, I am to work my way up again following electrons in their motion through more extensive systems, hoping to arrive, someday, at an understanding of the cellular level of organization. So the internal course of my life made a smooth sinusoid curve; not so the external course.

Lost in the 20th Century

Here are a few quotations from Albert Szent-Györgyi's autobiographical book, *Lost in the 20th Century*:

Overlooking my case history, I find a complete dichotomy. On the one hand, my inner story is exceedingly simple, if not indeed dull: my life has been devoted to science and my only real ambition has been to contribute to it and live up to its standards. In complete contradiction to this, the external course has been rather bumpy. I finished school in feudal Hungary as the son of a wealthy landowner and I had no worries about my future. A few

years later I find myself working in Hamburg, Germany, with a slight hunger edema. In 1942 I find myself in Istanbul, involved in secret diplomatic activÂ ity with a setting fit for a cheap and exciting spy story. Shortly after, I get a warning that Hitler had ordered the Governor of Hungary to appear before him, screaming my name at the top of his voice and demanding my delivery. Arrest warrants were passed out even against members of my family. In my pocket I find a Swedish passport, having been made a full Swedish citizen on the order of the King of Sweden-I am "Mr. Swenson," my wife, "M rs. Swenson." Sometime later I find myself in Moscow, treated in the most royal fashion by the Government (with caviar three times a day), but it does not take long before I am declared "a traitor of the people" and I play the role of the villain on the stages of Budapest. At the same time, I am refused entrance to the USA for my Soviet sympathies. Eventually, I find peace at Woods Hole, Massachusetts, working in a solitary corner of the Marine Biological Laboratory. After some nerve-racking complications, due to McCarthy, things straightened out, but the internal struggle is not comÂ pletely over . .I am troubled by grave doubts about the usefulness of scientific endeavor and have a whole drawer filled with treatises on politics and their relation to science, written for myself with the sole purpose of clarifying my mind, and finding an answer to the question: will science lead to the elevation or destruction of man, and has my scientific endeavor any sense? All this, in itself, would have no interest. There are many who did more for science, were braver, suffered more agony and even paid the penalty of death. What may lend interest to my story is that it reflects the turbulence of our days.

A fearless advocate of peace and rationality

Albert Szent-Györgyi spoke and wrote fearlessly against the institution of war. Here is a quotation from his writing:

The story of man consists of two parts, divided by the appearance of modern science... In the first period, man lived in the world in which his species was born and to which his senses were adapted. In the second, man stepped into a new, cosmic world to which he was a complete stranger... The forces at man's disposal were no longer terrestrial forces, of human dimension, but were cosmic forces, the forces which shaped the universe. The few hundred Fahrenheit degrees of our flimsy terrestrial fires were exchanged for the ten million degrees of the atomic reactions which heat the sun.

This is but a beginning, with endless possibilities in both directions; a building of a human life of undreamt of wealth and dignity, or a sudden end in utmost misery. Man lives in a new cosmic world for which he was not made. His survival depends on how well and how fast he can adapt himself to it, rebuilding all his ideas, all his social and political institutions.

...Modern science has abolished time and distance as factors separating nations. On our shrunken globe today, there is room for one group only: the family of man.

Suggestions for further reading

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5. Arabidopsis initiative (2000). *Analysis of the genome sequence of the flowering plant Arabidopsis thaliana.* Nature 408, 796-815.
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Chapter 11

CRICK AND WATSON

11.1 The structure of proteins

X-ray crystallography

In England, J.D. Bernal and Dorothy Crowfoot Hodgkin pioneered the application of X-ray diffraction methods to the study of complex biological molecules. In 1949, Hodgkin determined the structure of penicillin; and in 1955, she followed this with the structure of vitamin B12. In 1960, Max Perutz and John C. Kendrew obtained the structures of the blood proteins myoglobin and hemoglobin. This was an impressive achievement for the Cambridge crystallographers, since the hemoglobin molecule contains roughly 12,000 atoms.

The structure obtained by Perutz and Kendrew showed that hemoglobin is a long chain of amino acids, folded into a globular shape, like a small, crumpled ball of yarn. They found that the amino acids with an affinity for water were on the outside of the globular molecule; while the amino acids for which contact with water was energetically unfavorable were hidden on the inside. Perutz and Kendrew deduced that the conformation of the protein - the way in which the chain of amino acids folded into a 3-dimensional structure - was determined by the sequence of amino acids in the chain.

In 1966, D.C. Phillips and his co-workers at the Royal Institution in London found the crystallographic structure of the enzyme lysozyme (an egg-white protein which breaks down the cell walls of certain bacteria). Again, the structure showed a long chain of amino acids, folded into a roughly globular shape. The amino acids with hydrophilic groups were on the outside, in contact with water, while those with hydrophobic groups were on the inside. The structure of lysozyme exhibited clearly an active site, where sugar molecules of bacterial cell walls were drawn into a mouth-like opening and stressed by electrostatic forces, so that bonds between the sugars could easily be broken.

Meanwhile, at Cambridge University, Frederick Sanger developed methods for finding the exact sequence of amino acids in a protein chain. In 1945, he discovered a compound (2,4-dinitrofluorobenzene) which attaches itself preferentially to one end of a chain of amino acids. Sanger then broke down the chain into individual amino acids, and determined which

Figure 11.1: **Dorothy Crowfoot Hodgkin (1910-1994). She and her mentor J.D Bernal were a great pioneers in the application of X-ray crystallogrography to determination of the structure of biological molecules, such as proteins. She was awarded the Nobel Prize in Chemistry in 1964.**

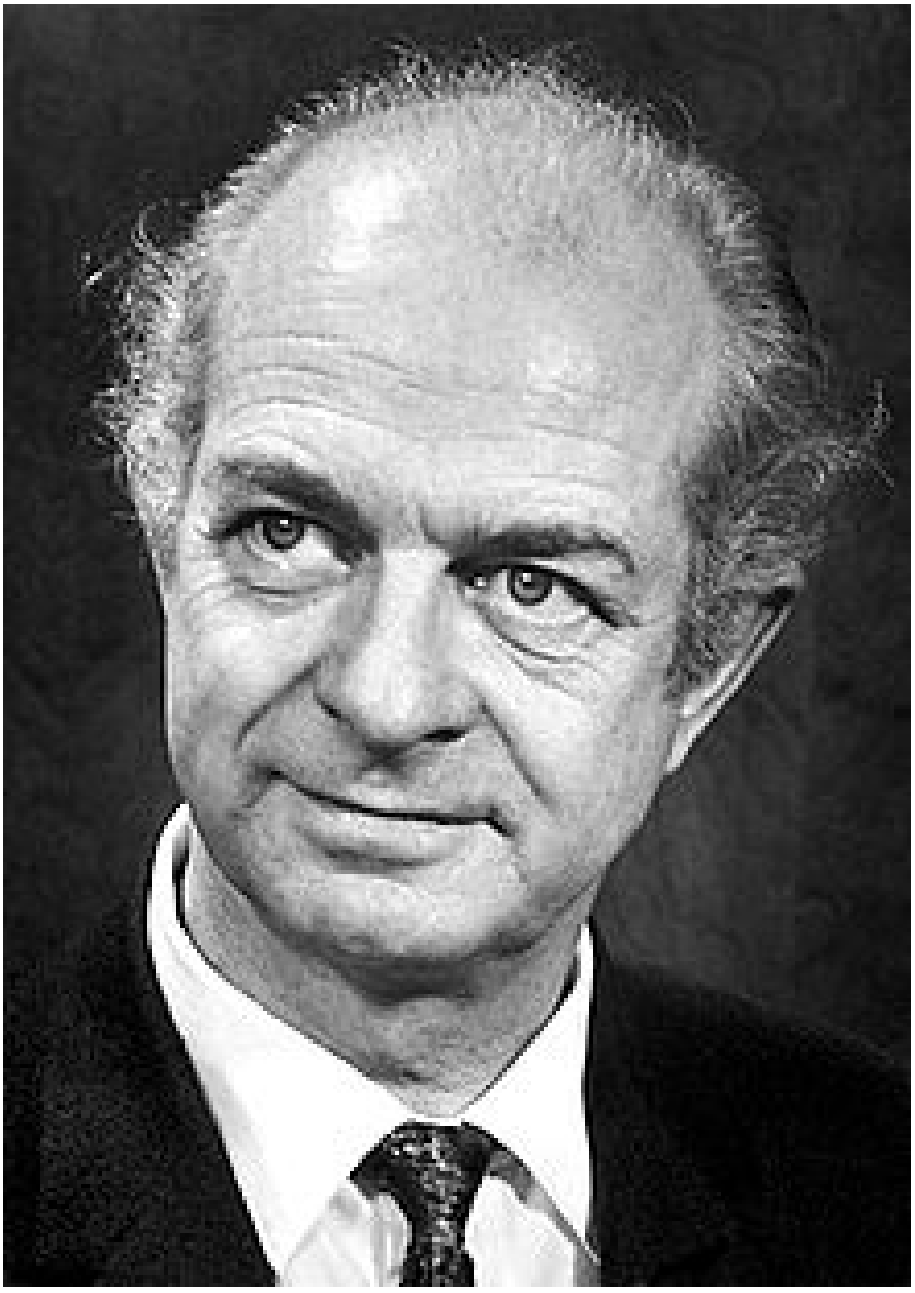

Figure 11.2: **Linus Pauling (1901-1994). The New Scientist called him one of the 20 most important scientists in history. He was awarded the Nobel Prize in Chemistry in 1954 and the Nobel Peace Prize in 1962.**

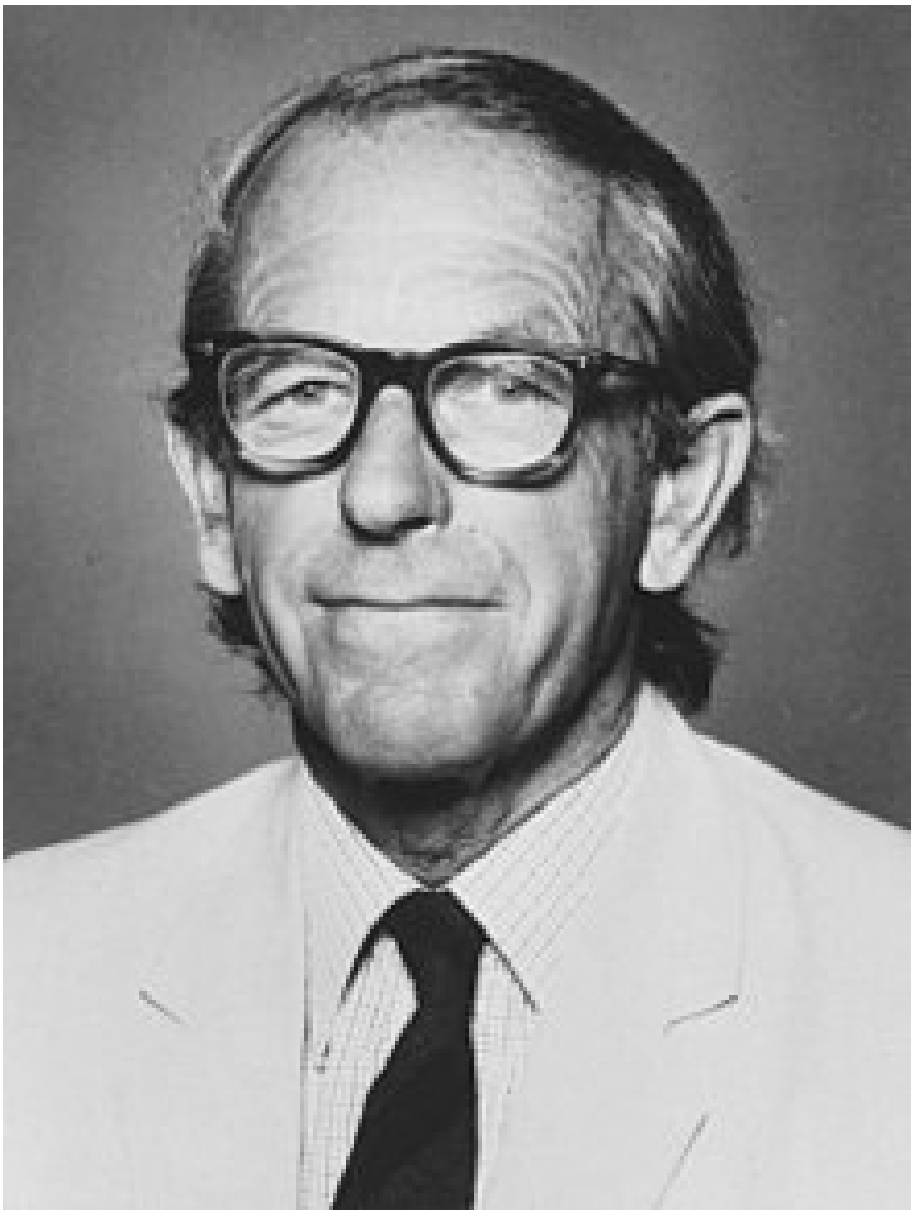

Figure 11.3: **Frederick Sanger (1918-2013) was one of the only two people in history have won two Nobel Prizes in the same field, in his case Chemistry. He won the first on 1958 for his work on the structure of proteins, and the second in 1980 for his method for determining the base sequences of nucleic acids.**

of them was connected to his reagent. By applying this procedure many times to fragments of larger chains, Sanger was able to deduce the sequence of amino acids in complex proteins. In 1953, he published the sequence of insulin. This led, in 1964, to the synthesis of insulin.

Linus Pauling also contributed importantly to our understanding of the structure of proteins. Wikipedia says of his work: "Pauling was one of the founders of the fields of quantum chemistry and molecular biology. His contributions to the theory of the chemical bond include the concept of orbital hybridisation and the first accurate scale of electronegativities of the elements. Pauling also worked on the structures of biological molecules, and showed the importance of the alpha helix and beta sheet in protein secondary structure. Pauling's approach combined methods and results from X-ray crystallography, molecular model building, and quantum chemistry. His discoveries inspired the work of James Watson, Francis Crick, and Rosalind Franklin on the structure of DNA, which in turn made it possible for geneticists to crack the DNA code of all organisms."

The biological role and structure of proteins which began to emerge was as follows: A mammalian cell produces roughly 10,000 different proteins. All enzymes are proteins; and the majority of proteins are enzymes - that is, they catalyze reactions involving other biological molecules. All proteins are built from chainlike polymers, whose monomeric sub-units are the following twenty amino acids: glycine, aniline, valine, isoleucine, leucine, serine, threonine, proline, aspartic acid, glutamic acid, lysine, arginine, asparagine, glutamine, cysteine, methionine, tryptophan, phenylalanine, tyrosine and histidine. These individual amino acid monomers may be connected together into a polymer (called a polypeptide) in any order - hence the great number of possibilities. In such a polypeptide, the backbone is a chain of carbon and nitrogen atoms showing the pattern ...-C-C-N-C-C-N-C-C-N-...and so on. The -C-C-N- repeating unit is common to all amino acids. Their individuality is derived from differences in the side groups which are attached to the universal -C-C-N- group.

Some proteins, like hemoglobin, contain metal atoms, which may be oxidized or reduced as the protein performs its biological function. Other proteins, like lysozyme, contain no metal atoms, but instead owe their biological activity to an active site on the surface of the protein molecule. In 1909, the English physician, Archibald Garrod, had proposed a one-gene-one-protein hypothesis. He believed that hereditary diseases are due to the absence of specific enzymes. According to Garrod's hypothesis, damage suffered by a gene results in the faulty synthesis of the corresponding enzyme, and loss of the enzyme ultimately results in the symptoms of the hereditary disease.

In the 1940's, Garrod's hypothesis was confirmed by experiments on the mold, Neurospora, performed at Stanford University by George Beadle and Edward Tatum. They demonstrated that mutant strains of the mold would grow normally, provided that specific extra nutrients were added to their diets. The need for these dietary supplements could in every case be traced to the lack of a specific enzyme in the mutant strains. Linus Pauling later extended these ideas to human genetics by showing that the hereditary disease, sickle-cell anemia, is due to a defect in the biosynthesis of hemoglobin.

11.2 What is Life?

What is Life? That was the title of a small book published by the physicist Erwin Schrödinger in 1944. Schrödinger (1887-1961) was born and educated in Austria. In 1926 he shared the Nobel Prize in Physics[1] for his contributions to quantum theory (wave mechanics). Schrödinger's famous wave equation is as fundamental to modern physics as Newton's equations of motion are to classical physics.

When the Nazis entered Austria in 1938, Schrödinger opposed them, at the risk of his life. To escape arrest, he crossed the Alps on foot, arriving in Italy with no possessions except his knapsack and the clothes which he was wearing. He traveled to England; and in 1940 he obtained a position in Ireland as Senior Professor at the Dublin Institute for Advanced Studies. There he gave a series of public lectures upon which his small book is based.

In his book, *What is Life?*, Schrödinger developed the idea that a gene is a very large information-containing molecule which might be compared to an aperiodic crystal. He also examined in detail the hypothesis (due to Max Delbrück) that X-ray induced mutations of the type studied by Hermann Muller can be thought of as photo-induced transitions from one isomeric conformation of the genetic molecule to another. Schrödinger's book has great historic importance, because Francis Crick (whose education was in physics) was one of the many people who became interested in biology as a result of reading it. Besides discussing what a gene might be in a way which excited the curiosity and enthusiasm of Crick, Schrödinger devoted a chapter to the relationship between entropy and life.

"What is that precious something contained in our food which keeps us from death? That is easily answered," Schrödinger wrote, "Every process, event, happening - call it what you will; in a word, everything that is going on in Nature means an increase of the entropy of the part of the world where it is going on. Thus a living organism continually increases its entropy - or, as you may say, produces positive entropy, which is death. It can only keep aloof from it, i.e. alive, by continually drawing from its environment negative entropy - which is something very positive as we shall immediately see. What an organism feeds upon is negative entropy. Or, to put it less paradoxically, the essential thing in metabolism is that the organism succeeds in freeing itself from all the entropy it cannot help producing while alive..."[2]

"Entropy, taken with a negative sign, is itself a measure of order. Thus the device by which an organism maintains itself stationary at a fairly high level of orderliness (= fairly low level of entropy) really consists in continually sucking orderliness from its environment. This conclusion is less paradoxical than it appears at first sight. Rather it could be blamed for triviality. Indeed, in the case of higher animals we know the kind of orderliness they feed upon well enough, viz. the extremely well-ordered state of matter state in more or less complicated organic compounds which serve them as foodstuffs. After utilizing it, they

[1] with P.A.M. Dirac

[2] The Hungarian-American biochemist Albert Szent-Györgyi, who won a Nobel prize for isolating vitamin C, and who was a pioneer of Bioenergetics, expressed the same idea in the following words: "We need energy to fight against entropy".

Figure 11.4: **The great Austrian physicist Erwin Schrödinger (1887-1961) was one of the principle founders of quantum theory. He fled from Austria over the mountains to Italy after the Nazis entered his country, and finally found refuge at the Institute for Advanced Studies in Ireland. It was there that he wrote his important book, "What is Life?". Reading Schrödinger's book, Francis Crick was inspired to look for the structure of DNA.**

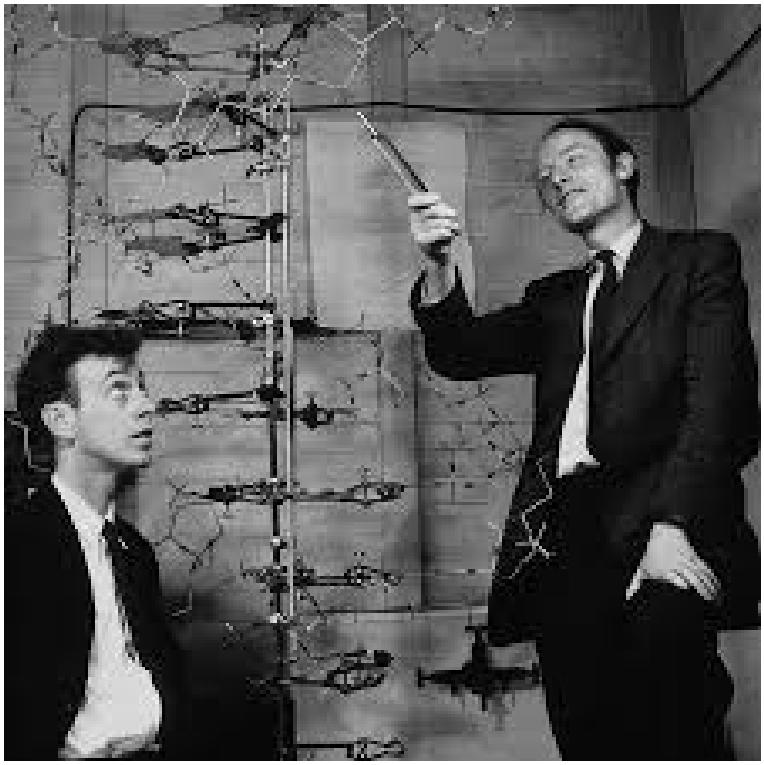

Figure 11.5: **Francis Crick (1916-2004) and James Dewey Watson (born 1928) at the Cavendish Laboratory with their model of DNA. After their discovery of the structure of DNA, it became clear that it was this molecule that carried genetic information between generations.**

return it in a very much degraded form - not entirely degraded, however, for plants can still make use of it. (These, of course, have their most powerful source of 'negative entropy' in the sunlight.)" At the end of the chapter, Schrödinger added a note in which he said that if he had been writing for physicists, he would have made use of the concept of free energy; but he judged that this concept might be difficult or confusing for a general audience.

All living organisms draw a supply of thermodynamic information from their environment, and they use it to "keep aloof" from the disorder which constantly threatens them. In the case of animals, the information-containing free energy comes in the form of food. In the case of green plants, it comes primarily from sunlight. The thermodynamic information thus gained by living organisms is used by them to create configurations of matter which are so complex and orderly that the chance that they could have arisen in a random way is infinitesimally small.

John von Neumann invented a thought experiment which illustrates the role which free energy plays in creating statistically unlikely configurations of matter. Von Neumann imagined a robot or automaton, made of wires, electrical motors, batteries, etc., constructed in such a way that when floating on a lake stocked with its component parts, it will reproduce itself. The important point about von Neumann's automaton is that it requires a source of

free energy (i.e., a source of energy from which work can be obtained) in order to function. We can imagine that the free energy comes from electric batteries which the automaton finds in its environment. (These are analogous to the food eaten by animals.) Alternatively we can imagine that the automaton is equipped with photocells, so that it can use sunlight as a source of free energy, but it is impossible to imagine the automaton reproducing itself without some energy source from which work can be obtained to drive its reproductive machinery. If it could be constructed, would von Neumann's automaton be alive? Few people would say yes. But if such a self-reproducing automaton could be constructed, it would have some of the properties which we associate with living organisms.

The autocatalysts which are believed to have participated in molecular evolution had some of the properties of life. They used "food" (i.e., energy-rich molecules in their environments) to reproduce themselves, and they evolved, following the principle of natural selection. The autocatalysts were certainly precursors of life, approaching the borderline between non-life and life.

Is a virus alive? We know, for example, that the tobacco mosaic virus can be taken to pieces. The proteins and RNA of which it is composed can be separated, purified, and stored in bottles on a laboratory shelf. At a much later date, the bottles containing the separate components of the virus can be taken down from the shelf and incubated together, with the result that the components assemble themselves in the correct way, guided by steric and electrostatic complementarity. New virus particles are formed by this process of autoassembly, and when placed on a tobacco leaf, the new particles are capable of reproducing themselves. In principle, the stage where the virus proteins and RNA are purified and placed in bottles could be taken one step further: The amino acid sequences of the proteins and the base sequence of the RNA could be determined and written down.

Later, using this information, the parts of the virus could be synthesized from amino acids and nucleotides. Would we then be creating life? Another question also presents itself: At a certain stage in the process just described, the virus seems to exist only in the form of information - the base sequence of the RNA and the amino acid sequence of the proteins. Can this information be thought of as the idea of the virus in the Platonic sense? (Pythagoras would have called it the "soul" of the virus.) Is a computer virus alive? Certainly it is not so much alive as a tobacco mosaic virus. But a computer virus can use thermodynamic information (supplied by an electric current) to reproduce itself, and it has a complicated structure, containing much cybernetic information.

Under certain circumstances, many bacteria form spores, which do not metabolize, and which are able to exist without nourishment for very long periods - in fact for millions of years. When placed in a medium containing nutrients, the spores can grow into actively reproducing bacteria. There are examples of bacterial spores existing in a dormant state for many millions of years, after which they have been revived into living bacteria. Is a dormant bacterial spore alive?

Clearly there are many borderline cases between non-life and life; and Aristotle seems to have been right when he said, "Nature proceeds little by little from lifeless things to animal life, so that it is impossible to determine either the exact line of demarcation, or on which side of the line an intermediate form should lie." However, one theme seems to characterize

life: It is able to convert the thermodynamic information contained in food or in sunlight into complex and statistically unlikely configurations of matter. A flood of information-containing free energy reaches the earth's biosphere in the form of sunlight. Passing through the metabolic pathways of living organisms, this information keeps the organisms far away from thermodynamic equilibrium ("which is death"). As the thermodynamic information flows through the biosphere, much of it is degraded into heat, but part is converted into cybernetic information and preserved in the intricate structures which are characteristic of life. The principle of natural selection ensures that as this happens, the configurations of matter in living organisms constantly increase in complexity, refinement and statistical improbability. This is the process which we call evolution, or in the case of human society, progress.

11.3 The structure of DNA

Until 1944, most scientists had guessed that the genetic message was carried by the proteins of the chromosome. In 1944, however, O.T. Avery and his co-workers at the laboratory of the Rockefeller Institute in New York performed a critical experiment, which proved that the material which carries genetic information is not protein, but deoxyribonucleic acid (DNA) - a giant chainlike molecule which had been isolated from cell nuclei by the Swiss chemist, Friedrich Miescher.

Avery had been studying two different strains of pneumococci, the bacteria which cause pneumonia. One of these strains, the S-type, had a smooth coat, while the other strain, the R-type, lacked an enzyme needed for the manufacture of a smooth carbohydrate coat. Hence, R-type pneumococci had a rough appearance under the microscope. Avery and his co-workers were able to show that an extract from heat-killed S-type pneumococci could convert the living R-type species permanently into S-type; and they also showed that this extract consisted of pure DNA.

In 1947, the Austrian-American biochemist, Erwin Chargaff, began to study the long, chainlike DNA molecules. It had already been shown by Levine and Todd that chains of DNA are built up of four bases: adenine (A), thymine (T), guanine (G) and cytosine (C), held together by a sugar-phosphate backbone. Chargaff discovered that in DNA from the nuclei of living cells, the amount of A always equals the amount of T; and the amount of G always equals the amount of C.

When Chargaff made this discovery, neither he nor anyone else understood its meaning. However, in 1953, the mystery was completely solved by Rosalind Franklin and Maurice Wilkins at Kings College, London, together with James Watson and Francis Crick at Cambridge University. By means of X-ray diffraction techniques, Wilkins and Franklin obtained crystallographic information about the structure of DNA. Using this information, together with Linus Pauling's model-building methods, Crick and Watson proposed a detailed structure for the giant DNA molecule.

The discovery of the molecular structure of DNA was an event of enormous importance for genetics, and for biology in general. The structure was a revelation! The giant, helical

Figure 11.6: **Sir Francis Crick (1916-2004). Besides being half of the team that determined the correct structure of DNA, he made many other extremely important contributions to molecular biology and neuroscience. He contributed importantly to the solution of the genetic code, and is known for his "central dogma": Information flows from DNA to RNA, and never backward. RNA codes the synthesis of proteins, and enzymes, which are proteins, catalyze the synthesis of smaller molecules.**

Figure 11.7: **James Dewey Watson (born in 1928) Crick's partner in solving the DNA structure. After serving for 35 years as Director and later President of the Cold Springs Harbor Laboratory and greatly expanding it facilities, he joined the US National Institutes of Health, where he has been the driving force behind the Human Genome Project.**

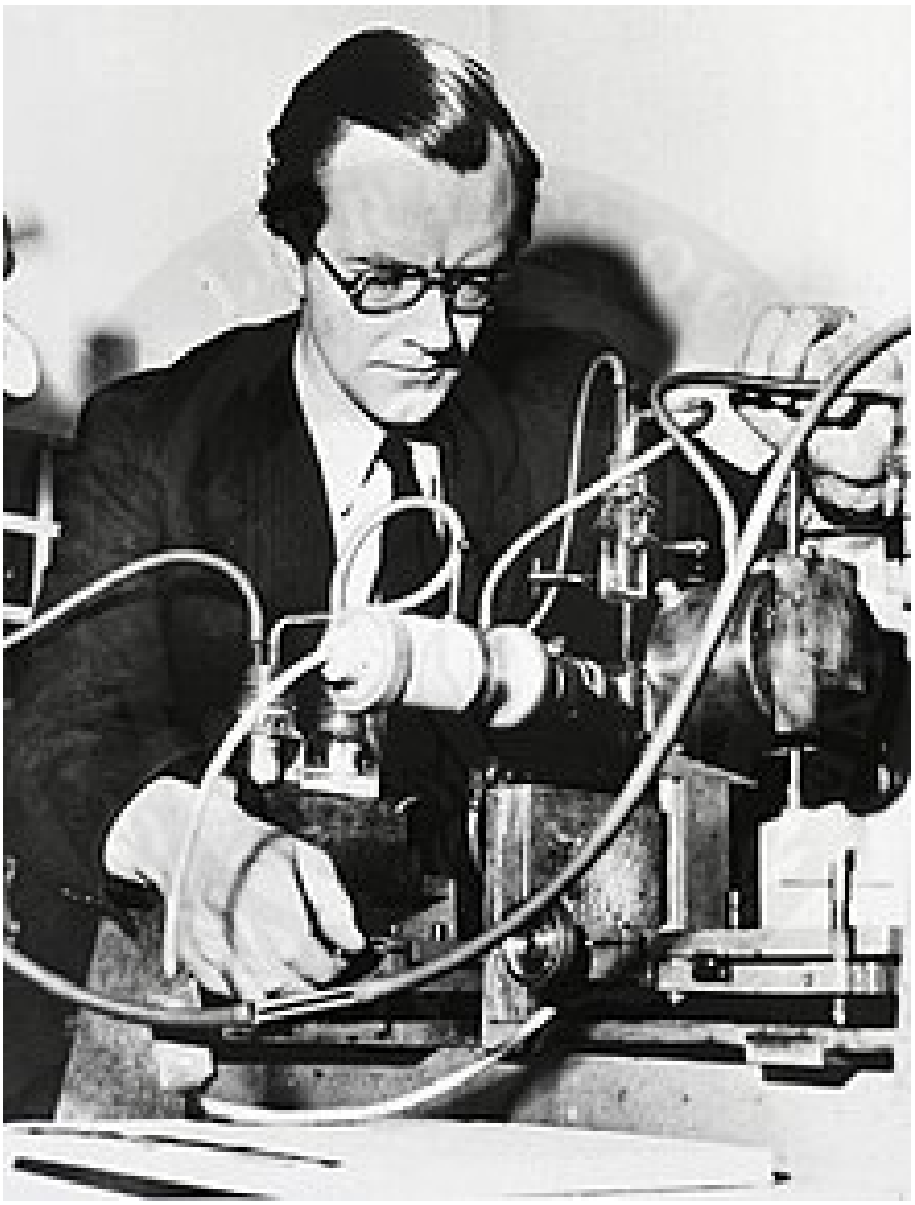

Figure 11.8: **Maurice Wilkins (1916-2004). He applied to DNA the X-ray diffraction methods pioneered by Dorothy Hodgkin. It was his work, and that of Rosalind Franklin, together with Linus Pauling's model-building methods, that enabled Crick and Watson to correctly solve the structure of DNA. He shared the 1962 Nobel Prize in Physiology or Medicine with them.**

Figure 11.9: **Rosalind Franklin (1920-1958). It was one of her high-quality diffraction photographs, taken in Maurice Wilkins' laboratory, that proved to be critical for the DNA structure. She might have shared the Nobel Prize with Wilkins, Crick and Watson, but before this could be considered by the committee, she died of overian cancer.**

Figure 11.10: **Oswald Theodore Avery (1877-1955). Together with his team at the Rockefeller University Hospital in New York City, he proved experimentally that DNA is the molecule that carries genetic information between generations.**

Figure 11.11: **The Austro-Hungarian biochemist Erwin Chargaff (1905-2002) found experimentally that in DNA from the nuclei of living cells, the amount of adenine always equals the amount of thiamine; and the amount of guanine always equals the amount of cytosine, but at the time of his discovery, neither he nor anyone else, understood the meaning of this rule.**

DNA molecule was like a twisted ladder: Two long, twisted sugar-phosphate backbones formed the outside of the ladder, while the rungs were formed by the base pairs, A, T, G and C. The base adenine (A) could only be paired with thymine (T), while guanine (G) fit only with cytosine (C). Each base pair was weakly joined in the center by hydrogen bonds - in other words, there was a weak point in the center of each rung of the ladder - but the bases were strongly attached to the sugar-phosphate backbone. In their 1953 paper, Crick and Watson wrote:

"It has not escaped our notice that the specific pairing we have postulated suggests a possible copying mechanism for genetic material". Indeed, a sudden blaze of understanding illuminated the inner workings of heredity, and of life itself.

If the weak hydrogen bonds in the center of each rung were broken, the ladderlike DNA macromolecule could split down the center and divide into two single strands. Each single strand would then become a template for the formation of a new double-stranded molecule.

Because of the specific pairing of the bases in the Watson-Crick model of DNA, the two strands had to be complementary. T had to be paired with A, and G with C. Therefore, if the sequence of bases on one strand was (for example) TTTGCTAAAGGTGAACCA... , then the other strand necessarily had to have the sequence AAACGATTTCCACTTGGT... The Watson-Crick model of DNA made it seem certain that all the genetic information needed for producing a new individual is coded into the long, thin, double-stranded DNA molecule of the cell nucleus, written in a four-letter language whose letters are the bases, adenine, thymine, guanine and cytosine.

The solution of the DNA structure in 1953 initiated a new kind of biology - molecular biology. This new discipline made use of recently-discovered physical techniques - X-ray diffraction, electron microscopy, electrophoresis, chromatography, ultracentrifugation, radioactive tracer techniques, autoradiography, electron spin resonance, nuclear magnetic resonance and ultraviolet spectroscopy. In the 1960's and 1970's, molecular biology became the most exciting and rapidly-growing branch of science.

11.4 The structure of DNA

The discovery of the molecular structure of DNA was an event of enormous importance for genetics, and for biology in general. The structure was a revelation! The giant, helical DNA molecule was like a twisted ladder: Two long, twisted sugar-phosphate backbones formed the outside of the ladder, while the rungs were formed by the base pairs, A, T, G and C. The base adenine (A) could only be paired with thymine (T), while guanine (G) fit only with cytosine (C). Each base pair was weakly joined in the center by hydrogen bonds - in other words, there was a weak point in the center of each rung of the ladder - but the bases were strongly attached to the sugar-phosphate backbone. In their 1953 paper, Crick and Watson wrote:

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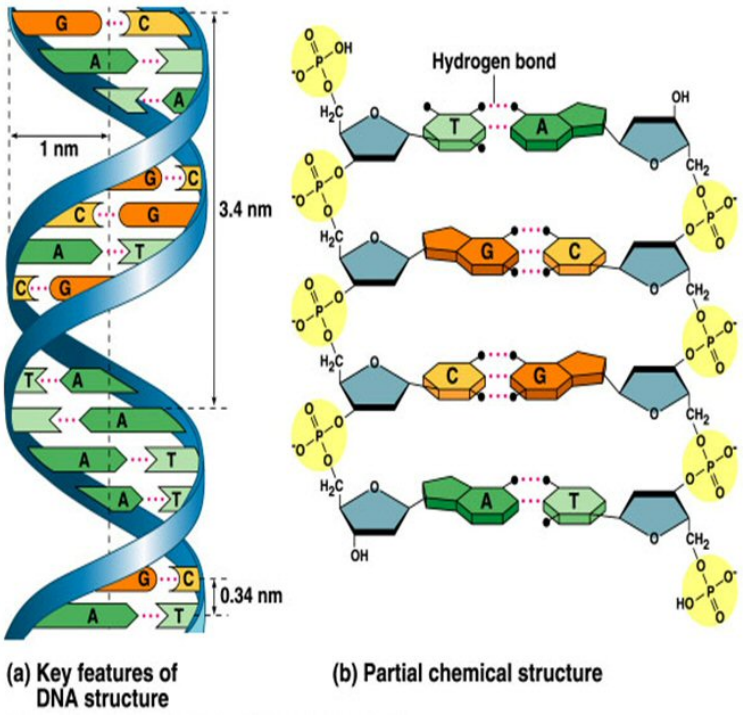

Figure 11.12: **Once the structure of DNA was known, it became clear that transgenerational information is transmitted in a chemical language based on a code with four letters, G, T, C and A.**

If the weak hydrogen bonds in the center of each rung were broken, the ladderlike DNA macromolecule could split down the center and divide into two single strands. Each single strand would then become a template for the formation of a new double-stranded molecule.

Because of the specific pairing of the bases in the Watson-Crick model of DNA, the two strands had to be complementary. T had to be paired with A, and G with C. Therefore, if the sequence of bases on one strand was (for example) TTTGCTAAAGGTGAACCA... , then the other strand necessarily had to have the sequence AAACGATTTCCACTTGGT... The Watson-Crick model of DNA made it seem certain that all the genetic information needed for producing a new individual is coded into the long, thin, double-stranded DNA molecule of the cell nucleus, written in a four-letter language whose letters are the bases, adenine, thymine, guanine and cytosine.

The solution of the DNA structure in 1953 initiated a new kind of biology - molecular biology. This new discipline made use of recently-discovered physical techniques - X-ray diffraction, electron microscopy, electrophoresis, chromatography, ultracentrifugation, radioactive tracer techniques, autoradiography, electron spin resonance, nuclear magnetic resonance and ultraviolet spectroscopy. In the 1960's and 1970's, molecular biology became the most exciting and rapidly-growing branch of science.

11.5 RNA and ribosomes

Since DNA was known to carry the genetic message, coded into the sequence of the four nucleotide bases, A, T, G and C, and since proteins were known to be composed of specific sequences of the twenty amino acids, it was logical to suppose that the amino acid sequence in a protein was determined by the base sequence of DNA. The information somehow had to be read from the DNA and used in the biosynthesis of the protein.

It was known that, in addition to DNA, cells also contain a similar, but not quite identical, polynucleotide called ribonucleic acid (RNA). The sugar-phosphate backbone of RNA was known to differ slightly from that of DNA; and in RNA, the nucleotide thymine (T) was replaced by a chemically similar nucleotide, uracil (U). Furthermore, while DNA was found only in cell nuclei, RNA was found both in cell nuclei and in the cytoplasm of cells, where protein synthesis takes place. Evidence accumulated indicating that genetic information is first transcribed from DNA to RNA, and afterwards translated from RNA into the amino acid sequence of proteins.

At first, it was thought that RNA might act as a direct template, to which successive amino acids were attached. However, the appropriate chemical complementarity could not be found; and therefore, in 1955, Francis Crick proposed that amino acids are first bound to an adaptor molecule, which is afterward bound to RNA.

In 1956, George Emil Palade of the Rockefeller Institute used electron microscopy to study subcellular particles rich in RNA (ribosomes). Ribosomes were found to consist of two subunits - a smaller subunit, with a molecular weight one million times the weight of a hydrogen atom, and a larger subunit with twice this weight.

It was shown by means of radioactive tracers that a newly synthesized protein molecule is attached temporarily to a ribosome, but neither of the two subunits of the ribosome seemed to act as a template for protein synthesis. Instead, Palade and his coworkers found that genetic information is carried from DNA to the ribosome by a messenger RNA molecule (mRNA). Electron microscopy revealed that mRNA passes through the ribosome like a punched computer tape passing through a tape-reader. It was found that the adapter molecules, whose existence Crick had postulated, were smaller molecules of RNA; and these were given the name “transfer RNA” (tRNA). It was shown that, as an mRNA molecule passes through a ribosome, amino acids attached to complementary tRNA adaptor molecules are added to the growing protein chain.

The relationship between DNA, RNA, the proteins and the smaller molecules of a cell was thus seen to be hierarchical: The cell’s DNA controlled its proteins (through the agency of RNA); and the proteins controlled the synthesis and metabolism of the smaller molecules.

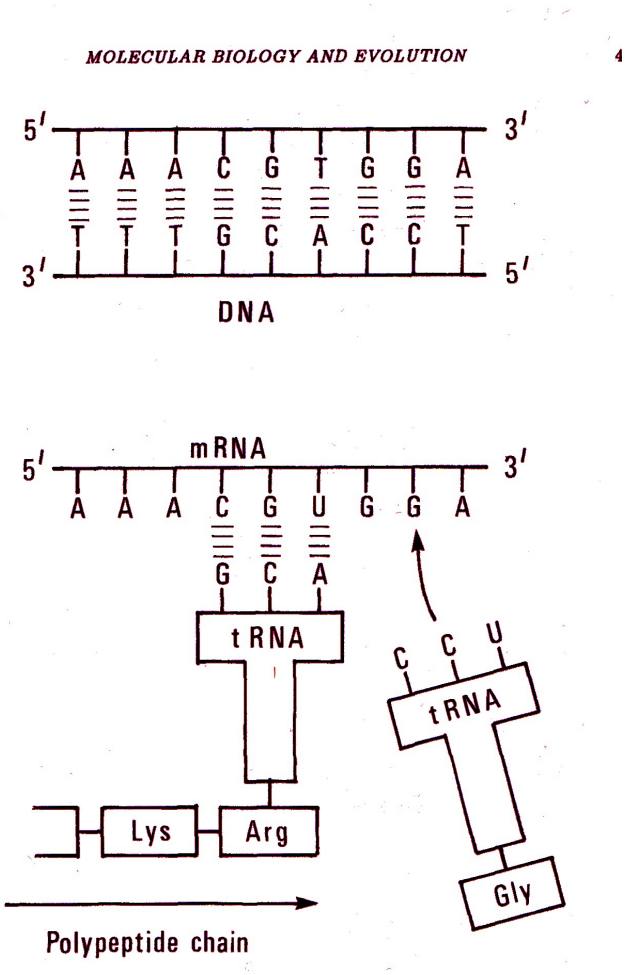

Figure 11.13: **Information coded on DNA molecules in the cell nucleus is transcribed to mRNA molecules. The messenger RNA molecules in turn provide information for the amino acid sequence in protein synthesis.**

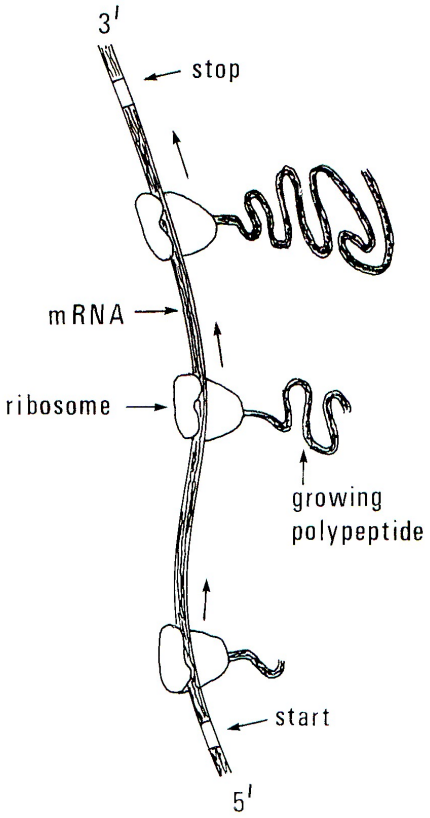

Figure 11.14: **mRNA passes through the ribosome like a punched computer tape passing through a tape-reader.**

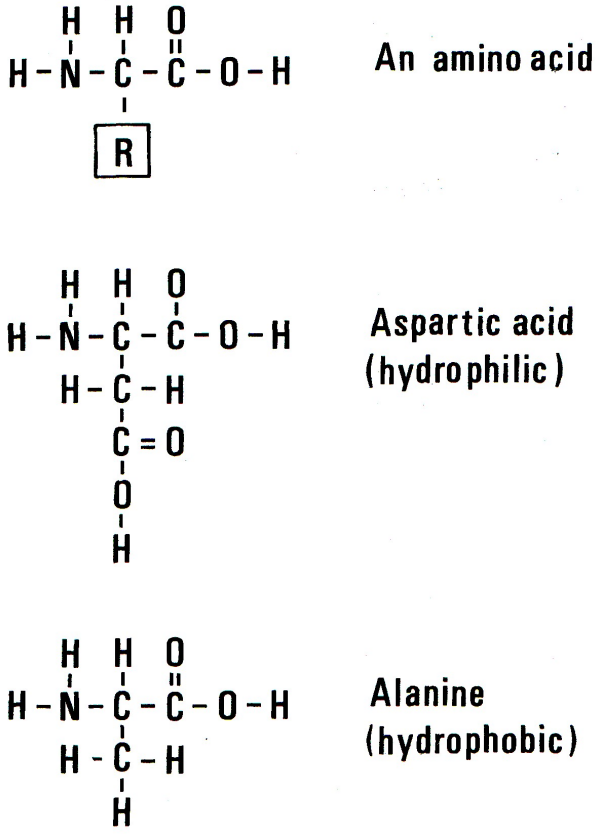

Figure 11.15: **This figure shows aspartic acid, whose residue (R) is hydrophilic, contrasted with alanine, whose residue is hydrophobic. A protein chain is formed from its constituent amino acids by removal of water so that a direct chain of the form -N-C-C-N-C-C-N-C-C-... is produced. The chain then folds in such a way that the hydrophilic residues are outermost while the hydrophobic residues are on the inside.**

11.6 The genetic code

In 1955, Severo Ochoa, at New York University, isolated a bacterial enzyme (RNA polymerase) which was able join the nucleotides A, G, U and C so that they became an RNA strand. One year later, this feat was repeated for DNA by Arthur Kornberg.

With the help of Ochoa's enzyme, it was possible to make synthetic RNA molecules containing only a single nucleotide - for example, one could join uracil molecules into the ribonucleic acid chain, ...U-U-U-U-U-U-... In 1961, Marshall Nirenberg and Heinrich Matthaei used synthetic poly-U as messenger RNA in protein synthesis; and they found that only polyphenylalanine was synthesized. In the same year, Sydney Brenner and Francis Crick reported a series of experiments on mutant strains of the bacteriophage, T4. The experiments of Brenner and Crick showed that whenever a mutation added or deleted either one or two base pairs, the proteins produced by the mutants were highly abnormal and non-functional. However, when the mutation added or subtracted three base pairs, the proteins often were functional. Brenner and Crick concluded that the genetic language has three-letter words (codons). With four different "letters", A, T, G and C, this gives sixty-four possible codons - more than enough to specify the twenty different amino acids.

In the light of the phage experiments of Brenner and Crick, Nirenberg and Matthaei concluded that the genetic code for phenylalanine is UUU in RNA and TTT in DNA. The remaining words in the genetic code were worked out by H. Gobind Khorana of the University of Wisconsin, who used other mRNA sequences (such as GUGUGU..., AAGAAGAAG... and GUUGUUGUU...) in protein synthesis. By 1966, the complete genetic code, specifying amino acids in terms of three-base sequences, was known. The code was found to be the same for all species studied, no matter how widely separated they were in form; and this showed that all life on earth belongs to the same family, as postulated by Darwin.

Table 11.1: **The genetic code**

TTT=Phe	TCT=Ser	TAT=Tyr	TGT=Cys
TTC=Phe	TCC=Ser	TAC=Tyr	TGC=Cys
TTA=Leu	TCA=Ser	TAA=Ter	TGA=Ter
TTG=Leu	TGC=Ser	TAG=Ter	TGG=Trp
CTT=Leu	CCT=Pro	CAT=His	CGT=Arg
CTC=Leu	CCC=Pro	CAC=His	CGC=Arg
CTA=Leu	CCA=Pro	CAA=Gln	CGA=Arg
CTG=Leu	CGC=Pro	CAG=Gln	CGG=Arg
ATT=Ile	ACT=Thr	AAT=Asn	AGT=Ser
ATC=Ile	ACC=Thr	AAC=Asn	AGC=Ser
ATA=Ile	ACA=Thr	AAA=Lys	AGA=Arg
ATG=Met	AGC=Thr	AAG=Lys	AGG=Arg
GTT=Val	GCT=Ala	GAT=Asp	GGT=Gly
GTC=Val	GCC=Ala	GAC=Asp	GGC=Gly
GTA=Val	GCA=Ala	GAA=Glu	GGA=Gly
GTG=Val	GGC=Ala	GAG=Glu	GGG=Gly

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Chapter 12

THE ORIGIN OF LIFE

12.1 Formation of the Sun and the Earth

Our local star, the Sun, was formed from molecular clouds in interstellar space, which had been produced by the explosion of earlier stars. Our Sun contains mainly hydrogen and a little helium, with very small amounts of heavier elements. The vast amounts of energy produced by the sun come mainly from a nuclear reaction in which hydrogen is converted into helium.

There were clouds of containing not only hydrogen and helium, but also heavier elements left swirling around the infant Sun. Gradually, over many millions of years, these condensed through a process of collision and accretion, to form the planets. In the four relatively small inner planets, Mercury, Venus, Earth and Mars, heavy elements predominate, while in the giants, Jupiter, Saturn, Uranus and Neptune, we find lighter elements.

The Sun accounts for 99.86% of the solar system's mass, while the four giant planets contain 99% of the remaining mass.

One *astronomical unit* (1 AU) is, by definition, the average distance of the earth from the sun, i.e. approximately 93 million miles or 150 million kilometers. In terms of this unit, the average distances of the planets from the sun are as follows: Mercury, 0.387 AU; Venus, 0.722 AU; Earth, 1.000 AU; Mars, 1.52 AU; Jupiter, 5.20 AU; Saturn, 9.58 AU; Uranus, 19.2 AU; Neptune, 30.1 AU.

The Solar System also includes the asteroid belt, which lies between the orbits of Mars and Jupiter; the Kuiper belt and scattered disc, which are populations of trans-Neptunian objects; the dwarf planets, Ceres, Pluto and Eris; and the comets. Many of the bodies in the solar system, including six of the planets, have natural satellites or moons. The Earth's moon was produced by collision with a Mars-sized body, soon after the formation of the Earth.

Of the four inner planets, the Earth is the only one that has large amounts of liquid water on its surface. When the Earth cooled sufficiently after the violent collision that gave us our Moon, oceans began to form, and life is believed to have originated in the oceans, approximately 3.8 billion years before the present.

Figure 12.1: **Much experimental evidence supports the Standard Model of cosmology, according to which our Universe began in an enormously hot and dense state 15.72 billion years ago, from which it is exploding outward. By 10 billion years before the present it had cooled enough for the first stars to form. Our own local star, the Sun, was formed 4.54 billion years ago from dust clouds left when earlier stars exploded.**

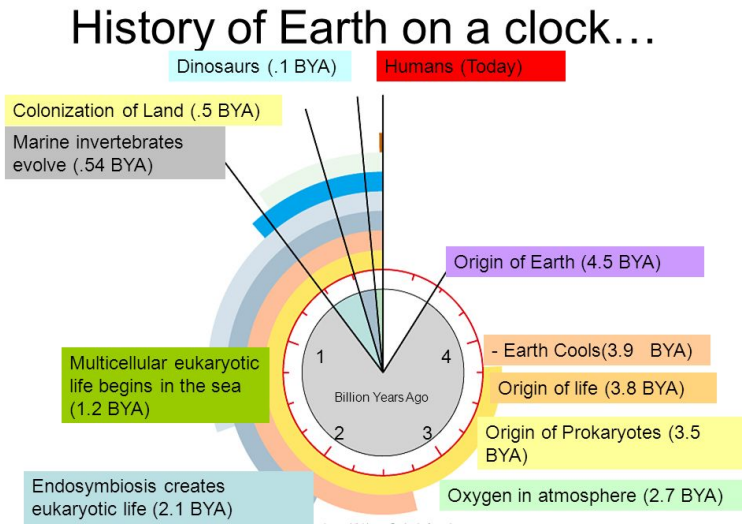

Figure 12.2: **The Earth was formed 4.54 billion years ago. Life on earth originated approximately 3.8 billion years ago (3.8 BYA).**

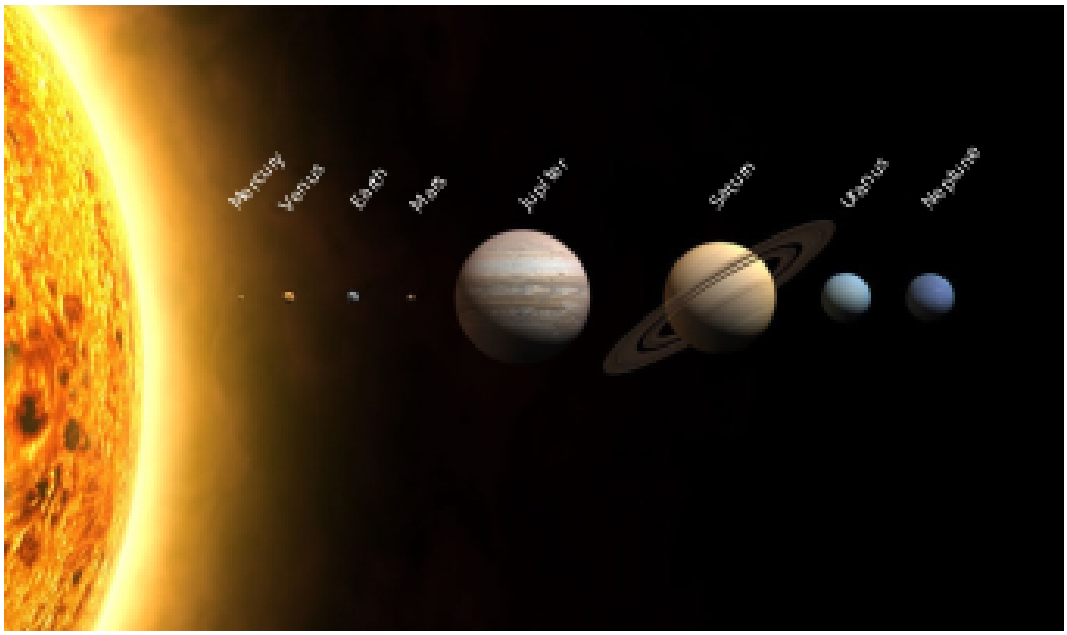

Figure 12.3: **This figure shows the relative sizes of the planets. Closest to the Sun are the relatively small terrestrial planets, Mercury, Venus, Earth and Mars, composed of metals and rock. Farther out are two gas giants, Jupiter and Saturn, which are composed mainly of hydrogen and helium. Still farther out are two ice giants, Uranus and Neptune, which are composed mainly of frozen water, frozen ammonia and frozen methane. The distances of the planets from the Sun shown in this figure are not realistic. The planetary orbits lie in roughly in the same plane, which is called the ecliptic, and all the planets circle the Sun in the same direction.**

12.2 Theories of chemical evolution towards the origin of life

The possibility of an era of chemical evolution prior to the origin of life entered the thoughts of Charles Darwin, but he considered the idea to be much too speculative to be included in his published papers and books. However, in February 1871, he wrote a letter to his close friend Sir Joseph Hooker containing the following words:

"It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sorts of ammonia and phosphoric salts, - light, heat, electricity etc. present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed."

The last letter which Darwin is known to have dictated and signed before his death in 1882 also shows that he was thinking about this problem: "You have expressed quite correctly my views", Darwin wrote, "where you said that I had intentionally left the question of the Origin of Life uncanvassed as being altogether ultra vires in the present state of our knowledge, and that I dealt only with the manner of succession. I have met with no evidence that seems in the least trustworthy, in favor of so-called Spontaneous Generation. (However) I believe that I have somewhere said (but cannot find the passage) that the principle of continuity renders it probable that the principle of life will hereafter be shown to be a part, or consequence, of some general law.."

Modern researchers, picking up the problem where Darwin left it, have begun to throw a little light on the problem of chemical evolution towards the origin of life. In the 1930's J.B.S. Haldane in England and A.I. Oparin in Russia put forward theories of an era of chemical evolution prior to the appearance of living organisms.

In 1924 Oparin published a pamphlet on the origin of life. An expanded version of this pamphlet was translated into English and appeared in 1936 as a book entitled *The Origin of Life on Earth*. In this book Oparin pointed out that the time when life originated, conditions on earth were probably considerably different than they are at present: The atmosphere probably contained very little free oxygen, since free oxygen is produced by photosynthesis which did not yet exist. On the other hand, he argued, there were probably large amounts of methane and ammonia in the earth's primitive atmosphere[1]. Thus, before the origin of life, the earth probably had a reducing atmosphere rather than an oxidizing one. Oparin believed that energy-rich molecules could have been formed very slowly by the action of light from the sun. On the present-day earth, bacteria quickly consume energy-rich molecules, but before the origin of life, such molecules could have accumulated, since there were no living organisms to consume them. (This observation is similar to the remark made by Darwin in his 1871 letter to Hooker.)

[1] It is now believed that the main constituents of the primordial atmosphere were carbon dioxide, water, nitrogen, and a little methane.

The first experimental work in this field took place in 1950 in the laboratory of Melvin Calvin at the University of California, Berkeley. Calvin and his co-workers wished to determine experimentally whether the primitive atmosphere of the earth could have been converted into some of the molecules which are the building-blocks of living organisms. The energy needed to perform these conversions they imagined to be supplied by volcanism, radioactive decay, ultraviolet radiation, meteoric impacts, or by lightning strokes.

The earth is thought to be approximately 4.6 billion years old. At the time when Calvin and his co-workers were performing their experiments, the earth's primitive atmosphere was believed to have consisted primarily of hydrogen, water, ammonia, methane, and carbon monoxide, with a little carbon dioxide. A large quantity of hydrogen was believed to have been initially present in the primitive atmosphere, but it was thought to have been lost gradually over a period of time because the earth's gravitational attraction is too weak to effectively hold such a light and rapidly-moving molecule. However, Calvin and his group assumed sufficient hydrogen to be present to act as a reducing agent. In their 1950 experiments they subjected a mixture of hydrogen and carbon dioxide, with a catalytic amount of Fe^{2+}, to bombardment by fast particles from the Berkeley cyclotron. Their experiments resulted in a good yield of formic acid and a moderate yield of formaldehyde. (The fast particles from the cyclotron were designed to simulate an energy input from radioactive decay on the primitive earth.)

Two years later, Stanley Miller, working in the laboratory of Harold Urey at the University of Chicago, performed a much more refined experiment of the same type. In Miller's experiment, a mixture of the gases methane, ammonia, water and hydrogen was subjected to an energy input from an electric spark. Miller's apparatus was designed so that the gases were continuously circulated, passing first through the spark chamber, then through a water trap which removed the non-volatile water soluble products, and then back again through the spark chamber, and so on. The resulting products are shown as a function of time in Figure 3.5.

The Miller-Urey experiment produced many of the building-blocks of living organisms, including glycine, glycolic acid, sarcosine, alanine, lactic acid, N-methylalanine, β-alanine, succinic acid, aspartic acid, glutamic acid, iminodiacetic acid, iminoacetic-propionic acid, formic acid, acetic acid, propionic acid, urea and N-methyl urea[2]. Another major product was hydrogen cyanide, whose importance as an energy source in chemical evolution was later emphasized by Calvin.

The Miller-Urey experiment was repeated and extended by the Ceylonese-American biochemist Cyril Ponnamperuma and by the American expert in planetary atmospheres, Carl Sagan. They showed that when phosphorus is made available, then in addition to amino acids, the Miller-Urey experiment produces not only nucleic acids of the type that join together to form DNA, but also the energy-rich molecule ATP (adenosine triphosphate). ATP is extremely important in biochemistry, since it is a universal fuel which drives chemical reactions inside present-day living organisms.

[2] The chemical reaction that led to the formation of the amino acids that Miller observed was undoubtedly the Strecker synthesis: $HCN + NH_3 + RC{=}O + H_2O \rightarrow RC(NH_2)COOH$.

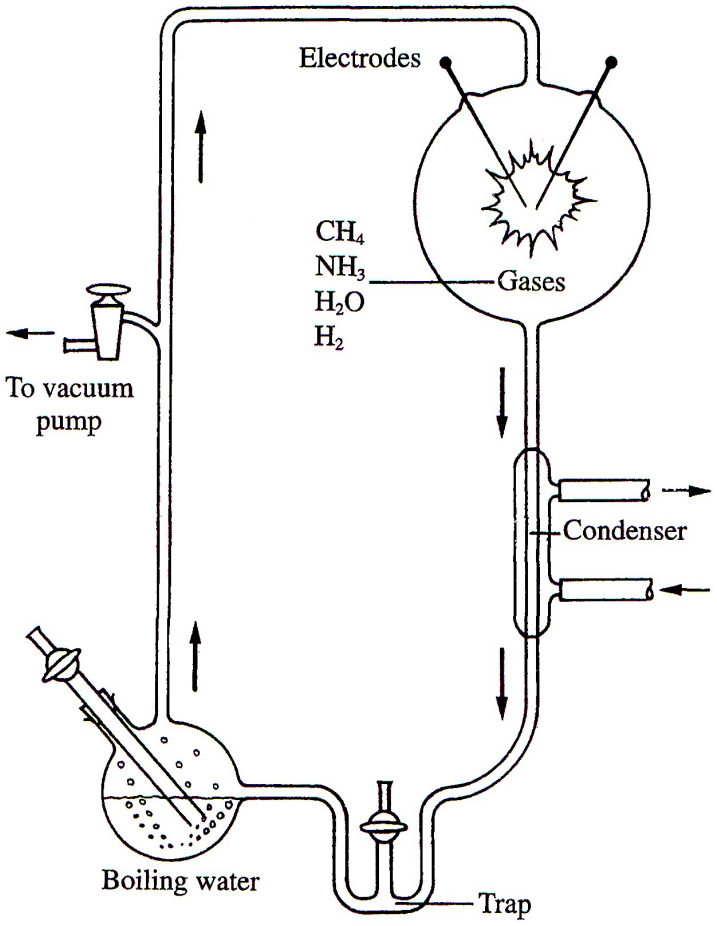

Figure 12.4: **Miller's apparatus.**

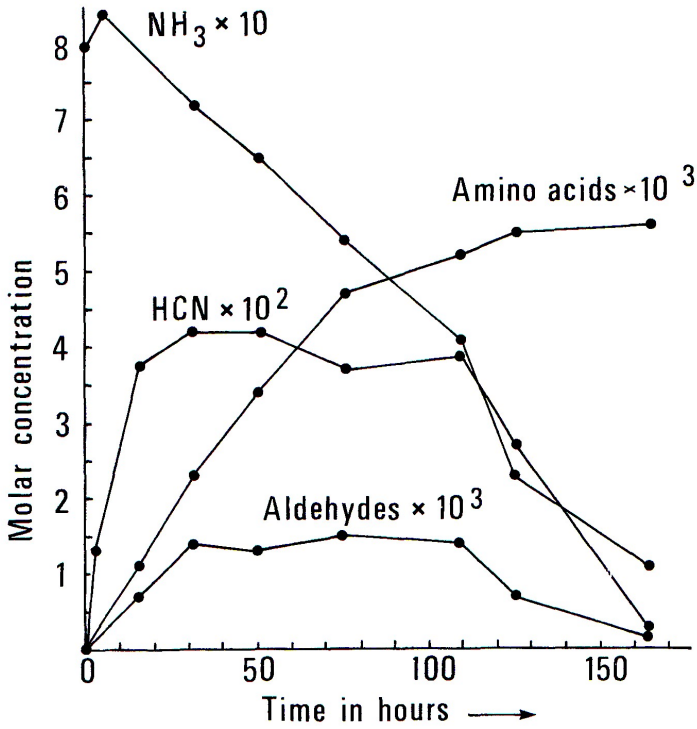

Figure 12.5: **Products as a function of time in the Miller-Urey experiment.**

Further variations on the Miller-Urey experiment were performed by Sydney Fox and his co-workers at the University of Miami. Fox and his group showed that amino acids can be synthesized from a primitive atmosphere by means of a thermal energy input, and that in the presence of phosphate esters, the amino acids can be thermally joined together to form polypeptides. However, some of the peptides produced in this way were cross linked, and hence not of biological interest.

In 1969, Melvin Calvin published an important book entitled *Chemical Evolution; Molecular Evolution Towards the Origin of Living Systems on Earth and Elsewhere*. In this book, Calvin reviewed the work of geochemists showing the presence in extremely ancient rock formations of molecules which we usually think of as being produced only by living organisms. He then discussed experiments of the Miller-Urey type - experiments simulating the first step in chemical evolution. According to Calvin, not only amino acids but also the bases adenine, thymine, guanine, cytosine and uracil, as well as various sugars, were probably present in the primitive ocean in moderate concentrations, produced from the primitive atmosphere by the available energy inputs, and not broken down because no organisms were present.

The next steps visualized by Calvin were dehydration reactions in which the building blocks were linked together into peptides, polynucleotides, lipids and porphyrins. Such dehydration reactions are in a thermodynamically uphill direction. In modern organisms, they are driven by a universally-used energy source, the high-energy phosphate bond of adenosine triphosphate (ATP). Searching for a substance present in the primitive ocean which could have driven the dehydrations, Calvin and his coworkers experimented with hydrogen cyanide (HC=N), and from the results of these experiments they concluded that the energy stored in the carbon-nitrogen triple bond of HC=N could indeed have driven the dehydration reactions necessary for polymerization of the fundamental building blocks. However, later work made it seem improbable that peptides could be produced from cyanide mixtures.

In Chemical Evolution, Calvin introduced the concept of autocatalysis as a mechanism for molecular selection, closely analogous to natural selection in biological evolution. Calvin proposed that there were a few molecules in the ancient oceans which could catalyze the breakdown of the energy-rich molecules present into simpler products. According to Calvin's hypothesis, in a very few of these reactions, the reaction itself produced more of the catalyst. In other words, in certain cases the catalyst not only broke down the energy-rich molecules into simpler products but also catalyzed their own synthesis. These autocatalysts, according to Calvin, were the first systems which might possibly be regarded as living organisms. They not only "ate" the energy-rich molecules but they also reproduced - i.e., they catalyzed the synthesis of molecules identical with themselves.

Autocatalysis leads to a sort of molecular natural selection, in which the precursor molecules and the energy-rich molecules play the role of "food", and the autocatalytic systems compete with each other for the food supply. In Calvin's picture of molecular evolution, the most efficient autocatalytic systems won this competition in a completely Darwinian way. These more efficient autocatalysts reproduced faster and competed more successfully for precursors and for energy-rich molecules. Any random change in the direc-

tion of greater efficiency was propagated by natural selection.

What were these early autocatalytic systems, the forerunners of life? Calvin proposed several independent lines of chemical evolution, which later, he argued, joined forces. He visualized the polynucleotides, the polypeptides, and the metallo-porphyrins as originally having independent lines of chemical evolution. Later, he argued, an accidental union of these independent autocatalysts showed itself to be a still more efficient autocatalytic system. He pointed out in his book that "autocatalysis" is perhaps too strong a word. One should perhaps speak instead of "reflexive catalysis" , where a molecule does not necessarily catalyze the synthesis of itself, but perhaps only the synthesis of a precursor. Like autocatalysis, reflexive catalysis is capable of exhibiting Darwinian selectivity.

The theoretical biologist, Stuart Kauffman, working at the Santa Fe Institute, has constructed computer models for the way in which the components of complex systems of reflexive catalysts may have been linked together. Kauffman's models exhibit a surprising tendency to produce orderly behavior even when the links are randomly programmed.

In 1967 and 1968, C. Woese, F.H.C. Crick and L.E. Orgel proposed that there may have been a period of chemical evolution involving RNA alone, prior to the era when DNA, RNA and proteins joined together to form complex self-reproducing systems. In the early 1980's, this picture of an "RNA world" was strengthened by the discovery (by Thomas R. Cech and Sydney Altman) of RNA molecules which have catalytic activity.

Today experiments aimed at throwing light on chemical evolution towards the origin of life are being performed in the laboratory of the Nobel Laureate geneticist Jack Sjostak at Harvard Medical School. The laboratory is trying to build a synthetic cellular system that undergoes Darwinian evolution.

In connection with autocatalytic systems, it is interesting to think of the polymerase chain reaction, which we discussed above. The target segment of DNA and the polymerase together form an autocatalytic system. The "food" molecules are the individual nucleotides in the solution. In the PCR system, a segment of DNA reproduces itself with an extremely high degree of fidelity. One can perhaps ask whether systems like the PCR system can have been among the forerunners of living organisms. The cyclic changes of temperature needed for the process could have been supplied by the cycling of water through a hydrothermal system. There is indeed evidence that hot springs and undersea hydrothermal vents may have played an important role in chemical evolution towards the origin of life. We will discuss this evidence in the next section.

Throughout this discussion of theories of chemical evolution, and the experiments which have been done to support these theories, energy has played a central role. None of the transformations discussed above could have taken place without an energy source, or to be more precise, they could not have taken place without a source of free energy. In Chapter 4 we will discuss in detail the reason why free energy plays a central role, not only in the origin of life but also in life's continuation. We will see that there is a connection between free energy and information, and that information-containing free energy is needed to produce the high degree of order which is characteristic of life.

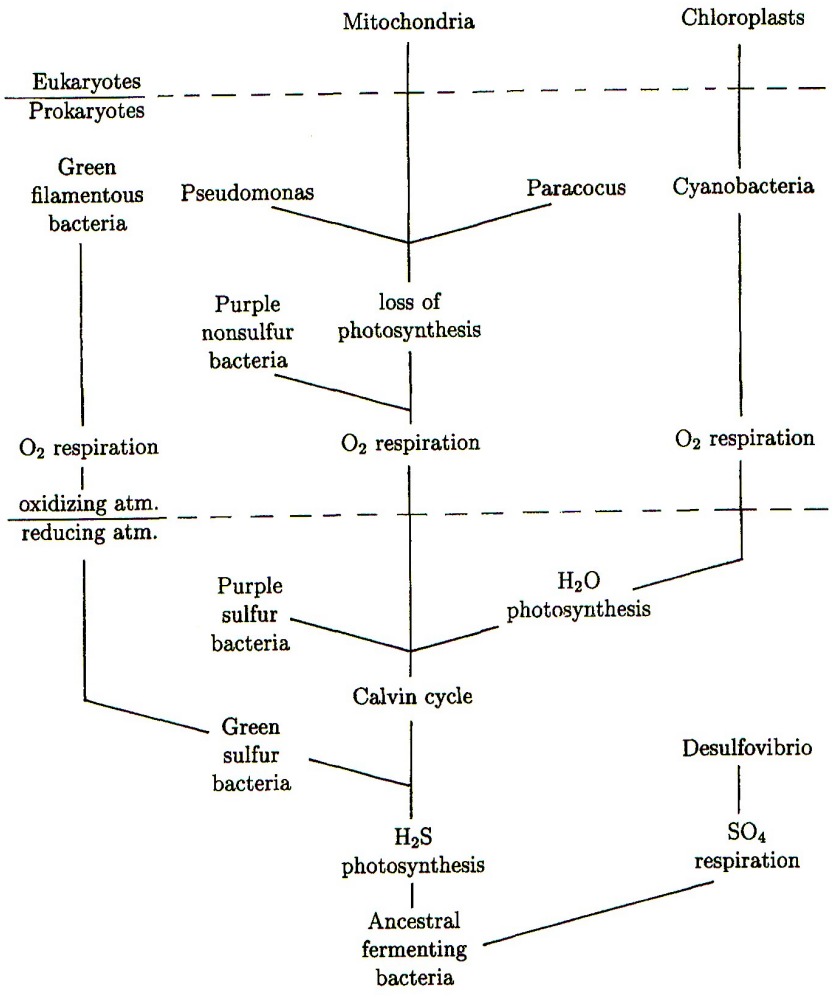

Figure 12.6: **Evolutionary relationships established by Dickerson and coworkers by comparing the amino acid sequences of Cytochrome C from various species.**

12.3 Molecular evidence establishing family trees in evolution

Starting in the 1970's, the powerful sequencing techniques developed by Sanger and others began to be used to establish evolutionary trees. The evolutionary closeness or distance of two organisms could be estimated from the degree of similarity of the amino acid sequences of their proteins, and also by comparing the base sequences of their DNA and RNA. One of the first studies of this kind was made by R.E. Dickerson and his coworkers, who studied the amino acid sequences in Cytochrome C, a protein of very ancient origin which is involved in the "electron transfer chain" of respiratory metabolism. Some of the results of Dickerson's studies are shown in Figure 12.6.

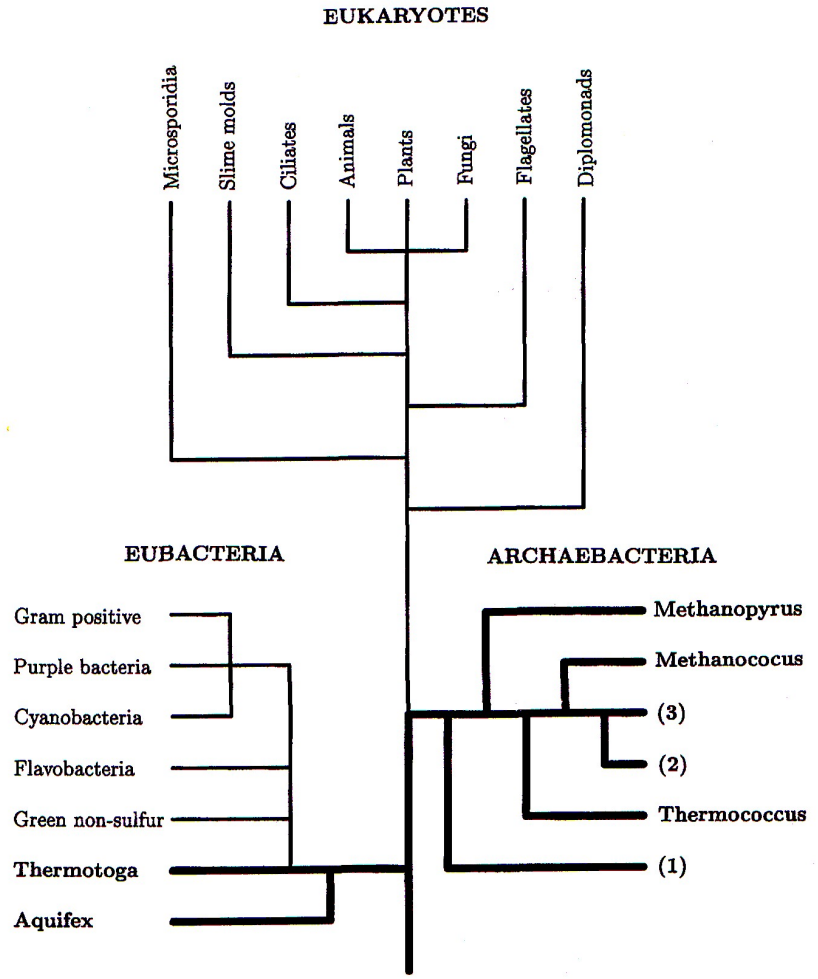

Figure 12.7: **This figure shows the universal phylogenetic tree, established by the work of Woese, Iwabe et al. Hyperthermophiles are indicated by bold lines and by bold type.**

Comparison of the base sequences of RNA and DNA from various species proved to be even more powerful tool for establishing evolutionary relationships. Figure 12.7 shows the universal phylogenetic tree established in this way by Iwabe, Woese and their coworkers.[3] In Figure 12.7, all presently living organisms are divided into three main kingdoms, Eukaryotes, Eubacteria, and Archaebacteria. Carl Woese, who proposed this classification on the basis of comparative sequencing, wished to call the three kingdoms "Eucarya, Bacteria and Archaea". However, the most widely accepted terms are the ones shown in capital letters on the figure. Before the comparative RNA sequencing work, which was performed on the ribosomes of various species, it had not been realized that there are two types of bacteria, so markedly different from each other that they must be classified as belonging to separate kingdoms. One example of the difference between archaebacteria and eubacteria is that the former have cell membranes which contain ether lipids, while the latter have ester lipids in their cell membranes. Of the three kingdoms, the eubacteria and the archaebacteria are "prokaryotes", that is to say, they are unicellular organisms having no cell nucleus. Most of the eukaryotes, whose cells contain a nucleus, are also unicellular, the exceptions being plants, fungi and animals.

One of the most interesting features of the phylogenetic tree shown in Figure 12.7 is that the deepest branches - the organisms with shortest pedigrees - are all hyperthermophiles, i.e. they live in extremely hot environments such as hot springs or undersea hydrothermal vents. The shortest branches represent the most extreme hyperthermophiles. The group of archaebacteria indicated by (1) in the figure includes **Thermofilum, Thermoproteus, Pyrobaculum, Pyrodictium, Desulfurococcus**, and **Sulfolobus** - all hypothermophiles[4]. Among the eubacteria, the two shortest branches, Aquifex and Thermatoga are both hyperthermophiles[5]

The phylogenetic evidence for the existence of hyperthermophiles at a very early stage of evolution lends support to a proposal put forward in 1988 by the German biochemist Günter Wächterhäuser. He proposed that the reaction for pyrite formation,

$$FeS + H_2S \rightarrow FeS_2 + 2H + +2e^-$$

which takes place spontaneously at high temperatures, supplied the energy needed to drive the first stages of chemical evolution towards the origin of life. Wächterhäuser pointed out that the surface of the mineral pyrite (FeS_2) is positively charged, and he proposed that, since the immediate products of carbon-dioxide fixation are negatively charged, they would be attracted to the pyrite surface. Thus, in Wächterhäuser's model, pyrite formation not only supplied the reducing agent needed for carbon-dioxide fixation, but also the pyrite

[3] "Phylogeny" means "the evolutionary development of a species". "Ontogeny" means "the growth and development an individual, through various stages, for example, from fertilized egg to embryo, and so on." Ernst Haeckel, a 19th century follower of Darwin, observed that, in many cases, "ontogeny recapitulates phylogeny."

[4] Group (2) in Figure 12.7 includes **Methanothermus**, which is hyperthermophilic, and Methanobacterium, which is not. Group (3) includes **Archaeoglobus**, which is hyperthermophilic, and Halococcus, Halobacterium, Methanoplanus, Methanospirilum, and Methanosarcina, which are not.

[5] Thermophiles are a subset of the larger group of extremophiles.

surface aided the process. Wächterhäuser further proposed an archaic autocatylitic carbon-dioxide fixation cycle, which he visualized as resembling the reductive citric acid cycle found in present-day organisms, but with all reducing agents replaced by FeS + H_2S, with thioester activation replaced by thioacid activation, and carbonyl groups replaced by thioenol groups. The interested reader can find the details of Wächterhäuser's proposals in his papers, which are listed at the end of this chapter.

A similar picture of the origin of life has been proposed by Michael J. Russell and Alan J. Hall in 1997. In this picture "...(i) life emerged as hot, reduced, alkaline, sulphide-bearing submarine seepage waters interfaced with colder, more oxidized, more acid, $Fe^{2+} >> Fe^{3+}$-bearing water at deep (*ca.* 4km) floors of the Hadean ocean *ca.* 4 Gyr ago; (ii) the difference in acidity, temperature and redox potential provided a gradient of pH (*ca.* 4 units), temperature (*ca.* 60°C) and redox potential (*ca.* 500 mV) at the interface of those waters that was sustainable over geological time-scales, providing the continuity of conditions conducive to organic chemical reactions needed for the origin of life..." [6]. Russell, Hall and their coworkers also emphasize the role that may have been played by spontaneously-formed 3-dimensional mineral chambers (bubbles). They visualize these as having prevented the reacting molecules from diffusing away, thus maintaining high concentrations.

Table 12.1 shows the energy-yielding reactions which drive the metabolisms of some organisms which are of very ancient evolutionary origin. All the reactions shown in the table make use of H_2, which could have been supplied by pyrite formation at the time when the organisms evolved. All these organisms are lithoautotrophic, a word which requires some explanation: A heterotrophic organism is one which lives by ingesting energy-rich organic molecules which are present in its environment. By contrast, an autotrophic organism ingests only inorganic molecules. The lithoautotrophs use energy from these inorganic molecules, while the metabolisms of photoautotrophs are driven by energy from sunlight.

Evidence from layered rock formations called "stromatolites", produced by colonies of photosynthetic bacteria, show that photoautotrophs (or phototrophs) appeared on earth at least 3.5 billion years ago. The geological record also supplies approximate dates for other events in evolution. For example, the date at which molecular oxygen started to become abundant in the earth's atmosphere is believed to have been 2.0 billion years ago, with equilibrium finally being established 1.5 billion years in the past. Multi-cellular organisms appeared very late on the evolutionary and geological time-scale - only 600 million years ago. By collecting such evidence, the Belgian cytologist Christian de Duve has constructed the phylogenetic tree shown in Figure 12.8, showing branching as a function of time. One very interesting feature of this tree is the arrow indicating the transfer of "endosymbionts" from the eubacteria to the eukaryotes. In the next section, we will look in more detail at this important event, which took place about 1.8 billion years ago.

[6] See W. Martin and M.J. Russell, *On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells*, Philos. Trans. R. Soc. Lond. B Biol. Sci., **358**, 59-85, (2003).

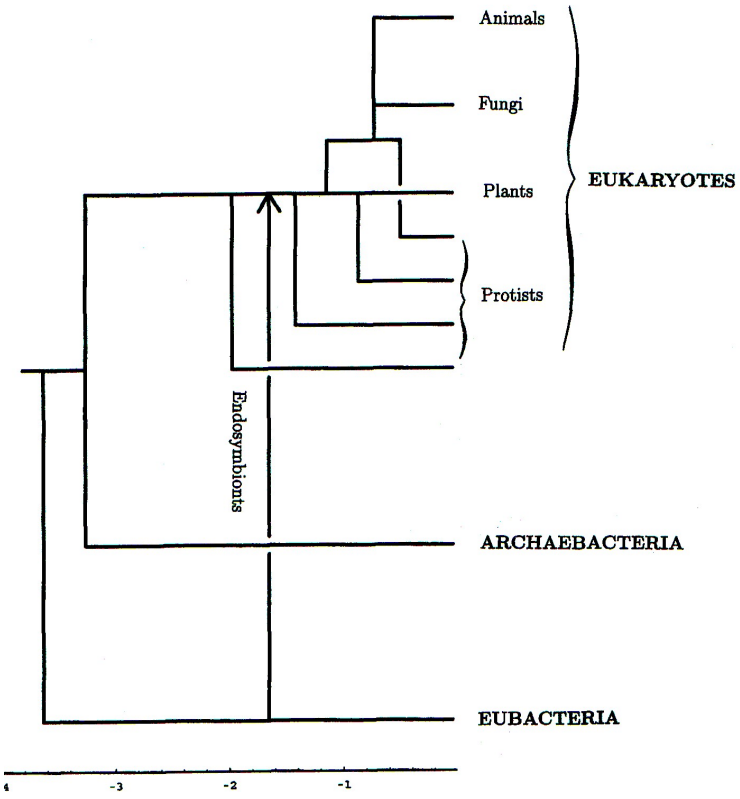

Figure 12.8: **Branching of the universal phylogenetic tree as a function of time. "Protists" are unicellular eukaryotes.**

Table 12.1: **Energy-yielding reactions of some lithoautotrophic hyperthermophiles. (After K.O. Setter)**

Energy-yielding reaction	Genera
$4H_2+CO_2 \rightarrow CH_4+2H_2O$	Methanopyrus, Methanothermus, Methanococcus
$H_2+S^\circ \rightarrow H_2S$	Pyrodictium, Thermoproteus, Pyrobaculum, Acidianus, Stygiolobus
$4H_2+H_2SO_4 \rightarrow H_2S+4H_2O$	Archaeoglobus

12.4 Symbiosis

The word "symbiosis" is derived from Greek roots meaning "living together". It was coined in 1877 by the German botanist Albert Bernard Frank. By that date, it had become clear that lichens are composite organisms involving a fungus and an alga; but there was controversy concerning whether the relationship was a parasitic one. Was the alga held captive and exploited by the fungus? Or did the alga and the fungus help each other, the former performing photosynthesis, and the latter leeching minerals from the lichen's environment? In introducing the word "symbiosis" (in German, "Symbiotismus"), Prank remarked that "We must bring all the cases where two different species live on or in one another under a comprehensive concept which does not consider the role which the two individuals play but is based on the mere coexistence, and for which the term symbiosis is to be recommended." Thus the concept of symbiosis, as defined by Frank, included all intimate relationships between two or more species, including parasitism at one extreme and "mutualism" at the other. However, as the word is used today, it usually refers to relationships which are mutually beneficial.

Charles Darwin himself had been acutely aware of close and mutually beneficial relationships between organisms of different species. For example, in his work on the fertilization of flowers,he had demonstrated the way in which insects and plants can become exquisitely adapted to each other's needs. However, T.H. Huxley, "Darwin's bulldog", emphasized competition as the predominant force in evolution. "The animal world is on about the same level as a gladiator's show", Huxley wrote in 1888, "The creatures are fairly well treated and set to fight - whereby the strongest, the swiftest and the cunningest live to

fight another day. The spectator has no need to turn his thumbs down, as no quarter is given." The view of nature as a sort of "gladiator's contest" dominated the mainstream of evolutionary thought far into the 20th century; but there was also a growing body of opinion which held that symbiosis could be an extremely important mechanism for the generation of new species.

Among the examples of symbiosis studied by Frank were the nitrogen-fixing bacteria living in nodules on the roots of legumes, and the mycorrhizal fungi which live on the roots of forest trees such as oaks, beech and conifers. Frank believed that the mycorrhizal fungi aid in the absorption of nutrients. He distinguished between "ectotrophic" fungi, which form sheaths around the root fibers, and "endotrophic" fungi, which penetrate the root cells. Other examples of symbiosis studied in the 19th century included borderline cases between plants and animals, for ex- ample, paramecia, sponges, hydra, planarian worms and sea anemones, all of which frequently contain green bodies capable of performing photosynthesis.

Writing in 1897, the American lichenologist Albert Schneider prophesied that "future studies may demonstrate that.., plasmic bodies (within the eukaryote cell), such as chlorophyll granules, leucoplastids, chromoplastids, chromosomes, centrosomes, nucleoli, etc., are perhaps symbionts comparable to those in less highly specialized symbiosis. Reinke expresses the opinion that it is not wholly unreasonable to suppose that some highly skilled scientist of the future may succeed in cultivating chlorophyll-bodies in artificial media."

19th century cytologists such as Robert Altman, Andreas Schimper and A. Benda focused attention on the chlorophyll-bodies of plants, which Schimper named chloroplasts, and on another type of subcellular granule, present in large numbers in all plant and animal cells, which Benda named mitochondria, deriving the name from the Greek roots mitos (thread) and chrondos (granule). They observed that these bodies seemed to reproduce themselves within the cell in very much the manner that might be expected if they were independent organisms. Schimper suggested that chloroplasts are symbionts, and that green plants owe their origin to a union of a colorless unicellular organism with a smaller chlorophyll-containing species.

The role of symbiosis in evolution continued to be debated in the 20th century. Mitochondria were shown to be centers of respiratory metabolism; and it was discovered that both mitochondria and chloroplasts contain their own DNA. However, opponents of their symbiotic origin pointed out that mitochondria alone cannot synthesize all their own proteins: Some mitochondrial proteins require information from nuclear DNA. The debate was finally settled in the 1970's, when comparative sequencing of ribosomal RNA in the laboratories of Carl Woese, W. Ford Doolittle and Michael Gray showed conclusively that both chloroplasts and mitochondria were originally endosymbionts. The ribosomal RNA sequences showed that chloroplasts had their evolutionary root in the cyanobacteria, a species of eubacteria, while mitochondria were traced to a group of eubacteria called the alpha-proteobacteria. Thus the evolutionary arrow leading from the eubacteria to the eukaryotes can today be drawn with confidence, as in Figure 3.8.

Cyanobacteria are bluish photosynthetic bacteria which often become linked to one another so as to form long chains. They can be found today growing in large colonies

on seacoasts in many parts of the world, for example in Baja California on the Mexican coast. The top layer of such colonies consists of the phototrophic cyanobacteria, while the organisms in underlying layers are heterotrophs living off the decaying remains of the cyanobacteria. In the course of time, these layered colonies can become fosilized, and they are the source of the layered rock formations called stromatolites (discussed above). Geological dating of ancient stromatolites has shown that cyanobacteria must have originated at least 3.5 billion years ago.

Cyanobacteria contain two photosystems, each making use of a different type of chlorophyll. Photosystem I, which is thought to have evolved first, uses the energy of light to draw electrons from inorganic compounds, and sometimes also from organic compounds (but never from water). Photosystem II, which evolved later, draws electrons from water. Hydrogen derived from the water is used to produce organic compounds from carbon-dioxide, and molecular oxygen is released into the atmosphere. Photosystem II never appears alone. In all organisms which possess it, Photosystem II is coupled to Photosystem I, and together the two systems raise electrons to energy levels that are high enough to drive all the processes of metabolism. Dating of ancient stromatolites makes it probable that cyanobacteria began to release molecular oxygen into the earth's atmosphere at least 3.5 billion years ago; yet from other geological evidence we know that it was only 2 billion years ago that the concentration of molecular oxygen began to rise, equilibrium being reached 1.5 billion years ago. It is believed that ferrous iron, which at one time was very abundant, initially absorbed the photosynthetically produced oxygen. This resulted in the time-lag, as well as the ferrous-ferric mixture of iron which is found in the mineral magnetite.

When the concentrations of molecular oxygen began to rise in earnest, most of the unicellular microorganisms living at the time found themselves in deep trouble, faced with extinction, because for them oxygen was a deadly poison; and very many species undoubtedly perished. However, some of the archaebacteria retreated to isolated anaerobic niches where we find them today, while others found ways of detoxifying the poisonous oxygen. Among the eubacteria, the ancestors of the alpha-proteobacteria were particularly good at dealing with oxygen and even turning it to advantage: They developed the biochemical machinery needed for respiratory metabolism.

Meanwhile, during the period between 3.5 and 2.0 billion years before the present, an extremely important evolutionary development had taken place: Branching from the archaebacteria, a line of large[7] heterotrophic unicellular organisms had evolved. They lacked rigid cell walls, and they could surround smaller organisms with their flexible outer membrane, drawing the victims into their interiors to be digested. These new heterotrophs were the ancestors of present-day eukaryotes, and thus they were the ancestors of all multicellular organisms.

Not only are the cells of present-day eukaryotes very much larger than the cells of archaebacteria and eubacteria; their complexity is also astonishing. Every eukaryote cell contains numerous intricate structures: a nucleus, cytoskeleton, Golgi apparatus, endoplas-

[7] not large in an absolute sense, but large in relation to the prokaryotes

mic reticulum, mitochondria, peroxisomes, chromosomes, the complex structures needed for mitotic cell division, and so on. Furthermore, the genomes of eykaryotes contain very much more information than those of prokaryotes. How did this huge and relatively sudden increase in complexity and information content take place? According to a growing body of opinion, symbiosis played an important role in this development.

The ancestors of the eukaryotes were in the habit of drawing the smaller prokaryotes into their interiors to be digested. It seems likely that in a few cases the swallowed prokaryotes resisted digestion, multiplied within the host, were transmitted to future generations when the host divided, and conferred an evolutionary advantage, so that the result was a symbiotic relationship. In particular, both mitochondria and chloroplasts have definitely been proved to have originated as endosymbionts. It is easy to understand how the photosynthetic abilities of the chloroplasts (derived from cyanobacteria) could have conferred an advantage to their hosts, and how mitochondria (derived from alpha-proteobacteria) could have helped their hosts to survive the oxygen crisis. The symbiotic origin of other sub-cellular organelles is less well understood and is currently under intense investigation.

If we stretch the definition of symbiosis a little, we can make the concept include cooperative relationships between organisms of the same species. For example, cyanobacteria join together to form long chains, and they live together in large colonies which later turn into stromatolites. Also, some eubacteria have a mechanism for sensing how many of their species are present, so that they know, like a wolf pack, when it is prudent to attack a larger organism. This mechanism, called "quorum sensing", has recently attracted much attention among medical researchers.

The cooperative behavior of a genus of unicellular eukaryotes called slime molds is particularly interesting because it gives us a glimpse of how multicellular organisms may have originated. The name of the slime molds is misleading, since they are not fungi, but heterotrophic protists similar to amoebae. Under ordinary circumstances, the individual cells wander about independently searching for food, which they draw into their interiors and digest, a process called "phagocytosis". However, when food is scarce, they send out a chemical signal of distress. Researchers have analyzed the molecule which expresses slime mold unhappiness, and they have found it to be cyclic adenosine monophosphate (cAMP). At this signal, the cells congregate and the mass of cells begins to crawl, leaving a slimy trail. At it crawls, the community of cells gradually develops into a tall stalk, surmounted by a sphere - the "fruiting body". Inside the sphere, spores are produced by a sexual process. If a small animal, for example a mouse, passes by, the spores may adhere to its coat; and in this way they may be transported to another part of the forest where food is more plentiful.

Thus slime molds represent a sort of missing link between unicellular and multicellular or organisms. Normally the cells behave as individualists, wandering about independently, but when challenged by a shortage of food, the slime mold cells join together into an entity which closely resembles a multicellular organism. The cells even seem to exhibit altruism, since those forming the stalk have little chance of survival, and yet they are willing to perform their duty, holding up the sphere at the top so that the spores will survive and carry the genes of the community into the future. We should especially notice the fact that

the cooperative behavior of the slime mold cells is coordinated by chemical signals.

Sponges are also close to the borderline which separates unicellular eukaryotes (protists) from multicellular organisms, but they are just on the other side of the border. Normally the sponge cells live together in a multicellular community, filtering food from water. However, if a living sponge is forced through a very fine cloth, it is possible to separate the cells from each other. The sponge cells can live independently for some time; but if many of them are left near to one another, they gradually join together and form themselves into a new sponge, guided by chemical signals. In a refinement of this experiment, one can take two living sponges of different species, separate the cells by passing the sponges through a fine cloth, and afterwards mix all the separated cells together. What happens next is amazing: The two types of sponge cells sort themselves out and become organized once more into two sponges - one of each species.

Slime molds and sponges hint at the genesis of multicellular organisms, whose evolution began approximately 600 million years ago. Looking at the slime molds and sponges, we can imagine how it happened. Some unicellular organisms must have experienced an enhanced probability of survival when they lived as colonies. Cooperative behavior and division of labor within the colonies were rewarded by the forces of natural selection, with the selective force acting on the entire colony of cells, rather than on the individual cell. This resulted in the formation of cellular societies and the evolution of mechanisms for cell differentiation. The division of labor within cellular societies (i.e., differentiation) came to be coordinated by chemical signals which affected the transcription of genetic information and the synthesis of proteins. Each cell within a society of cells possessed the entire genome characteristic of the colony, but once a cell had been assigned its specific role in the economy of the society, part of the information became blocked - that is, it was not expressed in the function of that particular cell. As multicellular organisms evolved, the chemical language of intercellular communication became very much more complex and refined. We will discuss the language of intercellular communication in more detail in a later section.

Geneticists have become increasingly aware that symbiosis has probably played a major role in the evolution of multicellular organisms. We mentioned above that, by means of genetic engineering techniques, transgenic plants and animals can be produced. In these chimeras, genetic material from a foreign species is incorporated into the chromosomes, so that it is inherited in a stable, Mendelian fashion. J.A. Shapiro, one of whose articles is referenced at the end of this chapter, believes that this process also occurs in nature, so that the conventional picture of evolutionary family trees needs to be corrected. Shapiro believes that instead of evolutionary trees, we should perhaps think of webs or networks.

For example, it is tempting to guess that symbiosis may have played a role in the development of the visual system of vertebrates. One of the archaebacteria, the purple halobacterium halobium (recently renamed halobacterium salinarum), is able to perform photosynthesis by means of a protein called bacterial rhodopsin, which transports hydrogen ions across the bacterial membrane. This protein is a near chemical relative of rhodopsin, which combines with a carotinoid to form the "visual purple" used in the vertebrate eye. It is tempting to think that the close similarity of the two molecules is not just a coincidence,

and that vertebrate vision originated in a symbiotic relationship between the photosynthetic halobacterium and an aquatic ancestor of the vertebrates, the host being able to sense when the halobacterium was exposed to light and therefore transporting hydrogen ions across its cell membrane.

In this chapter, we have looked at the flow of energy and information in the origin and evolution of life on earth. We have seen how energy-rich molecules were needed to drive the first steps in the origin of life, and how during the evolutionary process, information was preserved, transmitted, and shared between increasingly complex organisms, the whole process being driven by an input of energy. In the next chapter, we will look closely at the relationships between energy and information.

12.5 Timeline for the evolution of life on the Earth

The dates shown here are taken from the Wikipedia article entitled *Timeline of the evolutionary history of life*. The unit BYA means "Billion years ago", while MYA means "Million years ago".

- 4.540 BYA. Earliest Earth
- 4.404 BYA, First appearance of water on Earth.
- 4.280 BYA. Earliest appearance of life on Earth.[8]
- 3.900 BYA, Cells resembling prokaryotes appear. These first organisms use CO_2 as a source of carbon, and obtain energy by oxidizing inorganic materials.
- 3.500 BYA, Lifetime of the last universal common ancestor. The split between bacteria and archae occurs.
- 3.000 BYA, Photosynthetic cyanobacteria evolved. They used water as a reducing agent and produced oxygen as a waste product.
- 2.800 BYA, Earliest evidence of microbial life on land.
- 2.500 BYA, Great Oxygenation Event, produced by cyanobacteria's oxogenic photosynthesis.
- 1.850 BYA, Eukaryotic cells appear. They probably evolved from cooperative assemblages of prokaryotes (phagocytosis and symbiosis).
- 1.200 BYA, Sexual reproduction first appears in the fossil records. It may have existed earlier.
- 0.800 BYA, First multicellular organisms.
- 0.600 BYA, The ozone layer is formed, making landbased life more possible.
- 0.580-0.500 BYA, The Cambrian Explosion. Biodiversity quickly increases and most modern phyla of animals appear in the fossil record.
- 0.560 BYA, Fungi appear.
- 0.550 BYA, Comb jellies, sponges, sea anemones and corals evolved.
- 0.530 BYA, The first known fossilized footprints on land.

[8]This date for the first appearance of life on earth is earlier than previously thought possible. It is based on the ratio of carbon isotopes in zircon rocks recently found in Australia.

- 0.485 BYA, Jawless fishes.
- 0.434 BYA, The first primitive plants move onto land, accompanied by fungi which may have helped them.
- 0.420 BYA, Ray-finned fishes, arachnids, and land scorpions.
- 0.410 BYA, First signs of teeth in fish.
- 0.395 BYA, First lichens, stonewarts, harvestmen and springtails. The first known tracks of four-legged animals on land.
- 0.363 BYA, The Carboniferous Period starts. Insects appear on land and soon learn to fly. Seed-bearing plants and forests cover the land.
- 0.360 BYA, First crabs and ferns. Land flora dominated by ferns.
- 0.350 BYA, Large sharks, ratfishes and hagfish.
- 0.320 BYA, The precursors of mammals separate from the precursors to reptiles.
- 0.280 BYA, Earliest beetles, seed plants and conifers diversify.
- 0.2514 BYA, The Permian-Triassic extinction event eliminates 90-95% of marine species, and 70% of terrestrial vertebrates.[9]
- 0.245 BYA, Earliest icthyosaurs (i.e. seagoing dinosaurs).
- 0.225 BYA, Earliest dinosaurs. First mammals.
- 0.220 BYA, Seed-producing forests dominate the land. Herbivours grow to huge sizes. First flies and turtles.
- 0.155 BYA, First bloodsucking insects. Archaeopteryx, a possible ancestor of birds, appears.
- 0.130 BYA, Rise of the flowering plants. Coevolution of plants and their pollinators.
- 0.115 BYA, First monotreme (egg-laying) mammals.
- 0.110 BYA, Toothed diving birds.
- 0.100 BYA, Earliest bees.
- 0.090 BYA, Probable origin of placental mammals. However, the first undisputed fossil evidence is from 0.066 BYA.
- 0.080 BYA, First ants.
- 0.066 BYA, The Cretaceous-Paleogene extinction event wipes out about half of all animal species, including all of the dinosaurs except the birds. Afterwards, mammals become the dominant animal species. Conifers dominate northern forests.
- 0.060 BYA, Earliest true primates. Diversification of large, flightless birds. The ancestors of carnivorous mammals had appeared.
- 0.055 BYA, Diversification of birds. First songbirds, parrots, loons, swifts, and woodpeckers. First whale.
- 0.052 BYA, First bats appear in the fossil record.
- 0.050 BYA, Tapirs, rhinoceroses and camels appear. Diversification of primates.
- 0.040 BYA, Modern-type moths and butterflies were alive.
- 0.035 BYA, Grasses diversify. Many modern mammal groups appear.
- 0.030 BYA, Earliest pigs and cats.

[9]Today, there is a danger that human use of fossil fuels will initiate a very similar extinction event. This danger will be discussed in a later chapter.

- 0.025 BYA, First deer.
- 0.020 BYA, Giraffes, hyenas, bears, and giant anteaters appear. Birds increase in diversity.
- 0.015 BYA, First mastodons. Australian megafauna diversify. Kangaroos appear.
- 0.010 BYA, Grasslands and savannahs are established. Major diversification of grassland animals and snakes. Insects diversify, especially ants and termites.
- 0.0095 BYA = 9.50 MYA, Great American Interchange occurs. Armadillos, opossums, hummingbirds, "terror birds", and ground sloths were among the species that migrated from South America to North America after a land bridge formed between the previously isolated continents. Species moving in the opposite direction included horses, tapirs, saber-toothed cats, jaguars, bears, coaties, ferrets, otters, skunks and deer.
- 6.50 MYA, First homanins (our human ancestors diverging from the apes).
- 6.00 MYA, Australopithecines (extinct close relatives of humans after the split with chimpanzees) diversify.
- 5.00 MYA, First tree sloths and hippopotami. Diversification of grazing and carnivorous mammals.
- 4.00 MYA, Diversification of Australopithecines. The first modern elephants, giraffes, zebras, lions, rhinoceros and gazelles.
- 2.80 MYA, Appearance of a species intermediate between the Anthropithecines and Homo Habilis.
- 2.10 MYA, First member of the genus *Homo* appears, Homo habilis.

12.6 Life elsewhere in the universe

On December 18, 2017, scientists from the University of California published an article in *Science News* entitled *Ancient fossil microorganisms indicate that life in the universe is common.* According to the article:

"A new analysis of the oldest known fossil microorganisms provides strong evidence to support an increasingly widespread understanding that life in the universe is common.

"The microorganisms, from Western Australia, are 3.465 billion years old. Scientists from UCLA and the University of Wisconsin-Madison report today in the journal Proceedings of the National Academy of Sciences that two of the species they studied appear to have performed a primitive form of photosynthesis, another apparently produced methane gas, and two others appear to have consumed methane and used it to build their cell walls.

"The evidence that a diverse group of organisms had already evolved extremely early in the Earth's history, combined with scientists' knowledge of the vast number of stars in the universe and the growing understanding that planets orbit so many of them, strengthens the case for life existing elsewhere in the universe because it would be extremely unlikely that life formed quickly on Earth but did not arise anywhere else."

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Chapter 13

HODGKIN, HUXLEY AND ECCLES

13.1 The flow of information between and within cells

Information is transferred between cells in several ways. Among bacteria, in addition to the chronologically vertical transfer of genetic information directly from a single parent to its two daughter cells on cell division, there are mechanisms for the sharing of genetic information in a chronologically horizontal way, between cells of the same generation. These horizontal genetic information transfers can be thought of as being analogous to sex, as will be seen more clearly from some examples.

In the most primitive mechanism of horizontal information transfer, a bacterium releases DNA into its surroundings, and the DNA is later absorbed by another bacterium, not necessarily of the same species. For example, a loop or plasmid of DNA conferring resistance to an antibiotic (an "R-factor") can be released by a resistant bacterium and later absorbed by a bacterium of another species, which then becomes resistant[1].

A second mechanism for horizontal information transfer involves infection of a bacterium by a virus. As the virus reproduces itself inside the bacterium, some of the host's DNA can chance to be incorporated in the new virus particles, which then carry the extra DNA to other bacteria.

Finally, there is a third mechanism (discovered by J. Lederberg) in which two bacteria come together and construct a conjugal bridge across which genetic information can flow.

Almost all multicellular animals and plants reproduce sexually. In the case of sexual reproduction the genetic information of both parents is thrown into a lottery by means of special cells, the gametes. Gametes of each parent contain only half the genetic information

[1] The fact that this can happen is a strong reason for using antibiotics with great caution in agriculture. Resistance to antibiotics can be transferred from the bacteria commonly found in farm animals to bacteria which are dangerous for humans. Microbiologists have repeatedly warned farmers, drug companies and politicians of this danger, but the warnings have usually been ignored. Unfortunately there are now several instances of antibiotic-resistant human pathogens that have been produced by indiscriminate use of antibiotics in agriculture.

of the parent, and the exact composition of that half is determined by chance. Thus, when the gametes from two sexes fuse to form a new individual, the chances for variability are extremely large. This variability is highly valuable to multicellular organisms which reproduce sexually, not only because variability is the raw material of evolutionary adaption to changes in the environment, but also because the great variability of sexually-reproducing organisms makes them less likely to succumb to parasites. Infecting bacteria might otherwise deceive the immune systems of their hosts by developing cell-surface antigens which resemble those of the host, but when they infect sexually-reproducing organisms where each individual is unique, this is much less likely.

Within the cells of all organisms living today, there is a flow of information from polynucleotides (DNA and RNA) to proteins. As messenger RNA passes through a ribosome, like punched tape passing through a computer tapereader, the sequence of nucleotides in the mRNA is translated into the sequence of nucleic acids in the growing protein. The molecular mechanism of the reading and writing in this process involves not only spatial complementarity, but also complementarity of charge distributions.

As a protein grows, one amino acid at a time, it begins to fold. The way in which it folds (the "tertiary conformation") is determined both by spatial complementarity and by complementarity of charge distributions: Those amino acids which have highly polar groups, i.e., where several atoms have large positive or negative excess charges - "hydrophilic" amino acids - tend to be placed on the outside of the growing protein, while amino acids lacking large excess charges - "hydrophobic" amino acids - tend to be on the inside, away from water. Hydrophilic amino acids form hydrogen bonds with water molecules. Whenever there is a large negative charge on an atom of an amino acid, it attracts a positively-charged hydrogen from water, while positively-charged hydrogens on nucleic acids are attracted to negatively charged oxygens of water. Meanwhile, in the interior of the growing protein, non-polar amino acids are attracted to each other by so-called van der Waals forces, which do not require large excess charges, but only close proximity.

When a protein is complete, it is ready to participate in the activities of the cell, perhaps as a structural element or perhaps as an enzyme. Enzymes catalyze the processes by which carbohydrates, and other molecules used by the cell, are synthesized. Often an enzyme has an "active site", where such a process takes place. Not only the spatial conformation of the active site but also its pattern of excess charges must be right if the catalysis is to be effective. An enzyme sometimes acts by binding two smaller molecules to its active site in a proper orientation to allow a reaction between them to take place. In other cases, substrate molecules are stressed and distorted by electrostatic forces as they are pulled into the active site, and the activation energy for a reaction is lowered.

Thus, information is transferred first from DNA and RNA to proteins, and then from proteins to (for example) carbohydrates. Sometimes the carbohydrates then become part of surface of a cell. The information which these surface carbohydrates ("cell surface antigens") contain may be transmitted to other cells. In this entire information transfer process, the "reading" and "writing" depend on steric complementarity and on complementarity of molecular charge distributions.

Not only do cells communicate by touching each other and recognizing each other's cell

surface antigens - they also communicate by secreting and absorbing transmitter molecules. For example, the group behavior of slime mold cells is coordinated by the cyclic adenosine monophosphate molecules, which the cells secrete when distressed.

Within most multicellular organisms, cooperative behavior of cells is coordinated by molecules such as hormones - chemical messengers. These are recognized by "receptors", the mechanism of recognition once again depending on complementarity of charge distributions and shape. Receptors on the surfaces of cells are often membrane-bound proteins which reach from the exterior of the membrane to the interior. When an external transmitter molecule is bound to a receptor site on the outside part of the protein, it causes a conformational change which releases a bound molecule of a different type from a site on the inside part of the protein, thus carrying the signal to the cell's interior. In other cases the messenger molecule passes through the cell membrane.

In this way the individual cell in a society of cells (a multicellular organism) is told when to divide and when to stop dividing, and what its special role will be in the economy of the cell society (differentiation). For example, in humans, follicle-stimulating hormone, lutenizing hormone, prolactin, estrogen and progesterone are among the chemical messengers which cause the cell differentiation needed to create the secondary sexual characteristics of females.

Another role of chemical messengers in multicellular organisms is to maintain a reasonably constant internal environment in spite of drastic changes in the external environment of individual cells or of the organism as a whole (homeostasis). An example of such a homeostatic chemical messenger is the hormone insulin, which is found in humans and other mammals. The rate of its release by secretory cells in the pancreas is increased by high concentrations of glucose in the blood. Insulin carries the news of high glucose levels to target cells in the liver, where the glucose is converted to glycogen, and to other target cells in the muscles, where the glucose is burned.

13.2 Nervous systems

Hormones require a considerable amount of time to diffuse from the cells where they originate to their target cells; but animals often need to act very quickly, in fractions of seconds, to avoid danger or to obtain food. Because of the need for quick responses, a second system of communication has evolved - the system of neurons.

Neurons have a cell bodies, nuclei, mitochondria and other usual features of eukaryotic cells, but in addition they possess extremely long and thin tubelike extensions called axons and dendrites. The axons function as informational output channels, while the dendrites are inputs. These very long extensions of neurons connect them with other neurons which can be at distant sites, to which they are able to transmit electrical signals. The complex network of neurons within a multicellular organism, its nervous system, is divided into three parts. A sensory or input part brings in signals from the organism's interior or from its external environment. An effector or output part produces a response to the input signal, for example by initiating muscular contraction. Between the sensory and effector

parts of the nervous system is a message-processing (internuncial) part, whose complexity is not great in the jellyfish or the leech. However, the complexity of the internuncial part of the nervous system increases dramatically as one goes upward in the evolutionary order of animals, and in humans it is truly astonishing.

The small button-like connections between neurons are called synapses. When an electrical signal propagating along an axon reaches a synapse, it releases a chemical transmitter substance into the tiny volume between the synapse and the next neuron (the post-synaptic cleft). Depending on the nature of the synapse, this chemical messenger may either cause the next neuron to "fire" (i.e., to produce an electrical pulse along its axon) or it may inhibit the firing of the neuron. Furthermore, the question of whether a neuron will or will not fire depends on the past history of its synapses. Because of this feature, the internuncial part of an animal's nervous system is able to learn. There many kinds of synapses and many kinds of neurotransmitters, and the response of synapses is sensitive to the concentration of various molecules in the blood, a fact which helps to give the nervous systems of higher animals extraordinary subtlety and complexity.

The first known neurotransmitter molecule, acetylcholine, was discovered jointly by Sir Henry Dale in England and by Otto Loewi in Germany. In 1921 Loewi was able to show that nerve endings transmit information to muscles by means of this substance. The idea for the critical experiment occurred to him in a dream at 3 am. Otto Loewi woke up and wrote down the idea; but in the morning he could not read what he had written. Luckily he had the same dream the following night. This time he took no chances. He got up, drank some coffee, and spent the whole night working in his laboratory. By morning he had shown that nerve cells separated from the muscle of a frog's heart secrete a chemical substance when stimulated, and that this substance is able to cause contractions of the heart of another frog. Sir Henry Dale later showed that Otto Loewi's transmitter molecule was identical to acetylcholine, which Dale had isolated from the ergot fungus in 1910. The two men shared a Nobel Prize in 1936. Since that time, a large variety of neurotransmitter molecules have been isolated. Among the excitatory neurotransmitters (in addition to acetylcholine) are noradrenalin, norepinephrine, serotonin, dopamine, and glutamate, while gamma-amino-butyric acid is an example of an inhibitory neurotransmitter.

In 1953, Stephen W. Kuffler, working at Johns Hopkins University, made a series of discoveries which yielded much insight into the mechanisms by which the internuncial part of mammalian nervous systems processes information. Kuffler's studies showed that some degree of abstraction of patterns already takes place in the retina of the mammalian eye, before signals are passed on through the optic nerve to the visual cortex of the brain. In the mammalian retina, about 100 million light-sensitive primary light-receptor cells are connected through bipolar neurons to approximately a million retinal neurons of another type, called ganglions. Kuffler's first discovery (made using microelectrodes) was that even in total darkness, the retinal ganglions continue to fire steadily at the rate of about thirty pulses per second. He also found that diffuse light illuminating the entire retina does not change this steady rate of firing.

Kuffler's next discovery was that each ganglion is connected to an array of about 100 primary receptor cells, arranged in an inner circle surrounded by an outer ring. Kuffler

found the arrays to be of two types, which he called "on center arrays" and "off center arrays". In the "on center arrays", a tiny spot of light, illuminating only the inner circle, produces a burst of frequent firing of the associated ganglion, provided that cells in the outer ring of the array remain in darkness. However, if the cells in the outer ring are also illuminated, there is a cancellation, and there is no net effect. Exactly the opposite proved to be the case for the "off center arrays". As before, uniform illumination of both the inner circle and outer ring of these arrays produces a cancellation and hence no net effect on the steady background rate of ganglion firing. However, if the central circle by itself is illuminated by a tiny spot of light, the ganglion firing is inhibited, whereas if the outer ring alone is illuminated, the firing is enhanced. Thus Kuffler found that both types of arrays give no response to uniform illumination, and that both types of arrays measure, in different ways, the degree of contrast in the light falling on closely neighboring regions of the retina.

Kuffler's research was continued by his two associates, David H. Hubel and Torsten N. Wessel, at the Harvard Medical School, to which Kuffler had moved. In the late 1950's, they found that when the signals sent through the optic nerves reach the visual cortex of the brain, a further abstraction of patterns takes place through the arrangement of connections between two successive layers of neurons. Hubbel and Wessel called the cells in these two pattern-abstracting layers "simple" and "complex". The retinal ganglions were found to be connected to the "simple" neurons in such a way that a "simple" cell responds to a line of contrasting illumination of the retina. For such a cell to respond, the line has to be at a particular position and has to have a particular direction. However, the "complex" cells in the next layer were found to be connected to the "simple" cells in such a way that they respond to a line in a particular direction, even when it is displaced parallel to itself[2].

In analyzing their results, Kuffler, Hubel and Wessel concluded that pattern abstraction in the mammalian retina and visual cortex takes place through the selective destruction of information. This conclusion agrees with what we know in general about abstractions: They are always simpler than the thing which they represent.

13.3 The giant squid axon

The mechanism by which electrical impulses propagate along nerve ax- ons was clarified by the English physiologists Alan Lloyd Hodgkin and Andrew Fielding Huxley (a grandson of Darwin's defender, Thomas Henry Huxley). In 1952, working with the giant axon of the squid (which can be as large as a millimeter in diameter), they demonstrated that the electrical impulse propagating along a nerve is in no way similar to an electrical current in

[2] Interestingly, at about the same time, the English physiologist J.Z. Young came to closely analogous conclusions regarding the mechanism of pattern abstraction in the visual cortex of the octopus brain. However, the similarity between the image-forming eye of the octopus and the image-forming vertebrate eye and the rough similarity between the mechanisms for pattern abstraction in the two cases must both be regarded as instances of convergent evolution, since the mollusc eye and the vertebrate eye have evolved independently.

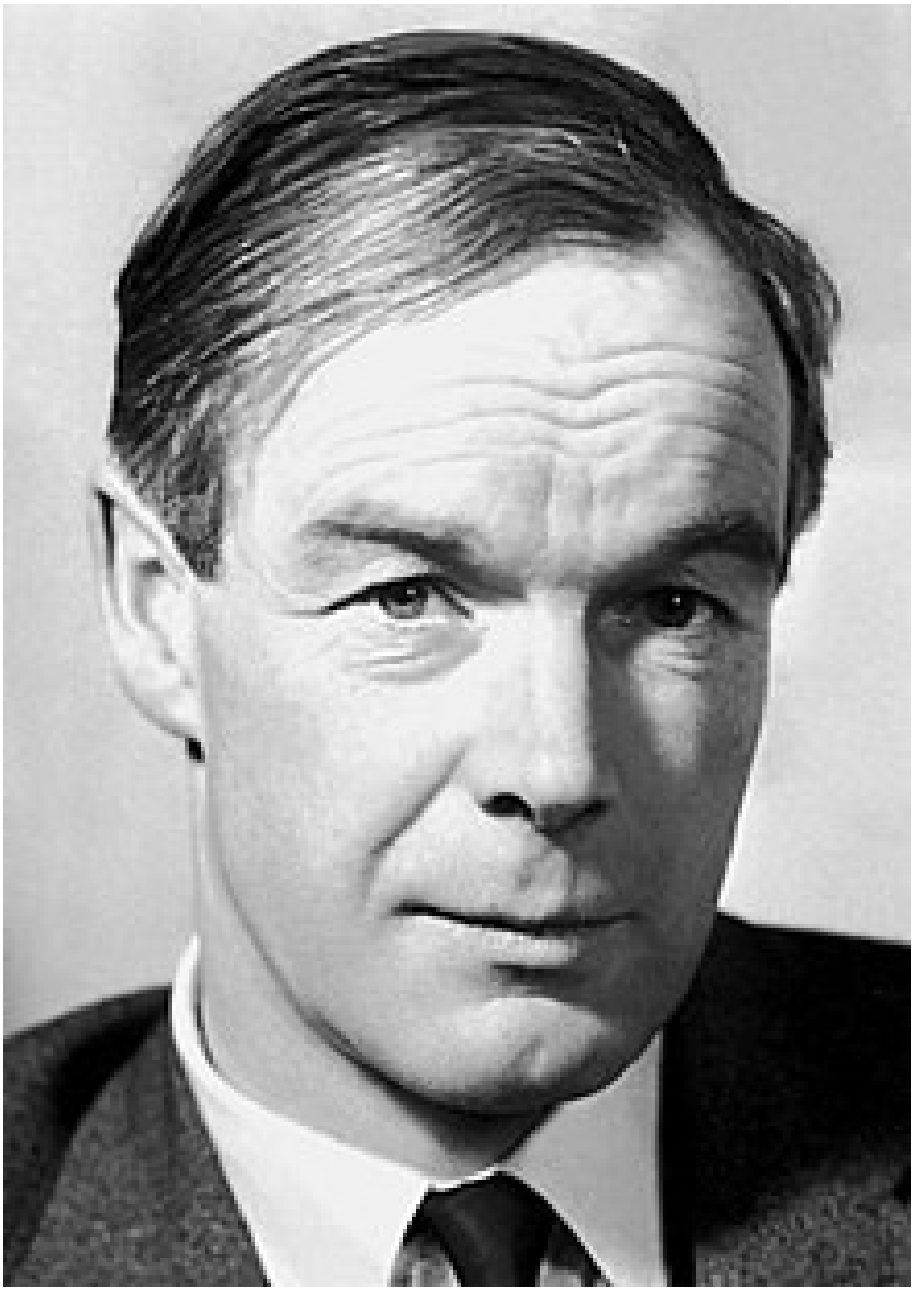

Figure 13.1: **Sir Alan Lloyd Hodgkin (1914-1998). He shared the 1963 Nobel Prize in Physiology or Medicine with Andrew Huxley and John Eccles.**

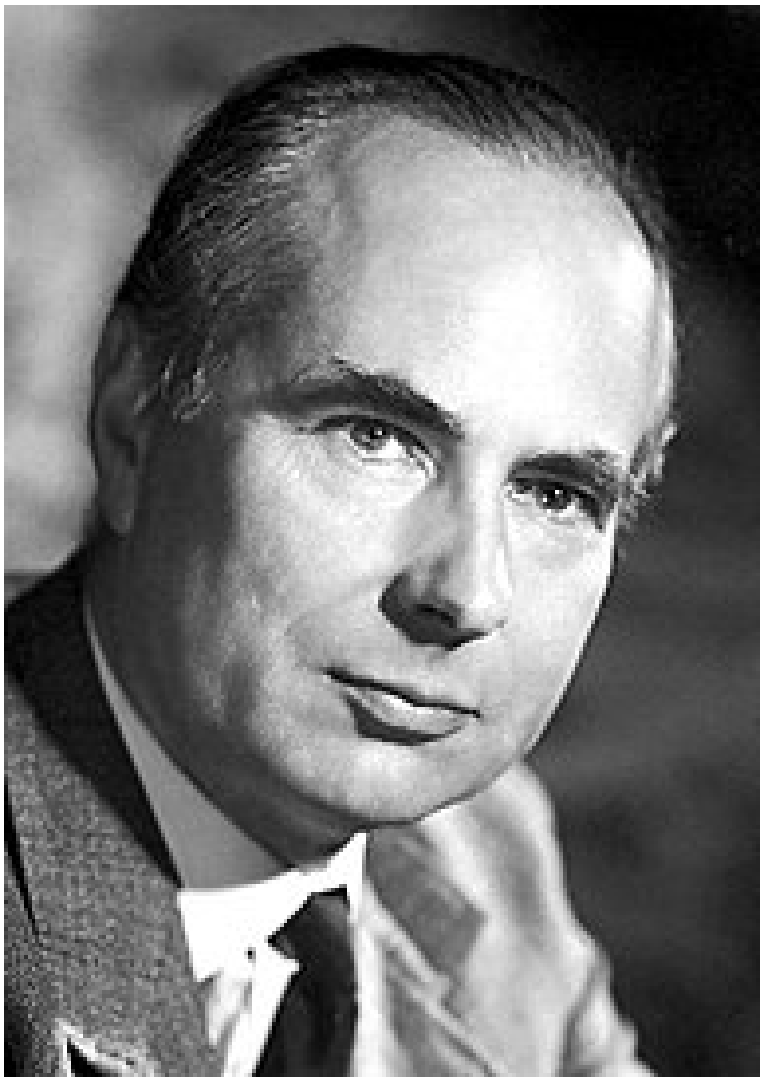

Figure 13.2: **Sir Andrew Fielding Huxley (1917-2012). He was a member of a famous family that included Thomas Henry Huxley ("Darwin's bulldog"), Aldous Huxley (author of *Brave New World*) and Sir Julian Huxley (a renowned evolutionary biologist, and the first director of UNESCO).**

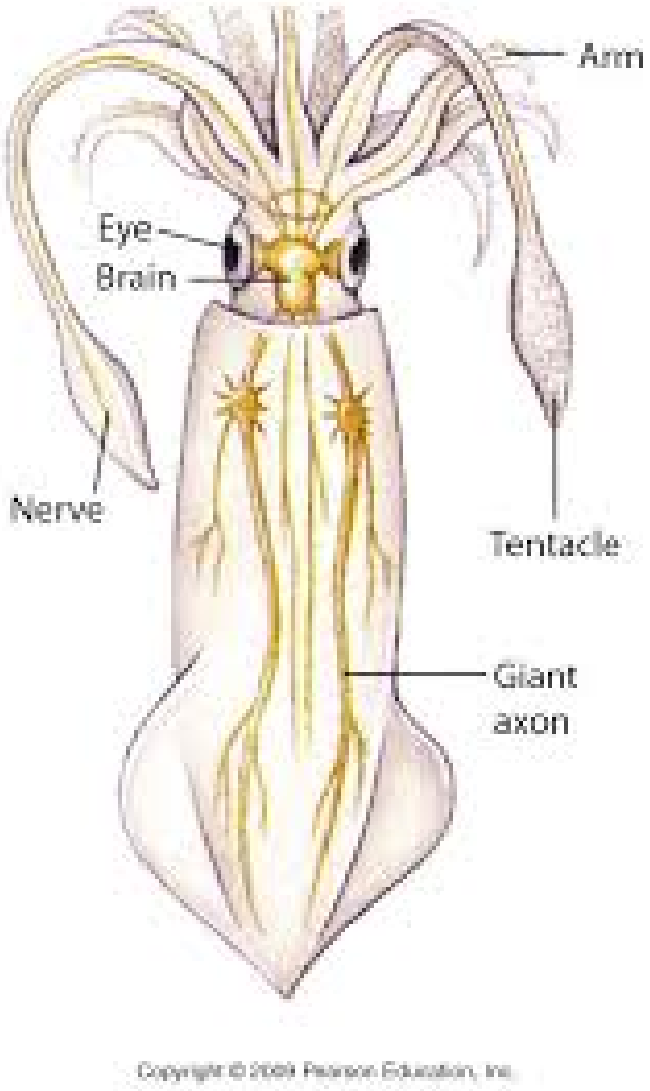

Figure 13.3: **The squid giant axon was large enough to allow Hodgkin and Huxley to perform their experiments demonstrating the mechanism of signal propagation in nerves. The squid giant axon was discovered by John Zachary Young (1907-1997) in the 1930's.**

Figure 13.4: **Hodgkin and Huxley working together.**

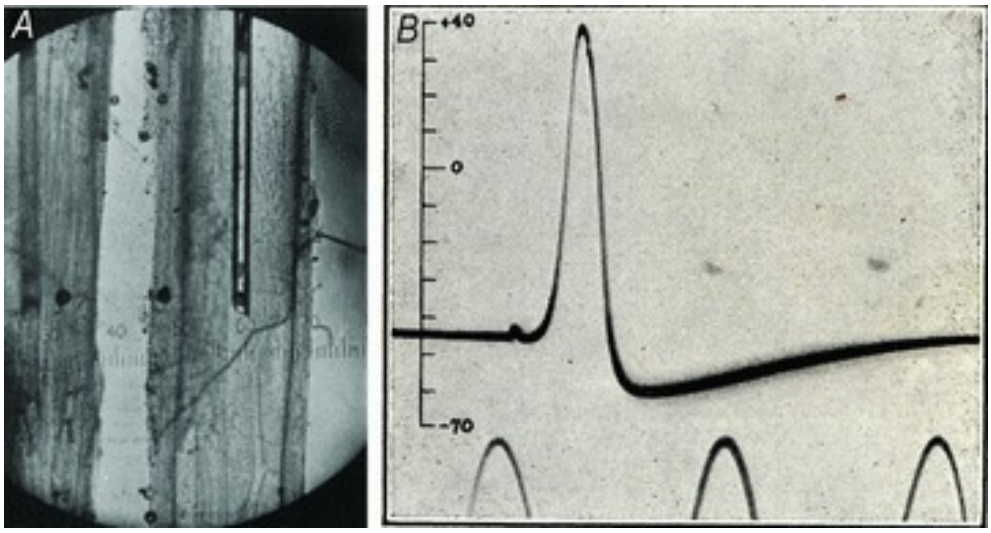

Figure 13.5: **Intracellular recording of the squid giant axon action potential.**

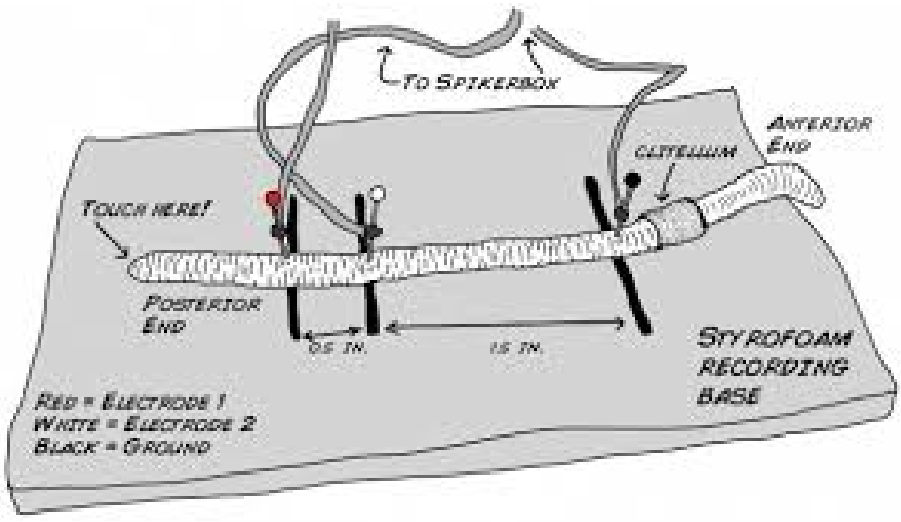

Figure 13.6: **A diagram of the Hodgkin-Huxley experiment with the giant squid axon.**

a conducting wire, but is more closely analogous to a row of dominoes knocking each other down. The nerve fiber, they showed, is like a long thin tube, within which there is a fluid containing K^+, and Na^+ ions, as well as anions. Inside a resting nerve, the concentration of K^+ is higher than in the normal body fluids outside, and the concentration of Na^+ is lower. These abnormal concentrations are maintained by an "ion pump", which uses the Gibbs free energy of adenosine triphosphate (ATP) to bring potassium ions into the nerve and to expel sodium ions.

The membrane surrounding the neural axon is more permeable to potassium ions than to sodium, and the positively charged potassium ions tend to leak out of the resting nerve, producing a small difference in potential between the inside and outside. This "resting potential" helps to hold the molecules of the membrane in an orderly layer, so that the membrane's permeability to ions is low.

Hodgkin and Huxley showed that when a neuron fires, the whole situation changes dramatically. Triggered by the effects of excitatory neurotransmitter molecules, sodium ions begin to flow into the axon, destroying the electrical potential which maintained order in the membrane. A wave of depolarization passes along the axon. Like a row of dominoes falling, the disturbance propagates from one section to the next: Sodium ions flow in, the order-maintaining electrical potential disappears, the next small section of the nerve membrane becomes permeable, and so on. Thus, Hodgkin and Huxley showed that when a neuron fires, a quick pulse-like electrical and chemical disturbance is transmitted along the axon.

Afterwards, the resting potential is restored by the sodium-potassium ion pump, later discovered by the Danish physiologist Jens Christian Skou. The pump consists of membrane-bound enzymes that use the energy of ATP to transport the ions across the electrochemical gradient.

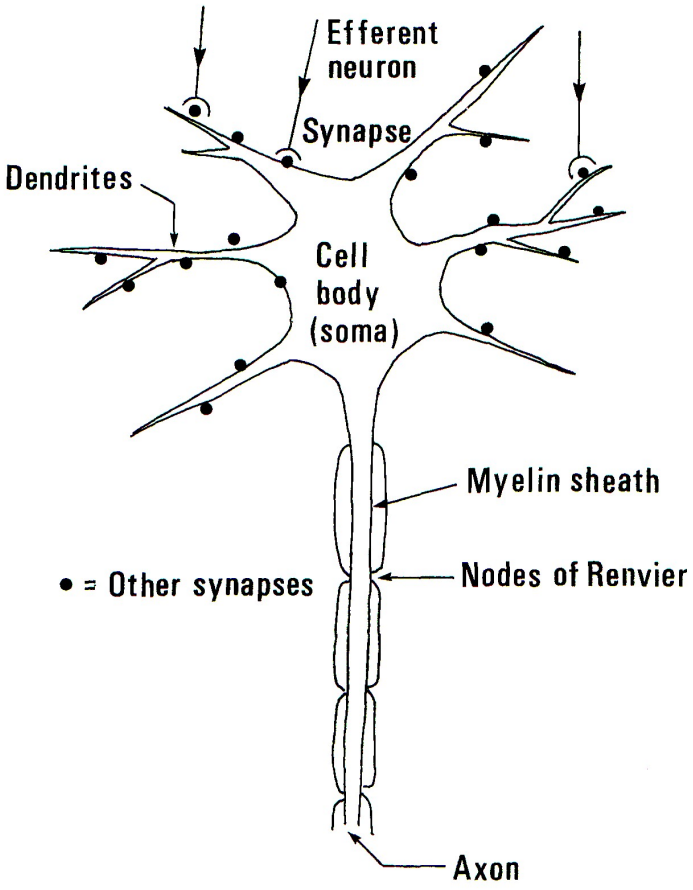

Figure 13.7: A schematic diagram of a neuron.

13.4 Chemical synapses

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Some important neurotransmitters

- **Glutamate**: This is the most abundant neurotransmitter in humans, used by about half of the neurons in the human brain. It is the primary excitatory transmitter in the central nervous system. One of its functions is to help form memories.

- **GABA**: The name GABA is an acronym for Gamma-aminobutyric acid. GABA is the primary inhibitory transmitter in the vertebrate brain. It helps to control anxiety, and it is sometimes used medically to treat anxiety and the associated sleeplessness.

- **Glycine**: This neurotransmitter is a single amino acid. It is the main inhibitory neurotransmitter in the vertebrate spinal cord. Glycine is important in the central nervous system, especially in the spinal cord, brainstem, and retina.

- **Acetylcholine**: An ester (the organic analogue of a salt) formed from the reaction between choline and acetic acid, acetylcholine stimulates muscles, functions in the autonomic nervous system and sensory neurons, and is associated with REM sleep. Alzheimer's disease is associated with a significant drop in acetylcholine levels.

- **Norepinepherine**: Also known as noradrenaline, norepinephorine increases heart rate and blood pressure. It is part of the body's "fight or flight" system. Norepinephrine is also needed to form memories. Stress depletes stores of this neurotransmitter.

- **Dopamine**: Dopamine is also synthesized in plants and most animals. It is an inhibitory transmitter associated with the reward center of the brain. Low dopamine levels are associated with social anxiety and Parkinson's disease, while excess dopamine is related to schizophrenia. The brain includes several distinct dopamine pathways, one of which plays a major role in reward-motivated behavior. Most types of rewards increase the level of dopamine in the brain, and many addictive drugs increase dopamine neuronal activity.

- **Serotonin**: Biochemically derived from the amino acid tryptophanis, serotonin an inhibitory neurotransmitter involved in mood, emotion, and perception. Low serotonin levels can lead to depression, suicidal tendencies, anger management issues, difficulty sleeping, migraines, and an increased craving for carbohydrates. It's functions include the regulation of mood, appetite, and sleep. Serotonin also has some cognitive functions, including memory and learning.

- **Endorphins**: The name of this class of neurotransmitters means "a class of a morphine-like substance originating from within the body". are a class of molecules similar to opioids (e.g., morphine, heroin) in terms of structure and function. The word "endorphin" is short for "endogenous morphine." Endorphins are inhibitory transmitters associated with pleasure and pain relief. In other animals, these chemicals slow metabolism and permit hibernation. The treatment of pain by means of acupuncture functions by releasing endorphines.

13.6 Transmission of signals across synapses

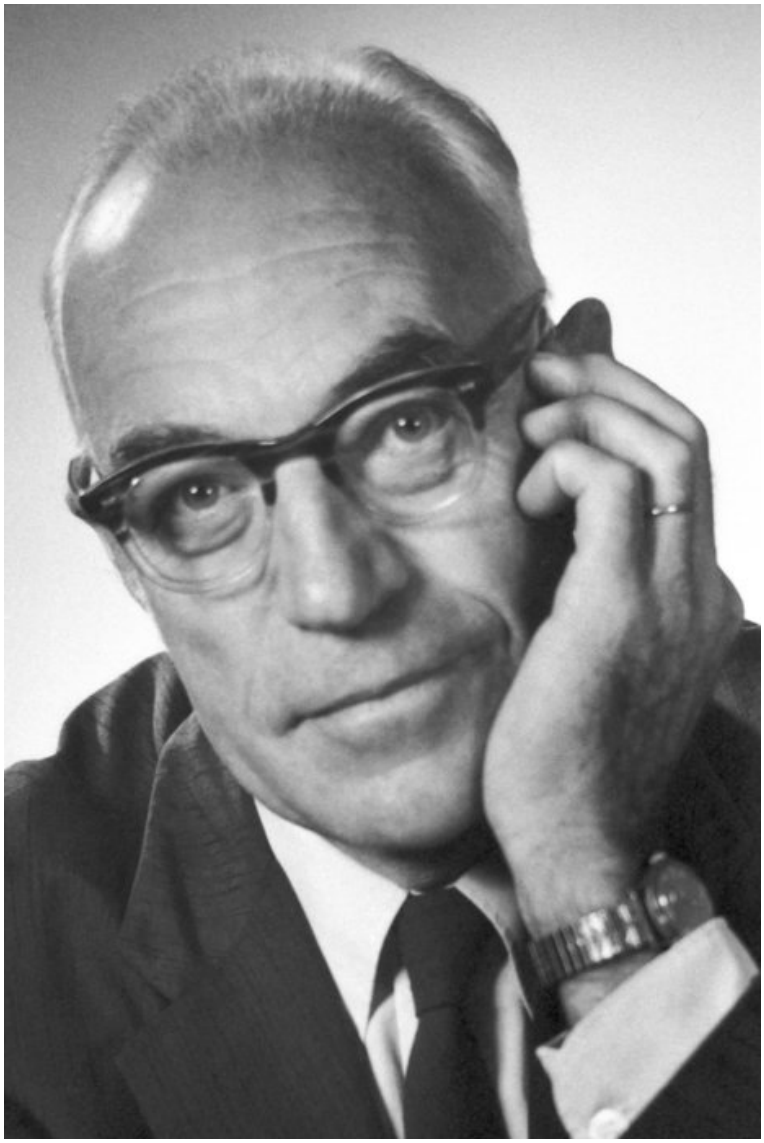

Figure 13.8: **Sir John Carew Eccles (1903-1997).**

Figure 13.9: **Jens Christian Skou (1908-2018). He received a Nobel Prize in Chemistry in in 1997 for his discovery of the K^+-Na^+ ion pump that uses energy from ATP to transport the ions across membranes against the electrochemical gradient. The photo shows him in 2008. He was born in Lemvig, Denmark.**

13.7 Are matter and mind separate?

One could, in principle, supply a computer with an input stream of sensory data, and program the computer to perform actions on the external world. In fact, the computer could be programmed in such a way that the actions taken would depend on the stored memory of previous sensory input. Could the computer then be said to be conscious? This depends on the way in which we define the word "conscious", and so the question is a semantic one, depending on our choice of a definition.

In any case, such a computer arrangement would be very closely analogous to the way in which living organisms experience their environment and act on it. Even the most primitive organisms receive a continuous stream of input data, and, if we choose, we can call this stream an elementary form of consciousness. Living organisms then react to the input stream, and their reactions may be modified by stored information of previous input data. The modification of response on the basis of previous experience is usually called "internuncial" modification, and it will be discussed below.

The pioneering Estonian scientist Jakob von Uexküll, whom we will discuss in detail below, introduced the word "Umwelt", which he defined to be the stream of sensory input data experienced by an organism. For example, speaking of a tick, he wrote: "...this eyeless animal finds the way to her watchpoint [at the top of a tall blade of grass] with the help of only its skin's general sensitivity to light. The approach of her prey becomes apparent to this blind and deaf bandit only through her sense of smell. The odor of butyric acid, which emanates from the sebaceous follicles of all mammals, works on the tick as a signal that causes her to abandon her post (on top of the blade of grass/bush) and fall blindly downward toward her prey. If she is fortunate enough to fall on something warm (which she perceives by means of an organ sensible to a precise temperature) then she has attained her prey, the warm-blooded animal, and thereafter needs only the help of her sense of touch to find the least hairy spot possible and embed herself up to her head..."

Figure 13.10: **The French philosopher, mathematician and scientist René Descartes (1596-1650) advocated mind-matter dualism. Descartes thought that nerves bring sensory inputs to the brain, where the data are then transferred to the "soul". After some time, he thought, the soul tells the brain how how the human should respond. Descartes did not discuss the question of whether organisms very low on the evolutionary scale have souls. Darwin visualized a continuous evolutionary progression from lower forms of life to ourselves. At what point did these less developed organisms obtain souls? Everyone must find his or her own opinion on this question.**

13.8 Jakob von Uexküll and Umwelt

Jakob Johann, Baron von Uexküll (1864-1944) was born in Estonia, on the estate of his aristocratic parents, Alexander, Baron von Uexküll and Sophie von Hahn. The family lost most of their wealth by expropriation during the Russian Revolution, and Jakob was forced to earn a living. He studied zoology at the University of Tartu. After graduation, he worked at the Institute of Physiology at the University of Heidelberg, and later at the Zoological Station in Naples. In 1907, he was given an honorary doctorate by Heidelberg for his studies of the physiology of muscles. Among his discoveries in this field was the first recognized instance of negative feedback in an organism.

Later work was concerned with the way in which animals experience the world around them. To describe the animal's subjective perception of its environment he introduced the word Umwelt; and in 1926 he founded the Institut für Umweltforschung at the University of Hamburg. Von Uexküll visualized an animal - for example a mouse - as being surrounded by a world of its own - the world conveyed by its own special senses organs, and processed by its own interpretative systems. Obviously, the Umwelt will differ greatly depending on the organism. For example, bees are able to see polarized light and ultraviolet light; electric eels are able to sense their environment through their electric organs; many insects are extraordinarily sensitive to pheromones; and a dog's Umwelt far richer in smells than that of most other animals. The Umwelt of a jellyfish is very simple, but nevertheless it exists.

It is interesting to ask to what extent the concept of Umwelt can be equated to that of consciousness. To the extent that these two concepts can be equated, von Uexküll's Umweltforschung offers us the opportunity to explore the phylogenetic evolution of the phenomenon of consciousness.

Von Uexküll's Umwelt concept can even extend to one-celled organisms, which receive chemical and tactile signals from their environment, and which are often sensitive to light. The ideas and research of Jakob von Uexküll inspired the later work of the Nobel Laureate ethologist Konrad Lorenz, and thus von Uexküll can be thought of as one of the founders of ethology as well as of biosemiotics. Indeed, ethology and biosemiotics are closely related. Because of his work on feedback loops in living organisms, von Uexküll can also be thought of as an early pioneer of cybernetics. His work influenced the philosophers Max Scheler, Ernst Cassirer, Martin Heidegger, Maurice Merleau-Ponty, Humberto Maturana, Georges Canguilhem, Michel Foucault, Gilles Deleuze and Félix Guattari.

Interestingly, his grandson, Carl Wolmar Jakob, Baron von Uexküll (born 1944) became a member of the European Parliament and contributed the funds for the Right Livelihood Award, which has been called the "Alternative Nobel Prize". Carl Wolmer Jakob is also the co-founder of the World Future Council and the Other Economic Summit.

Amoebae, slime molds and sponges

Amoebae are eukaryotes that have the ability to alter their shape. Like other eukaryotes they have a cell nucleus and other organelles, such as mitochondria, surrounded by an

Figure 13.11: **Jakob Johann, Baron von Uexküll (1864-1944) was the founder of Umwelt research. He was also an early pioneer of Cybernetics and Biosemiotics.**

Figure 13.12: **Carl Wolmar Jakob, Baron von Uexküll (born 1944) co-founded the World Future Council and the Other Economic Summit, as well as contributing the money needed to fund the Right Livelihood Award.**

Figure 13.13: **The Copenhagen-Tartu school of biosemiotics is a network of scholars working in the field of biosemiotics at the University of Tartu and the University of Copenhagen. An important member of the group is Center Leader Claus Emmeche of the Niels Bohr Institute (shown here). Other members include Kalevi Kull, Jesper Hoffmeyer, Peeter Torop, Timo Maran and Mikhail Lotman.**

outer membrane. Amoebae often eat bacteria by engulfing them.

More than 900 species of slime molds exist in various parts of the world. They are very common on the floors of tropical rain forests, where they perform the valuable service of helping to recycle nutrients.

Slime molds are particularly interesting because they gives us a glimpse of how multicellular organisms may have originated. The name of the slime molds is misleading, since they are not fungi, but heterotrophic protists similar to amoebae. Under ordinary circumstances, the individual cells wander about independently searching for food, which they draw into their interiors and digest, a process called "phagocytosis". However, when food is scarce, they send out a chemical signal of distress. Researchers have analyzed the molecule which expresses slime mold unhappiness, and they have found it to be cyclic adenosine monophosphate (cAMP). At this signal, the cells congregate and the mass of cells begins to crawl, leaving a slimy trail. At it crawls, the community of cells gradually develops into a tall stalk, surmounted by a sphere - the "fruiting body". Inside the sphere, spores are produced by a sexual process. If a small animal, for example a mouse, passes by, the spores may adhere to its coat; and in this way they may be transported to another part of the forest where food is more plentiful.

Thus slime molds represent a sort of missing link between unicellular and multicellular or organisms. Normally the cells behave as individualists, wandering about independently, but when challenged by a shortage of food, the slime mold cells join together into an entity which closely resembles a multicellular organism. The cells even seem to exhibit altruism, since those forming the stalk have little chance of survival, and yet they are willing to perform their duty, holding up the sphere at the top so that the spores will survive and carry the genes of the community into the future. We should especially notice the fact that the cooperative behavior of the slime mold cells is coordinated by chemical signals.

Sponges are also close to the borderline which separates unicellular eukaryotes (protists) from multicellular organisms, but they are just on the other side of the border. Normally the sponge cells live together in a multicellular community, filtering food from water. However, if a living sponge is forced through a very fine cloth, it is possible to separate the cells from each other. The sponge cells can live independently for some time; but if many of them are left near to one another, they gradually join together and form themselves into a new sponge, guided by chemical signals. In a refinement of this experiment, one can take two living sponges of different species, separate the cells by passing the sponges through a fine cloth, and afterwards mix all the separated cells together. What happens next is amazing: The two types of sponge cells sort themselves out and become organized once more into two sponges - one of each species.

Slime molds and sponges hint at the genesis of multicellular organisms, whose evolution began approximately 600 million years ago. Looking at the slime molds and sponges, we can imagine how it happened. Some unicellular organisms must have experienced an enhanced probability of survival when they lived as colonies. Cooperative behavior and division of labor within the colonies were rewarded by the forces of natural selection, with the selective force acting on the entire colony of cells, rather than on the individual cell. This resulted in the formation of cellular societies and the evolution of mechanisms for cell

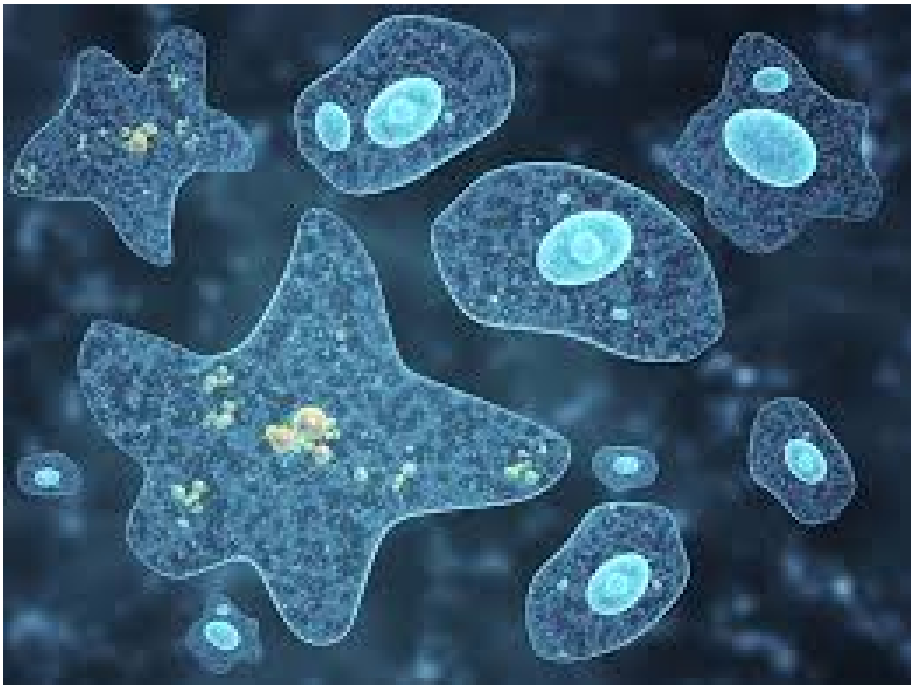

Figure 13.14: **Amoebae are eukaryotes, with a nucleus and other organelles, such as mitochondria, contained within a cell membrane. They are able to change their shapes, and often eat bacteria by engulfing them.**

differentiation. The division of labor within cellular societies (i.e., differentiation) came to be coordinated by chemical signals which affected the transcription of genetic information and the synthesis of proteins. Each cell within a society of cells possessed the entire genome characteristic of the colony, but once a cell had been assigned its specific role in the economy of the society, part of the information became blocked - that is, it was not expressed in the function of that particular cell. As multicellular organisms evolved, the chemical language of intercellular communication became very much more complex and refined. later section.

The world as seen by a jellyfish

Not all jellyfish are alike. Some species have much more highly-developed sensory perception than others. Jellyfish can swim, and their motions are coordinated by a rudimentary nervous system.

According to Wikipedia, "Jellyfish employ a loose network of nerves, located in the epidermis, which is called a 'nerve net'. Although traditionally thought not to have a central nervous system, nerve net concentration and ganglion-like structures could be considered to constitute one in most species. A jellyfish detects various stimuli including the touch of other animals via this nerve net, which then transmits impulses both throughout the nerve net and around a circular nerve ring, through the rhopalial lappet, located at the rim of

Figure 13.15: **The fruiting bodies of a slime mold.**

Figure 13.16: **Like slime molds, sponges are close to the borderline between single-celled and multi-cellular organisms.**

Figure 13.17: **How does a jellyfish experience the world around it?**

the jellyfish body, to other nerve cells.

"Some jellyfish have ocelli: light-sensitive organs that do not form images but which can detect light and are used to determine up from down, responding to sunlight shining on the water's surface. These are generally pigment spot ocelli, which have some cells (not all) pigmented.

"Certain species of jellyfish, such as the box jellyfish, have more advanced vision than their counterparts. The box jellyfish has 24 eyes, two of which are capable of seeing color, and four parallel information processing areas or rhopalia that act in competition, supposedly making it one of the few creatures to have a 360-degree view of its environment.

"The eyes are suspended on stalks with heavy crystals on one end, acting like a gyroscope to orient the eyes skyward. They look upward to navigate from roots in mangrove swamps to the open lagoon and back, watching for the mangrove canopy, where they feed."

13.9 Biosemiotics

The Oxford Dictionary of Biochemistry and Molecular Biology (Oxford University Press, 1997) defines biosemiotics as "the study of signs, of communication, and of information in living organisms". The biologists Claus Emmeche and K. Kull offer another definition of biosemiotics: "biology that interprets living systems as sign systems".

The American philosopher Charles Sanders Peirce (1839-1914) is considered to be one of the founders of semiotics (and hence also of biosemiotics). Peirce studied philosophy and

chemistry at Harvard, where his father was a professor of mathematics and astronomy. He wrote extensively on philosophical subjects, and developed a theory of signs and meaning which anticipated many of the principles of modern semiotics. Peirce built his theory on a triad: (1) the sign, which represents (2) something to (3) somebody. For example, the sign might be a broken stick, which represents a trail to a hunter, it might be the arched back of a cat, which represents an aggressive attitude to another cat, it might be the waggle-dance of a honey bee, which represents the coordinates of a source of food to her hive-mates, or it might be a molecule of trans-10-cis-hexadecadienol, which represents irresistible sexual temptation to a male moth of the species Bombyx mori. The sign might be a sequence of nucleotide bases which represents an amino acid to the ribosome-transfer-RNA system, or it might be a cell-surface antigen which represents self or non-self to the immune system. In information technology, the sign might be the presence or absence of a pulse of voltage, which represents a binary digit to a computer. Semiotics draws our attention to the sign and to its function, and places much less emphasis on the physical object which forms the sign. This characteristic of the semiotic viewpoint has been expressed by the Danish biologist Jesper Hoffmeyer in the following words: "The sign, rather than the molecule, is the basic unit for studying life."

A second important founder of biosemiotics was Jakob von Uexküll (1864-1944). He was born in Estonia, and studied zoology at the University of Tartu. After graduation, he worked at the Institute of Physiology at the University of Heidelberg, and later at the Zoological Station in Naples. In 1907, he was given an honorary doctorate by Heidelberg for his studies of the physiology of muscles. Among his discoveries in this field was the first recognized instance of negative feedback in an organism. Von Uexküll's later work was concerned with the way in which animals experience the world around them. To describe the animal's subjective perception of its environment he introduced the word Umwelt; and in 1926 he founded the Institut fur Umweltforschung at the University of Heidelberg. Von Uexküll visualized an animal - for example a mouse - as being surrounded by a world of its own - the world conveyed by its own special senses organs, and processed by its own interpretative systems. Obviously, the Umwelt will differ greatly depending on the organism. For example, bees are able to see polarized light and ultraviolet light; electric eels are able to sense their environment through their electric organs; many insects are extraordinarily sensitive to pheromones; and a dog's Umwelt far richer in smells than that of most other animals. The Umwelt of a jellyfish is very simple, but nevertheless it exists.[3] Von Uexküll's Umwelt concept can even extend to one-celled organisms, which receive chemical and tactile signals from their environment, and which are often sensitive to light. The ideas and research of Jakob von Uexk"ull inspired the later work of the Nobel Laureate ethologist Konrad Lorenz, and thus von Uexküll can be thought of as one of the founders of ethology as well as of biosemiotics. Indeed, ethology and biosemiotics are closely related.

Biosemiotics also values the ideas of the American anthropologist Gregory Bateson

[3] It is interesting to ask to what extent the concept of Umwelt can be equated to that of consciousness. To the extent that these two concepts can be equated, von Uexküll's Umweltforschung offers us the opportunity to explore the phylogenetic evolution of the phenomenon of consciousness.

(1904-1980), who was mentioned in Chapter 7 in connection with cybernetics and with the Macy Conferences. He was married to another celebrated anthropologist, Margaret Mead, and together they applied Norbert Wiener's insights concerning feedback mechanisms to sociology, psychology and anthropology. Bateson was the originator of a famous epigrammatic definition of information: "..a difference which makes a difference" . This definition occurs in Chapter 3 of Bateson's book, Mind and Nature: A Necessary Unity, Bantam, (1980), and its context is as follows: "To produce news of a difference, i.e. information", Bateson wrote, "there must be two entities... such that news of their difference can be represented as a difference inside some information-processing entity, such as a brain or, perhaps, a computer. There is a profound and unanswerable question about the nature of these two entities that between them generate the difference which becomes information by making a difference. Clearly each alone is - for the mind and perception - a non-entity, a non-being... the sound of one hand clapping. The stuff of sensation, then, is a pair of values of some variable, presented over time to a sense organ, whose response depends on the ratio between the members of the pair."

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Chapter 14

PATHFINDING

14.1 The 2014 Nobel Prize in Physiology or Medicine

Some excerpts from Edvard L. Moser's Nobel lecture

All three 2014 Nobel Prize winners in Physiology or Medicine stand on the shoulders of E.C. Tolman. Based on experiments on rats running in various types of mazes, Tolman suggested from the 1930s to the 1950s that animals form internal maps of the external environment. He referred to such maps as cognitive maps and considered them as mental knowledge structures in which information was stored according to its position in the environment (Tolman, 1948). In this sense, Tolman was not only one of the first cognitive psychologists but he also directly set the stage for studies of how space is represented in the brain. Tolman himself avoided any reference to neural structures and neural activity in his theories, which was understandable at a time when neither concepts nor methods had been developed for investigations at the brain-behaviour

Figure 14.1: **The three winners of the 2014 Nobel Prize in Physiology or Medicine**

Figure 14.2: **Edward Chace Tolman (1886-1959). He founded a branch of psychology known as *perposive behaviourism*.**

interface. However, at the end of his life he expressed strong hopes for a neuroscience of behaviour. In 1958, after the death of Lashley, he wrote the following in a letter to Donald O. Hebb when Hebb asked him about his view of physiological explanations of behaviour in the early days of behaviourism: "I certainly was an anti-physiologist at that time and am glad to be considered as one then. Today, however, I believe that this ('physiologising') is where the great new break-throughs are coming."

The psychology-physiology boundary was broken from the other side by two pioneers of physiology, David Hubel and Torsten Wiesel, who in the late 1950s bravely started to record activity from single neurons in the cortex, the origin of most of our intellectual activity. Inserting electrodes into the primary visual cortex of awake animals, they discovered how activity of individual neurons could be related to specific elements of the visual image. This work set the stage for decades of investigation of the neural basis for vision and helped the emergence of a new field of cortical computation. Their insights at the low levels of the visual cortex provided a window into how the cortex might work. As a result of Hubel and Wiesel's work, parts of the coding mechanism for vision are now understood, almost 60 years after they started their investigations...

The potential for understanding a higher brain function brought May-Britt and me to John O'Keefe's lab in 1996. During a period of three months, John generously taught us everything about place cells and how they were studied and we then went back to Norway, to Trondheim, to set up our own new lab. One of our hopes was to find out how the place signal was generated.

In this overview, I will first review the events that led up to the discovery of grid cells and the organisation of a grid cell-based map of space in the medial

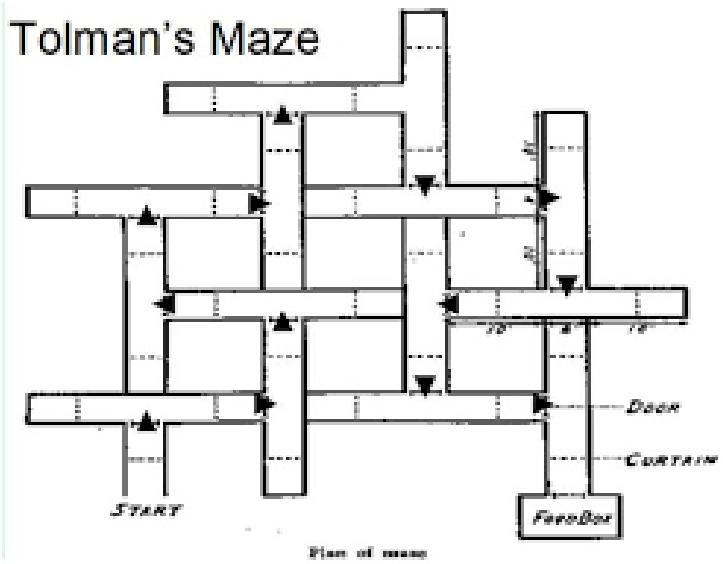

Figure 14.3: **Tolmen's experiments with animals learning to run through a maze form the foundation on which the work of John O'Keefe, May-Britt Moser and Edvard Moser was built.**

Figure 14.4: **David H. Hubel and Torsten N. Wiesel broke the physiology-psychology boundary from the physiology side. By identifying the elementary neural components of the visual image at low levels of the visual cortex, they showed that psychological concepts, such as sensation and perception, could be understood through elementary interactions between cells with specific functions.**

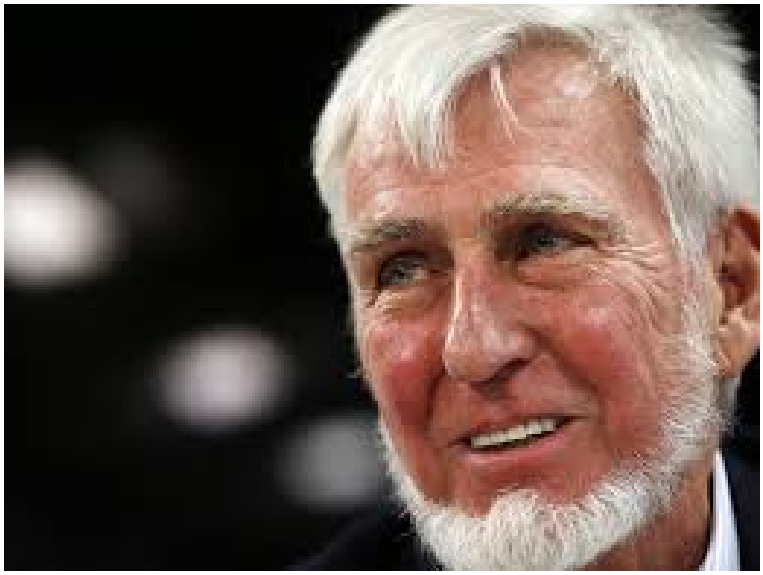

Figure 14.5: **A photo of John O'Keefe, who discovered place cells in the hippocampus. Place cells are cells that fire specifically when an animal is at a certain location in its local environment.**

entorhinal cortex. Then, in the second part, I will present recent work on the interactions between grid cells and the geometry of the external environment, the topography of the grid-cell map, and the mechanisms underlying the hexagonal symmetry of the grid cells.

To determine if place fields were formed in the intrahippocampal circuit, we worked together with neuroanatomist Menno Witter, then at the Free University of Amsterdam...

In 2005, with our students Torkel Hafting, Marianne Fyhn and Sturla Molden, we were able to describe the structure of the firing pattern. Using larger environments than in the past, we could clearly see that the firing pattern was periodic. The multiple firing fields of the cell formed a hexagonal grid that tiled the entire surface space available to the animal, much like the holes in a bee hive or a Chinese checkerboard. Many entorhinal cells fired like this, and we named them grid cells. We were excited about the grid- like firing pattern, both because nothing like it exists in the sensory inputs to the animal, suggesting that the pattern is generated intrinsically in the entorhinal cortex or neighbouring structures, and because such a regular pattern provides a metric to the brain's spatial map, a metric that had been missing in the place map of the hippocampus.

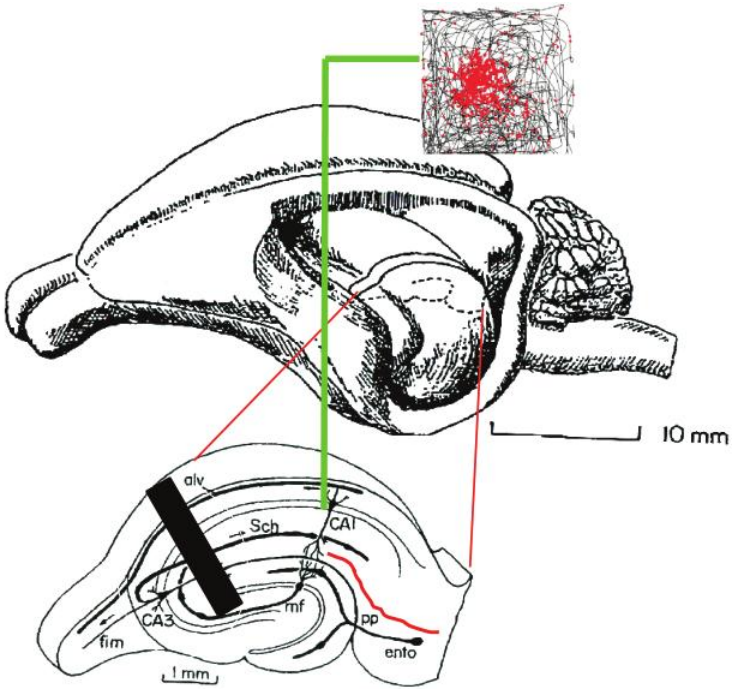

Figure 14.6: **Location of recording electrode and lesion in the experiment that led us to move out of the hippocampus, to the entorhinal cortex.**

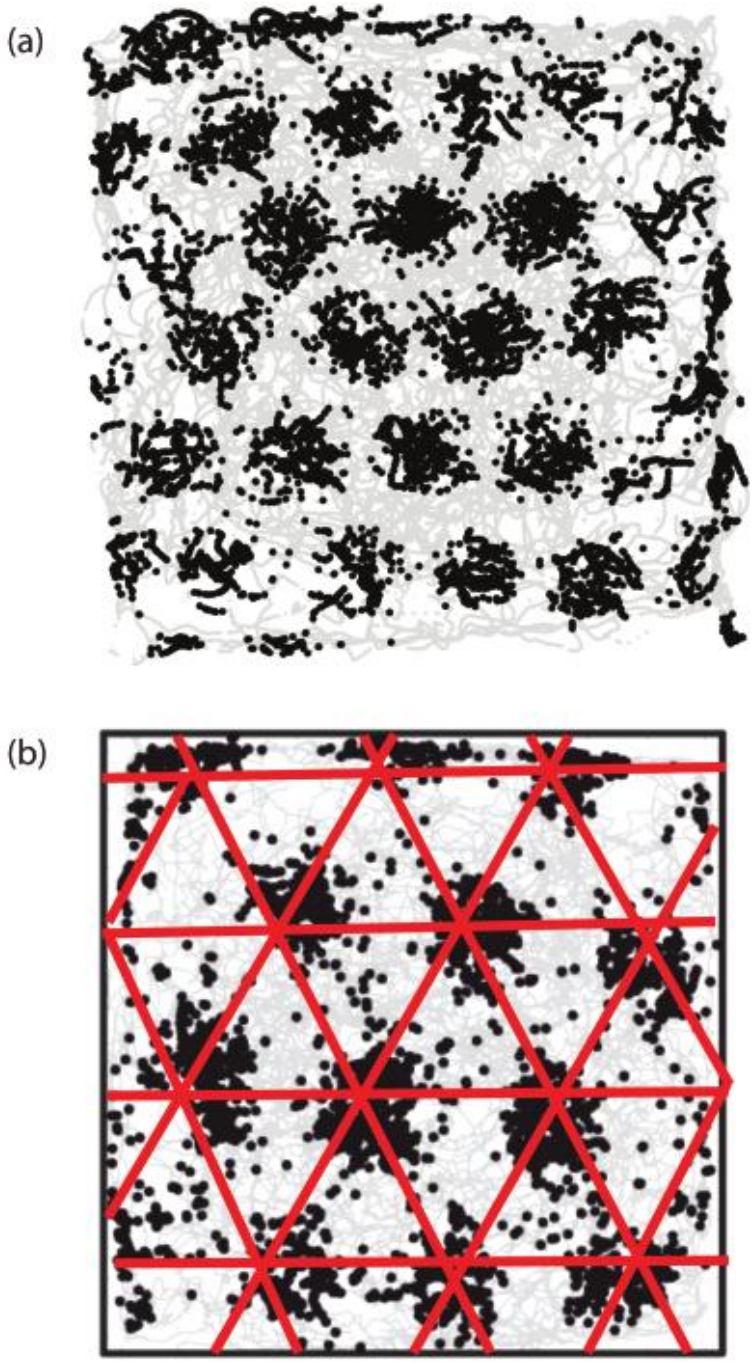

Figure 14.7: **Firing pattern of grid cells. (a) Spatially periodic firing pattern of an entorhinal grid cell during 30 min of foraging in a 220 cm wide square enclosure. The trajectory of the rat is shown in grey, individual spike locations in black. (b) Firing pattern of a grid cell in a 1 m wide enclosure. Symbols as in (a) but with red lines superimposed to indicate the hexagonal structure of the grid. Modified from Stensola et al. (2012) and Hafting et al. (2005), respectively.**

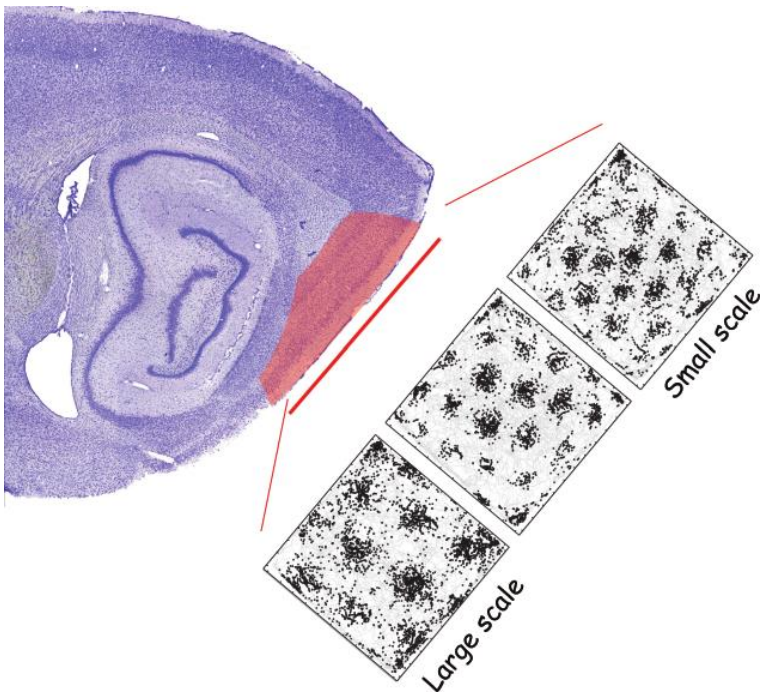

Figure 14.8: **Topographical organisation of grid scale. The figure shows a sagittal brain section with medial entorhinal cortex indicated in red. Firing maps are shown for three grid cells recorded at successive dorso-ventral levels in medial entorhinal cortex. Note change from small scale to large scale along the dorso-ventral axis. Modified from Stensola et al. (2012).**

14.2 Paths in cell differentiation

In animals, the fertilized egg cell divides a number of times to form the blastula. At this stage of development, the cells are unspecialized. However, as they continue to divide, the cells become increasingly specialized. First they are totipotent, then pluripotent, then multipotent, then oligopotent and finally unipotent. The increasingly specialized differentiation of cells is closely analogous to the increasingly specialized classification of destinations in package address systems, which will be discussed in the next section.

14.3 Paths in package address systems

The history of the Internet and World Wide Web

The history of the Internet began in 1961, when Leonard Kleinrock, a student at MIT, submitted a proposal for Ph.D. thesis entitled "Information Flow in Large Communication Nets". In his statement of the problem, Kleinrock wrote: "The nets under consideration consist of nodes, connected to each other by links. The nodes receive, sort, store, and transmit messages that enter and leave via the links. The links consist of one-way channels, with fixed capacities. Among the typical systems which fit this description are the Post Office System, telegraph systems, and satellite communication systems." Kleinrock's theoretical treatment of package switching systems anticipated the construction of computer networks which would function on a principle analogous to a post office rather than a telephone exchange: In a telephone system, there is a direct connection between the sender and receiver of information. But in a package switching system, there is no such connection - only the addresses of the sender and receiver on the package of information, which makes its way from node to node until it reaches its destination.

Further contributions to the concept of package switching systems and distributed communications networks were made by J.C.R. Licklider and W. Clark of MIT in 1962, and by Paul Baran of the RAND corporation in 1964. Licklider visualized what he called a "Galactic Network", a globally interconnected network of computers which would allow social interactions and interchange of data and software throughout the world. The distributed computer communication network proposed by Baran was motivated by the desire to have a communication system that could survive a nuclear war. The Cold War had also provoked the foundation (in 1957) of the Advanced Research Projects Agency (ARPA) by the U.S. government as a response to the successful Russian satellite "Sputnik".

In 1969, a 4-node network was tested by ARPA. It connected computers at the University of California divisions at Los Angeles and Santa Barbara with computers at the Stanford Research Institute and the University of Utah. Describing this event, Leonard Kleinrock said in an interview: "We set up a telephone connection between us and the guys at SRI. We typed the L and we asked on the phone 'Do you see the L?' 'Yes we see the L', came the response. We typed the 0 and we asked 'Do you see the 0?' 'Yes we see the O.' Then we typed the G and the system crashed." The ARPANET (with 40 nodes)

performed much better in 1972 at the Washington Hilton Hotel where the participants at a Conference on Computer Communications were invited to test it.

Although the creators of ARPANET visualized it as being used for long- distance computations involving several computers, they soon discovered that social interactions over the Internet would become equally important if not more so. An electronic mail system was introduced in the early 1970's, and in 1976 Queen Elizabeth II of the United Kingdom became one of the increasing number of e-mail users.

In September, 1973, Robert F. Kahn and Vinton Cerf presented the basic ideas of the Internet at a meeting of the International Network Working Group at the University Sussex in Brighton, England. Among these principles was the rule that the networks to be connected should not be changed internally. Another rule was that if a packet did not arrive at its destination, it would be retransmitted from its original source. No information was to be retained by the gateways used to connect networks; and finally there was to be no global control of the Internet at the operations level.

Computer networks devoted to academic applications were introduced in the 1970's and 1980's, both in England, the United States and Japan. The Joint Academic Network (JANET) in the U.K. had its counterpart in the National Science Foundation's network (NSFNET) in America and Japan's JUNET (Japan Unix Network). Internet traffic is approximately doubling each year,[1] and it is about to overtake voice communication in the volume of information transferred.

In March, 2011, there were more than two billion Internet users in the world. In North America they amounted to 78.3 % of the total population, in Europe 58.3 % and worldwide, 30.2 %. Another index that can give us an impression of the rate of growth of digital data generation and exchange is the "digital universe", which is defined to be the total volume of digital information that human information technology creates and duplicates in a year. In 2011 the digital universe reached 1.2 zettabytes, and it is projected to quadruple by 2015. A zettabyte is 10^{21} bytes, an almost unimaginable number, equivalent to the information contained in a thousand trillion books, enough books to make a pile that would stretch twenty billion kilometers.

Postal addresses

A second example of package address systems can be found in postal addresses. Here the coarsest category is country. Within a particular country the city or town is the next part of the address. Next, the street is specified; then the street number, and finally (in some cases), the number labeling the room or flat within a building. This progression from course categorization to progressively finer specification of the address can be seen in all types of classification.

[1] In the period 1995-1996, the rate of increase was even faster - a doubling every four months

14.4 Paths in the organization of computer memories

Most of us use directories to organize the data on our computers. For example, on my own PC, the address of the file on which I am working at the moment is "home/work/books/languages". There is a directory called "home". Within "home" there are many sub-directories, one of which is called "work". Suppose that we click on "work". We find within this sub-directory many sub-sub-directories, one of which is called "books". If, among the many options, we click on "books", we find that it contains many sub-sub-sub-directories, one of which is called "languages".

We can visualize the process of starting in the home directory and finally reaching the sub-sub-sub-directory "languages" as a process of pathfinding. At each point where the paths branch, we make a choice, just as an animal does when finding its way through a forest or maze. At each choice, the destination reached becomes more specific; the classification of destinations becomes more refined.

One is reminded of the postal address system, within which the destination of a letter becomes more refined at each branch: First the country is specified, then the city or town, then the street, then the house number, and finally (in some cases) the apartment or room. Here too, the destination becomes progressively more refined as one progresses through a set of choices.

One may even be reminded of the existentialist philosophy of Jean-Paul Sartre and others, which has the motto "existence is prior to essence". As we progress through life, we make choices, and within each choice, we make sub-choices which define more and more specifically our final destination, i.e. our destiny or "essence".

14.5 Pattern abstraction

Pattern abstraction in the octopus brain

J.Z. Young lectures to the Wells Society at Imperial College

I vividly remember a lecture that Prof. J.Z. Young delivered to the Wells Society[2] of London's Imperial College of Science and Technology. It was during the early 1960's, and at that time I was writing my Ph.D. thesis in theoretical chemistry.

Professor Young told us of his research on the visual cortex of the octopus. Being a mollusc, the octopus is lucky to have eyes at all, but in fact its eyes are very similar to our own, a striking example of convergent evolution. Young's research combined microscopic examination of extremely thin slices of the octopus brain with experiments on the extent to which the octopus is able to learn, and to profit from past experience.

Each image on the retina of the octopus eye is directly mapped in a one to one manner onto the outer layer of the animal's visual cortex. But as the signal propagated inwards towards the center of the visual cortex, the arrangement of dendrites and axons insures

[2]H.G. Wells had once been a student at Imperial College, London. and the Wells Society was named after him.

that synapses would only fire if activated by a specific pattern. The specificity of the pattern becomes progressively more refined as it propagates more deeply into the cortex.

Finally a "grandmother's face cell" is reached, a cell which can only be activated by a specific pattern. At this point in the visual cortex of the octopus, neural pathways to to parts of the brain controlling muscular actions are activated. The paths branched, with one leading towards an attack response and the other towards retreat. There is a bias towards the attack pathway, so that initially, any pattern observed by the eyes of the animal will produce an attack.

Professor Young told us that he could actually see the arrangements of dendrites and axons in his histological studies of the visual cortex of the octopus. These histological studies were supplemented by behavioral experiments, in which the octopus was either rewarded for the attack, or else punished with a mild electric shock. If rewarded, the animal would continue to attack when again presented with the same pattern. If punished, the animal would always retreat when presented with the same stimulus. Prof. Young explained this behaviour by postulating the existence of a feedback neural circuit which blocked the attack pathway if the animal was punished. When the signal subsequently passed the "grandmother's face cell", only the retreat pathway remained. The octopus had learned.

Figure 14.9: **Prof. John Zachary Young, FRS, in 1978. He has been described as "one of the most influential biologists of the 20th century". His studies of pattern abstraction in the visual cortex of the octopus combined examination of histological microsections with experimental studies of octopus learning.**

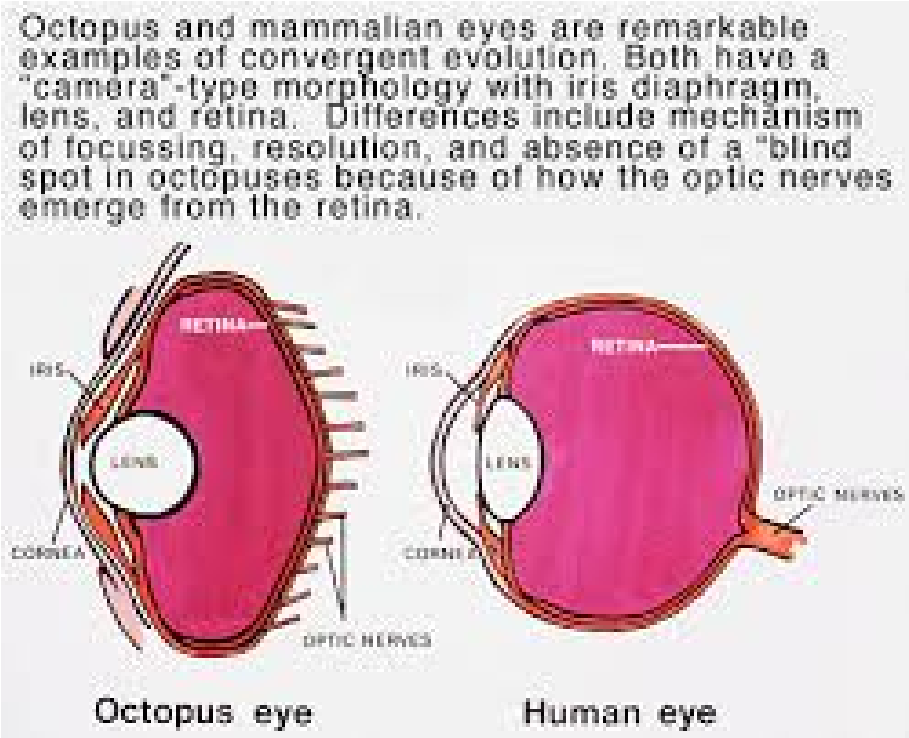

Figure 14.10: **The octopus eye, like the human eye, has an image-forming lens and a retina. This similarity is a striking example of convergent evolution. The common ancestor of humans and molluscs had no eye at all.**

14.6 Abstraction of concepts and natural laws

Can two contradictory statements both be true? The physicist Niels Bohr thought that this could happen, and he called such an occurrence "complementarity". I think that I understand what Niels Bohr meant: Whenever we make a statement about the real world we are making a model which is simpler than what it is supposed to represent. Therefore every statement must to some extent be false because it is an oversimplification. In fact, a model of the world is an abstraction, and it is possible to make two conflicting abstractions, starting with the same real object.

If you say, "The eye is like a camera", you are making an abstraction by concentrating on the way that the eye works and the way that a camera works. Both use a lens to form an image. If you say "The eye is like a small onion", you are again making an abstraction, but this time concentrating the size and texture of the eye. It is somewhat round, elastic and damp. If you drop it on a stone floor, it will bounce rather than breaking. Both these abstractions have a certain degree of truth, although they are contradictory.

Similarly, science and ethics are both abstractions, and both oversimplify the real world, which is much more complex than either of them. Which abstraction we should use depends on the problem that we wish to discuss. If we are talking about atomic spectra, then Schrödinger and Dirac should be our guides. But if the lecture is on how to achieve peace in the world, I would far rather hear it from Mahatma Gandhi than from either Schrödinger or Dirac.

In his autobiography, Charles Darwin says that "Science consists in arranging facts in such a way that general conclusions may be drawn from them". At the lowest level of abstraction, we have a very large number of individual observations. A number of these observations may be gathered together to form a low-level generalization. The low-level generalizations may in turn be coordinated into a somewhat more general law, and so on. Today one hears that physicists are aiming at a "theory of everything", which, if could ever be achieved, would coordinate all individual observations of every kind.

Suggestions for further reading

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Chapter 15

SOME MODERN DEVELOPMENTS

15.1 The World Health Organization

Gro Harlem Brundtland (born 1939)

Gro Harlem Brundtland is the daughter of Gudmund Harlem, a physician who was also Norway's Minister of Social Affairs. Like her father she became a physician and a high-ranking politician associated with Norway's Labor Party. From 1974 to 1979 she was Norway's Minister for Environmental Affairs. In 1981, she became the first female Prime Minister of Norway. Although her first term in this position only lasted a year, she was later Prime Minister for longer periods, from 1986 to 1989 and from 1990 to 1996.

In 1983, Gro Harlem Brundtland was invited by United Nations Secretary-General Javier Pérez du Cuéllar to set up and chair a World Commission on Environment and Development. This later became known as the Brundtland Commission. The Commission's report, entitled *Our Common Future* was published in 1987, and it provided a basis for the 1992 Earth Summit, which was held in Rio de Janeiro.

In order to obtain broad political support, Brundtland was forced to introduce the concept of "sustainable development", which is dangerously close to the self-contradictory concept of "sustainable growth". Nevertheless, the Brundtland report, *Our Common Future*, and the Rio Earth Summit, were enormously valuable in spreading awareness of the problems involved in achieving sustainability. An important achievement of the Rio Earth Summit was the *Climate Change Convention*, which later led to the *Kyoto Protocol*, and more recently to the *Paris Agreement*.

In 1998, Brundtland (whose third term as Prime Minister of Norway had come to an end) was elected Director-General of the World Health Organization. On of her notable achievements was a campaign to reduce cigarette smoking worldwide through education, persuasion, and increased taxation.

Gro Harlem Brundtland is currently engaged in many organizations working to find solutions to the serious problems facing us today. For example she works with The Elders,

Figure 15.1: **Gro Harlem Brundtland (born in 1939).**

and with the Council of Women World Leaders.

15.2 Gene splicing

In 1970, Hamilton Smith of Johns Hopkins University observed that when the bacterium *Haemophilus influenzae* is attacked by a bacteriophage (a virus parasitic on bacteria), it can defend itself by breaking down the DNA of the phage. Following up this observation, he introduced DNA from the bacterium *E. coli* into *H. influenzae*. Again the foreign DNA was broken down.

Further investigation revealed that *H. influenzae* produced an enzyme, later named *Hin* dII, which cut a DNA strand only when it recognized a specific sequence of bases: The DNA was cut only if one strand contained the sequence GTPyPuAC, where Py stands for C or T, while Pu stands for A or G. The other strand, of course, contained the complementary sequence, CAPuPyTG. The enzyme *Hin* dII cut both strands in the middle of the six-base sequence.

Smith had, in fact, discovered the first of a class of bacterial enzymes which came to be called "restriction enzymes" or "restriction nucleases". Almost a hundred other restriction enzymes were subsequently discovered; and each was found to cut DNA at a specific base sequence. Smith's colleague, Daniel Nathans, used the restriction enzymes *Hin* dII and *Hin* dIII to produce the first "restriction map" of the DNA in a virus.

In 1971 and 1972, Paul Berg, and his co-workers Peter Lobban, Dale Kaiser and David Jackson at Stanford University, developed methods for adding cohesive ends to DNA fragments. Berg and his group used the calf thymus enzyme, terminal transferase, to add short, single-stranded polynucleotide segments to DNA fragments. For example, if they added the single-stranded segment AAAA to one fragment, and TTTT to another, then the two ends joined spontaneously when the fragments were incubated together. In this way Paul Berg and his group made the first recombinant DNA molecules.

The restriction enzyme *Eco* RI, isolated from the bacterium *E. coli*, was found to recognize the pattern, GAATTC, in one strand of a DNA molecule, and the complementary pattern, CTTAAG, in the other strand. Instead of cutting both strands in the middle of the six-base sequence, *Eco* RI was observed to cut both strands between G and A. Thus, each side of the cut was left with a "sticky end" - a four-base single-stranded segment, attached to the remainder of the double-stranded DNA molecule.

In 1972, Janet Mertz and Ron Davis, working at Stanford University, demonstrated that DNA strands cut with *Eco* RI could be rejoined by means of another enzyme - a DNA ligase. More importantly, when DNA strands from two different sources were cut with *Eco* RI, the sticky end of one fragment could form a spontaneous temporary bond with the sticky end of the other fragment. The bond could be made permanent by the addition of DNA ligase, even when the fragments came from different sources. Thus, DNA fragments from different organisms could be joined together.

Bacteria belong to a class of organisms (prokaryotes) whose cells do not have a nucleus. Instead, the DNA of the bacterial chromosome is arranged in a large loop. In the early 1950's, Joshua Lederberg had discovered that bacteria can exchange genetic information. He found that a frequently-exchanged gene, the F-factor (which conferred fertility), was not linked to other bacterial genes; and he deduced that the DNA of the F-factor was not physically a part of the main bacterial chromosome. In 1952, Lederberg coined the word "plasmid" to denote any extrachromosomal genetic system.

In 1959, it was discovered in Japan that genes for resistance to antibiotics can be exchanged between bacteria; and the name "R-factors" was given to these genes. Like the F-factors, the R-factors did not seem to be part of the main loop of bacterial DNA.

Because of the medical implications of this discovery, much attention was focused on the R-factors. It was found that they were plasmids, small loops of DNA existing inside the bacterial cell, but not attached to the bacterial chromosome. Further study showed that, in general, between one percent and three percent of bacterial genetic information is carried by plasmids, which can be exchanged freely even between different species of bacteria.

In the words of the microbiologist, Richard Novick, "Appreciation of the role of plasmids has produced a rather dramatic shift in biologists' thinking about genetics. The traditional view was that the genetic makeup of a species was about the same from one cell to another, and was constant over long periods of time. Now a significant proportion of genetic traits are known to be variable (present in some individual cells or strains, absent in others), labile (subject to frequent loss or gain) and mobile - all because those traits are associated with plasmids or other atypical genetic systems."

In 1973, Herbert Boyer, Stanley Cohen and their co-workers at Stanford University and the University of California carried out experiments in which they inserted foreign DNA segments, cut with *Eco* RI, into plasmids (also cut with *Eco* RI). They then resealed the plasmid loops with DNA ligase. Finally, bacteria were infected with the gene-spliced plasmids. The result was a new strain of bacteria, capable of producing an additional protein coded by the foreign DNA segment which had been spliced into the plasmids.

Cohen and Boyer used plasmids containing a gene for resistance to an antibiotic, so that

a few gene-spliced bacteria could be selected from a large population by treating the culture with the antibiotic. The selected bacteria, containing both the antibiotic-resistance marker and the foreign DNA, could then be cloned on a large scale; and in this way a foreign gene could be "cloned". The gene-spliced bacteria were chimeras, containing genes from two different species.

The new recombinant DNA techniques of Berg, Cohen and Boyer had revolutionary implications: It became possible to produce many copies of a given DNA segment, so that its base sequence could be determined. With the help of direct DNA-sequencing methods developed by Frederick Sanger and Walter Gilbert, the new cloning techniques could be used for mapping and sequencing genes.

Since new bacterial strains could be created, containing genes from other species, it became possible to produce any protein by cloning the corresponding gene. Proteins of medical importance could be produced on a large scale. Thus, the way was open for the production of human insulin, interferon, serum albumin, clotting factors, vaccines, and protein hormones such as ACTH, human growth factor and leuteinizing hormone.

It also became possible to produce enzymes of industrial and agricultural importance by cloning gene-spliced bacteria. Since enzymes catalyze reactions involving smaller molecules, the production of these substrate molecules through gene-splicing also became possible.

It was soon discovered that the possibility of producing new, transgenic organisms was not limited to bacteria. Gene-splicing was also carried out on higher plants and animals as well as on fungi. It was found that the bacterium *Agrobacterium tumefaciens* contains a tumor-inducing (Ti) plasmid capable of entering plant cells and producing a crown gall. Genes spliced into the Ti plasmid frequently became incorporated in the plant chromosome, and afterwards were inherited in a stable, Mendelian fashion.

Transgenic animals were produced by introducing foreign DNA into embryo-derived stem cells (ES cells). The gene-spliced ES cells were then selected, cultured and introduced into a blastocyst, which afterwards was implanted in a foster-mother. The resulting chimeric animals were bred, and stable transgenic lines selected.

Thus, for the first time, humans had achieved direct control over the process of evolution. Selective breeding to produce new plant and animal varieties was not new - it was one of the oldest techniques of civilization. However, the degree and speed of intervention which recombinant DNA made possible was entirely new. In the 1970's it became possible to mix the genetic repetoires of different species: The genes of mice and men could be spliced together into new, man-made forms of life!

The Asilomar Conference

In the summer of 1971, Janet Mertz, who was then a student in Paul Berg's laboratory, gave a talk at Cold Spring Harbor. She discussed some proposed experiments applying recombinant techniques to the DNA of the tumor-inducing virus SV40.

This talk worried the cell biologist, Richard Pollack. He was working with SV40 and was already concerned about possible safety hazards in connection with the virus. Pollack

telephoned to Berg, and asked whether it might not be dangerous to clone a gene capable of producing human cancer. As a result of this call, Berg decided not to clone genes from tumor-inducing viruses.

Additional concern over the safety of recombinant DNA experiments was expressed at the 1973 Gordon Conference on Nucleic Acids. The scientists attending the conference voted to send a letter to the President of the U.S. National Academy of Sciences:

"...We presently have the technical ability", the letter stated, "to join together, covalently, DNA molecules from diverse sources... This technique could be used, for example, to combine DNA from animal viruses with bacterial DNA... In this way, new kinds of hybrid plasmids or viruses, with biological activity of unpredictable nature, may eventually be created. These experiments offer exciting and interesting potential, both for advancing knowledge of fundamental biological processes, and for alleviation of human health problems."

"Certain such hybrid molecules may prove hazardous to laboratory workers and to the public. Although no hazard has yet been established, prudence suggests that the potential hazard be seriously considered."

"A majority of those attending the Conference voted to communicate their concern in this matter to you, and to the President of the Institute of Medicine... The conferees suggested that the Academies establish a study committee to consider this problem, and to recommend specific actions and guidelines."

As a result of this letter, the National Academy of Sciences set up a Committee on Recombinant DNA, chaired by Paul Berg. The Committee's report, published in July, 1974, contained the following passage:

"...There is serious concern that some of these artificial recombinant DNA molecules could prove biologically hazardous. One potential hazard in current experiments derives from the need to use a bacterium like *E. coli* to clone the recombinant DNA molecules and to amplify their number. Strains of *E. coli* commonly reside in the human intestinal tract, and they are capable of exchanging genetic information with other types of bacteria, some of which are pathogenic to man. Thus, new DNA elements introduced into *E. coli* might possibly become widely disseminated among human, bacterial, plant, or animal populations, with unpredictable effects."

The Committee on Recombinant DNA recommended that scientists throughout the world should join in a voluntary postponement of two types of experiments: Type 1, introduction of antibiotic resistance factors into bacteria not presently carrying the R-factors; and Type 2, cloning of cancer-producing plasmids or viruses.

The Committee recommended caution in experiments linking DNA from animal cells to bacterial DNA, since animal-derived DNA can carry cancer-inducing base sequences. Finally, the Committee recommended that the National Institutes of Health establish a permanent advisory group to supervise experiments with recombinant DNA, and that an international meeting be held to discuss the biohazards of the new techniques.

In February, 1975, more than 100 leading molecular biologists from many parts of the world met at the Asilomar Conference Center near Monterey, California, to discuss safety guidelines for recombinant DNA research. There was an almost unanimous consensus at

the meeting that, until more was known about the dangers, experiments involving cloning of DNA should make use of organisms and vectors incapable of living outside a laboratory environment.

The Asilomar Conference also recommended that a number of experiments be deferred. These included cloning of recombinant DNA derived from highly pathogenic organisms, or containing toxin genes, as well as large-scale experiments using recombinant DNA able to make products potentially harmful to man, animals or plants.

The Asilomar recommendations were communicated to a special committee appointed by the U.S. National Institutes of Health; and the committee drew up a set of guidelines for recombinant DNA research. The NIH Guidelines went into effect in 1976; and they remained in force until 1979. They were stricter than the Asilomar recommendations regarding cloning of DNA from cancer-producing viruses; and this was effectively forbidden by the NIH until 1979. (Of course, the NIH Guidelines were effective only for research conducted within the United States and funded by the U.S. government.)

In 1976, the first commercial genetic engineering company (Genentech) was founded. In 1980, the initial public offering of Genentech stock set a Wall Street record for the fastest increase of price per share. In 1981, another genetic engineering company (Cetus) set a Wall Street record for the largest amount of money raised in an initial public offering (125 million U.S. dollars). During the same years, Japan's Ministry of International Trade and Technology declared 1981 to be "The Year of Biotechnology"; and England, France and Germany all targeted biotechnology as an area for special development.

A number of genetic-engineering products reached the market in the early 1980's. These included rennin, animal growth hormones, foot and mouth vaccines, hog diarrhea vaccine, amino acids, antibiotics, anabolic steroids, pesticides, pesticide-resistant plants, cloned livestock, improved yeasts, cellulose-digesting bacteria, and a nitrogen-fixation enzyme.

Recently the United States and Japan have initiated large-scale programs whose aim is to map the entire human genome; and the European Economic Community is considering a similar program. The human genome project is expected to make possible prenatal diagnosis of many inherited diseases. For example, the gene for cystic fibrosis has been found; and DNA technology makes it possible to detect the disease prenatally.

The possibility of extensive genetic screening raises ethical problems which require both knowledge and thought on the part of the public. An expectant mother, in an early stage of pregnancy, often has an abortion if the foetus is found to carry a serious genetic defect. But with more knowledge, many more defects will be found. Where should the line be drawn between a serious defect and a minor one?

The cloning of genes for lethal toxins also needs serious thought and public discussion. From 1976 to 1982, this activity was prohibited in the United States under the NIH Guidelines. However, in April, 1982, the restriction was lifted, and by 1983, the toxins being cloned included several aflatoxins, lecithinase, cytochalasins, ochratoxins, sporidesmin, T-2 toxin, ricin and tremogen. Although international conventions exist under which chemical and biological weapons are prohibited, there is a danger that nations will be driven to produce and stockpile such weapons because of fear of what other nations might do.

Finally, the release of new, transgenic species into the environment requires thought

and caution. Much benefit can come, for example, from the use of gene-spliced bacteria for nitrogen fixation or for cleaning up oil spills. However, once a gene-spliced microorganism has been released, it is virtually impossible to eradicate it; and thus the change produced by the release of a new organism is permanent. Permanent changes in the environment should not be made on the basis of short-term commercial considerations, nor indeed on the basis of short-term considerations of any kind; nor should such decisions be made unilaterally by single nations, since new organisms can easily cross political boundaries.

The rapid development of biotechnology has given humans enormous power over the fundamental mechanisms of life and evolution. But is society mature enough to use this power wisely and compassionately?

The Polymerase Chain Reaction

One day in the early 1980's, an American molecular biologist, Kary Mullis, was driving to his mountain cabin with his girl friend. The journey was a long one, and to pass the time, Kary Mullis turned over and over in his mind a problem which had been bothering him: He worked for a California biotechnology firm, and like many other molecular biologists he had been struggling to analyze very small quantities of DNA. Mullis realized that it would be desirable have a highly sensitive way of replicating a given DNA segment - a method much more sensitive than cloning. As he drove through the California mountains, he considered many ways of doing this, rejecting one method after the other as impracticable. Finally a solution came to him; and it seemed so simple that he could hardly believe that he was the first to think of it. He was so excited that he immediately pulled over to the side of the road and woke his sleeping girlfriend to tell her about his idea. Although his girlfriend was not entirely enthusiastic about being wakened from a comfortable sleep to be presented with a lecture on biochemistry, Kary Mullis had in fact invented a technique which was destined to revolutionize DNA technology: the Polymerase Chain Reaction (PCR)[1].

The technique was as follows: Begin with a small sample of the genomic DNA to be analyzed. (The sample may be extremely small - only a few molecules.) Heat the sample to 95 °C to separate the double-stranded DNA molecule into single strands. Suppose that on the long DNA molecule there is a target segment which one wishes to amplify. If the target segment begins with a known sequence of bases on one strand, and ends with a known sequence on the complementary strand, then synthetic "primer" oligonucleotides[2] with these known beginning ending sequences are added in excess. The temperature is then lowered to 50-60 °C, and at the lowered temperature, the "start" primer attaches itself to one DNA strand at the beginning of the target segment, while the "stop" primer becomes attached to the complementary strand at the other end of the target segment. Polymerase (an enzyme which aids the formation of double-stranded DNA) is then added, together with a supply of nucleotides. On each of the original pieces of single-stranded DNA, a new complementary strand is generated with the help of the polymerase. Then

[1] The flash of insight didn't take long, but at least six months of hard work were needed before Mullis and his colleagues could convert the idea to reality.

[2] Short segments of single-stranded DNA.

the temperature is again raised to 95 °C, so that the double-stranded DNA separates into single strands, and the cycle is repeated.

In the early versions of the PCR technique, the polymerase was destroyed by the high temperature, and new polymerase had to be added for each cycle. However, it was discovered that polymerase from the bacterium Thermus aquaticus would withstand the high temperature. (Thermus aquaticus lives in hot springs.) This discovery greatly simplified the PCR technique. The temperature could merely be cycled between the high and low temperatures, and with each cycle, the population of the target segment doubled, concentrations of primers, deoxynucleotides and polymerase being continuously present.

After a few cycles of the PCR reaction, copies of copies begin to predominate over copies of the original genomic DNA. These copies of copies have a standard length, always beginning on one strand with the start primer, and ending on that strand with the complement of the stop primer.

15.3 Bioinformation technology and artificial life

The merging of information technology and biotechnology

Information technology and biology are today the two most rapidly developing fields of science. Interestingly, these two fields seem to be merging, each gaining inspiration and help from the other. For example, computer scientists designing both hardware and software are gaining inspiration from physiological studies of the mechanism of the brain; and conversely, neurophysiologists are aided by insights from the field of artificial intelligence. Designers of integrated circuits wish to prolong the period of validity of Moore's law; but they are rapidly approaching physical barriers which will set limits to the miniaturization of conventional transistors and integrated circuits. They gain inspiration from biology, where the language of molecular complementarity and the principle of autoassembly seem to offer hope that molecular switches and self-assembled integrated circuits may one day be constructed.

Geneticists, molecular biologists, biochemists and crystallographers have now obtained so much information about the amino acid sequences and structures of proteins and about the nucleotide sequences in genomes that the full power of modern information technology is needed to store and to analyze this information. Computer scientists, for their part, turn to evolutionary genetics for new and radical methods of developing both software and hardware - genetic algorithms and simulated evolution.

Self-assembly of supramolecular structures; Nanoscience

In previous chapters, we saw that the language of molecular complementarity (the "lock and key" fitting discovered by Paul Ehrlich) is the chief mechanism by which information is stored and transferred in biological systems. Biological molecules have physical shapes

and patterns of excess charge[3] which are recognized by complementary molecules because they fit together, just as a key fits the shape of a lock. Examples of biological "lock and key" fitting are the fit between the substrate of an enzyme and the enzyme's active site, the recognition of an antigen by its specific antibody, the specificity of base pairs in DNA and RNA, and the autoassembly of structures such as viruses and subcellular organelles.

One of the best studied examples of autoassembly through the mechanism of molecular complementarity is the tobacco mosaic virus. The assembled virus has a cylindrical form about 300 nm long (1 nm = 1 nanometer = 10^{-9} meters = 10 Ångstroms), with a width of 18 nm. The cylindrically shaped virus is formed from about 2000 identical protein molecules. These form a package around an RNA molecule with a length of approximately 6400 nucleotides. The tobacco mosaic virus can be decomposed into its constituent molecules in vitro, and the protein and RNA can be separated and put into separate bottles, as was discussed in Chapter 4.

If, at a later time, one mixes the protein and RNA molecules together in solution, they spontaneously assemble themselves into new infective tobacco mosaic virus particles. The mechanism for this spontaneous autoassembly is a random motion of the molecules through the solvent until they approach each other in such a way that a fit is formed. When two molecules fit closely together, with their physical contours matching, and with complementary patterns of excess charge also matching, the Gibbs free energy of the total system is minimized. Thus the self-assembly of matching components proceeds spontaneously, just as every other chemical reaction proceeds spontaneously when the difference in Gibbs free energy between the products and reactants is negative. The process of autoassembly is analogous to crystallization, except that the structure formed is more complex than an ordinary crystal.

A second very well-studied example of biological autoassembly is the spontaneous formation of bilayer membranes when phospholipid molecules are shaken together in water. Each phospholipid molecule has a small polar (hydrophilic) head, and a long nonpolar (hydrophobic) tail. The polar head is hydrophilic - water-loving - because it has large excess charges with which water can form hydrogen bonds. By contrast, the non-polar tail of a phospholipid molecule has no appreciable excess charges. The tail is hydrophobic - it hates water - because to fit into the water structure it has to break many hydrogen bonds to make a hole for itself, but it cannot pay for these broken bonds by forming new hydrogen bonds with water.

There is a special configuration of the system of water and phospholipid molecules which has a very low Gibbs free energy - the lipid bilayer. In this configuration, all the hydrophilic polar heads are in contact with water, while the hydrophobic nonpolar tails are in the interior of the double membrane, away from the water, and in close contact with each other, thus maximizing their mutual Van der Waals attractions. (The basic structure of biological membranes is the lipid bilayer just described, but there are also other components, such as membrane-bound proteins, caveolae, and ion pores.)

[3] They also have patterns of polarizable groups and reactive groups, and these patterns can also play a role in recognition.

The mechanism of self-organization of supramolecular structures is one of the most important universal mechanisms of biology. Chemical reactions take place spontaneously when the change in Gibbs free energy produced by the reaction is negative, i.e., chemical reactions take place in such a direction that the entropy of the universe increases. When spontaneous chemical reactions take place, the universe moves from a less probable configuration to a more probable one. The same principle controls the motion of larger systems, where molecules arrange themselves spontaneously to form supramolecular structures. Self-assembling collections of molecules move in such a way as to minimize their Gibbs free energy, thus maximizing the entropy of the universe.

Biological structures of all kinds are formed spontaneously from their components because assembly information is written onto their joining surfaces in the form of complementary surface contours and complementary patterns of excess charge[4]. Matching pieces fit together, and the Gibbs free energy of the system is minimized. Virtually every structure observed in biology is formed in this way - by a process analogous to crystallization, except that biological structures can be far more complex than ordinary crystals.

Researchers in microelectronics, inspired by the self-assembly of biological structures, dream of using the same principles to generate self-organizing integrated circuits with features so small as to approach molecular dimensions. As we mentioned in Chapter 7, the speed of a computing operation is limited by the time that it takes an electrical signal (moving at approximately the speed of light) to traverse a processing unit. The desire to produce ever greater computation speeds as well as ever greater memory densities, motivates the computer industry's drive towards ultraminiaturization.

Currently the fineness of detail in integrated circuits is limited by diffraction effects caused by the finite wavelength of the light used to project an image of the circuit onto a layer of photoresist covering the chip where the circuit is being built up. For this reason, there is now very active research on photolithography using light sources with extremely short wavelengths, in the deep ultraviolet, or even X-ray sources, synchrotron radiation, or electron beams. The aim of this research is to produce integrated circuits whose feature size is in the nanometer range - smaller than 100 nm. In addition to these efforts to create nanocircuits by "top down" methods, intensive research is also being conducted on "bottom up" synthesis, using principles inspired by biological self-assembly. The hope to make use of "the spontaneous association of molecules, under equilibrium conditions, into stable, structurally well-defined aggregates, joined by non-covalent bonds"[5]

The Nobel Laureate Belgian chemist J.-M. Lehn pioneered the field of supramolecular chemistry by showing that it is possible to build nanoscale structures of his own design. Lehn and his coworkers at the University of Strasbourg used positively-charged metal ions as a kind of glue to join larger structural units at points where the large units exhibited excess negative charges. Lehn predicts that the supramolecular chemistry of the future will follow the same principles of self-organization which underlie the growth of biological structures, but with a greatly expanded repertory, making use of elements (such as silicon)

[4] Patterns of reactive or polarizable groups also play a role.

[5] G.M. Whiteside et al., Science, **254**, 1312-1314, (1991).

that are not common in carbon-based biological systems.

Other workers in nanotechnology have concentrated on the self-assembly of two-dimensional structures at water-air interfaces. For example, Thomas Bjørnholm, working at the University of Copenhagen, has shown that a nanoscale wire can be assembled spontaneously at a water-air interface, using metal atoms complexed with DNA and a DNA template. The use of a two-dimensional template to reproduce a nanostructure can be thought of as "microprinting". One can also think of self-assembly at surfaces as the two-dimensional version of the one-dimensional copying process by which a new DNA or RNA strand assembles itself spontaneously, guided by the complementary strand.

In 1981, Gerd Binning and Heinrich Rohrer of IBM's Research Center in Switzerland announced their invention of the scanning tunneling microscope. The new microscope's resolution was so great that single atoms could be observed. The scanning tunneling microscope consists of a supersharp conducting tip, which is brought near enough to a surface so that quantum mechanical tunneling of electrons can take place between tip and surface when a small voltage is applied. The distance between the supersharp tip and the surface is controlled by means of a piezoelectric crystal. As the tip is moved along the surface, its distance from the surface (and hence the tunneling current) is kept constant by applying a voltage to the piezoelectric crystal, and this voltage as a function of position gives an image of the surface.

Variations on the scanning tunneling microscope allow single atoms to be deposited or manipulated on a surface. Thus there is a hope that nanoscale circuit templates can be constructed by direct manipulation of atoms and molecules, and that the circuits can afterwards be reproduced using autoassembly mechanisms.

The scanning tunneling microscope makes use of a quantum mechanical effect: Electrons exhibit wavelike properties, and can tunnel small distances into regions of negative kinetic energy - regions which would be forbidden to them by classical mechanics. In general it is true that for circuit elements with feature sizes in the nanometer range, quantum effects become important. For conventional integrated circuits, the quantum effects which are associated with this size-range would be a nuisance, but workers in nanotechnology hope to design integrated circuits which specifically make use of these quantum effects.

Molecular switches; bacteriorhodopsin

The purple, salt-loving archaebacterium Halobacterium halobium (recently renamed Halobacterium salinarum) possesses one of the simplest structures that is able to perform photosynthesis. The purple membrane subtraction of this bacterium's cytoplasmic membrane contains only two kinds of molecules - lipids and bacteriorhodopsin. Nevertheless, this simple structure is able to trap the energy of a photon from the sun and to convert it into chemical energy.

The remarkable purple membrane of Halobacterium has been studied in detail by Walter Stoeckenius, D. Osterhelt[6], Lajos Keszthelyi and others.

[6] D. Osterhelt and Walter Stoeckenius, Nature New Biol. **233**, 149-152 (1971); D. Osterhelt et al.,

It can be decomposed into its constituent molecules. The lipids from the membrane and the bacteriorhodopsin can be separated from each other and put into different bottles. At a later time, the two bottles can be taken from the laboratory shelf, and their contents can be shaken together in water. The result is the spontaneous formation of tiny vesicles of purple membrane.

In the self-organized two-component vesicles, the membrane-bound protein bacteriorhodopsin is always correctly oriented, just as it would be in the purple membrane of a living Halobacterium. When the vesicles are illuminated, bacteriorhodopsin absorbs H^+ ions from the water on the inside, and releases them outside.

Bacteriorhodopsin consists of a chain of 224 amino acids, linked to the retinal chromophore. The amino acids are arranged in 7 helical segments, each of which spans the purple membrane, and these are joined on the membrane surface by short nonhelical segments of the chain. The chromophore is in the middle of the membrane, surrounded by a-helical segments. When the chromophore is illuminated, its color is temporarily bleached, and it undergoes a cis-trans isomerization which disrupts the hydrogen-bonding network of the protein. The result is that a proton is released on the outside of the membrane. Later, a proton is absorbed from the water in the interior of the membrane vesicle, the hydrogen-bonding system of the protein is reestablished, and both the protein and the chromophore return to their original conformations. In this way, bacteriorhodopsin functions as a proton pump. It uses the energy of photons to transport H^+ ions across the membrane, from the inside to the outside, against the electrochemical gradient. In the living Halobacterium, this H^+ concentration difference would be used to drive the synthesis of the high-energy phosphate bond of adenosine triphosphate (ATP), the inward passage of H^+ through other parts of the cytoplasmic membrane being coupled to the reaction $ADP + P_i \rightarrow ATP$ by membrane-bound reversible ATPase.

Bacteriorhodopsin is interesting as a component of one of the simplest known photosynthetic systems, and because of its possible relationship to the evolution of the eye (as was discussed in Chapter 3). In addition, researchers like Lajos Keszthelyi at the Institute of Biophysics of the Hungarian Academy of Sciences in Szeged are excited about the possible use of bacteriorhodopsin in optical computer memories[7]. Arrays of oriented and partially dehydrated bacteriorhodopsin molecules in a plastic matrix can be used to construct both 2-dimensional and 3-dimensional optical memories using the reversible color changes of the molecule. J. Chen and coworkers[8] have recently constructed a prototype 3-dimensional optical memory by orienting the proteins and afterwards polymerizing the solvent into a solid polyacrylamide matrix. Bacteriorhodopsin has extraordinary stability, and can tolerate as many as a million optical switching operations without damage.

Quart. Rev. Biophys. **24**, 425-478 (1991); W. Stoeckenius and R. Bogomolni, Ann. Rev. Biochem. **52**, 587-616 (1982).

[7] A. Der and L. Keszthelyi, editors, Bioelectronic Applications of Photochromic Pigments, IOS Press, Amsterdam, Netherlands, (2001).

[8] J. Chen et al., Biosystems **35**, 145-151 (1995).

Neural networks, biological and artificial

In 1943, W. McCulloch and W. Pitts published a paper entitled *A Logical Calculus of the Ideas Immanent in Nervous Activity*. In this pioneering paper, they proposed the idea of a Threshold Logic Unit (TLU), which they visualized not only as a model of the way in which neurons function in the brain but also as a possible subunit for artificial systems which might be constructed to perform learning and pattern-recognition tasks. Problems involving learning, generalization, pattern recognition and noisy data are easily handled by the brains of humans and animals, but computers of the conventional von Neumann type find such tasks especially difficult.

Conventional computers consist of a memory and one or more central processing units (CPUs). Data and instructions are repeatedly transferred from the memory to the CPUs, where the data is processed and returned to the memory. The repeated performance of many such cycles requires a long and detailed program, as well as high-quality data. Thus conventional computers, despite their great speed and power, lack the robustness, intuition, learning powers and powers of generalization which characterize biological neural networks. In the 1950's, following the suggestions of McCulloch and Pitts, and inspired by the growing knowledge of brain structure and function which was being gathered by histologists and neurophysiologists, computer scientists began to construct artificial neural networks - massively parallel arrays of TLU's.

The analogy between a TLU and a neuron can be seen by comparing Figure 5.2, which shows a neuron, with Figure 8.1, which shows a TLU. As we saw in Chapter 5, a neuron is a specialized cell consisting of a cell body (*soma*) from which an extremely long, tubelike fiber called an *axon* grows. The axon is analogous to the output channel of a TLU. From the soma, a number of slightly shorter, rootlike extensions called *dendrites* also grow. The dendrites are analogous to the input channels of a TLU.

In a biological neural network, branches from the axon of a neuron are connected to the dendrites of many other neurons; and at the points of connection there are small, knoblike structures called synapses. As was discussed in Chapter 5, the "firing" of a neuron sends a wave of depolarization out along its axon. When the pulselike electrical and chemical disturbance associated with the wave of depolarization (the action potential) reaches a synapse, where the axon is connected with another neuron, transmitter molecules are released into the post-synaptic cleft. The neurotransmitter molecules travel across the post-synaptic cleft to receptors on a dendrite of the next neuron in the net, where they are bound to receptors. There are many kinds of neurotransmitter molecules, some of which tend to make the firing of the next neuron more probable, and others which tend to inhibit its firing. When the neurotransmitter molecules are bound to the receptors, they cause a change in the dendritic membrane potential, either increasing or decreasing its polarization. The post-synaptic potentials from the dendrites are propagated to the soma; and if their sum exceeds a threshold value, the neuron fires. The subtlety of biological neural networks derives from the fact that there are many kinds of neurotransmitters and synapses, and from the fact that synapses are modified by their past history.

Turning to Figure 8.1, we can compare the biological neuron with the Threshold Logic

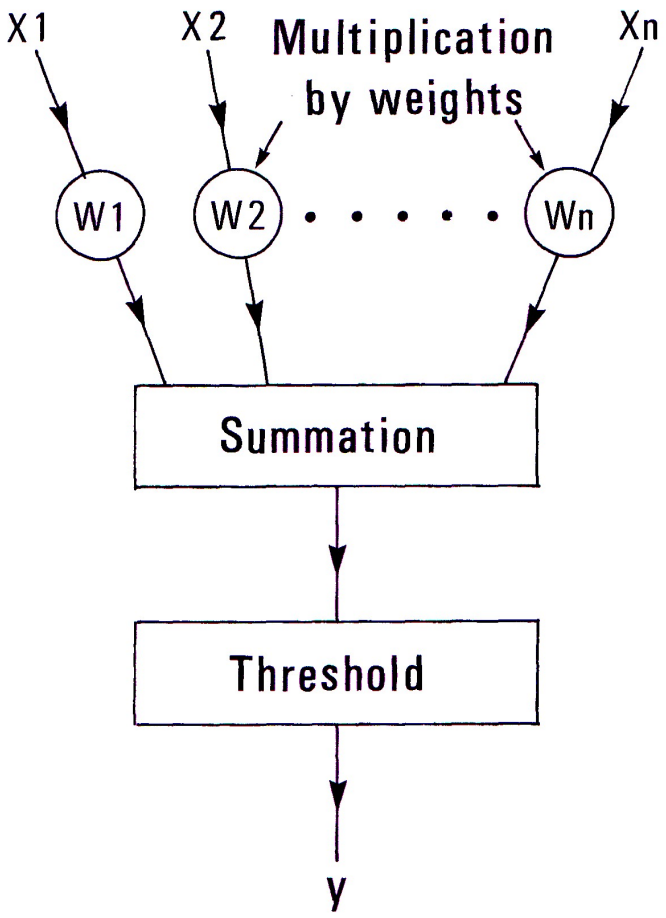

Figure 15.2: A Threshold Logic Unit (TLU) of the type proposed by McCulloch and Pitts.

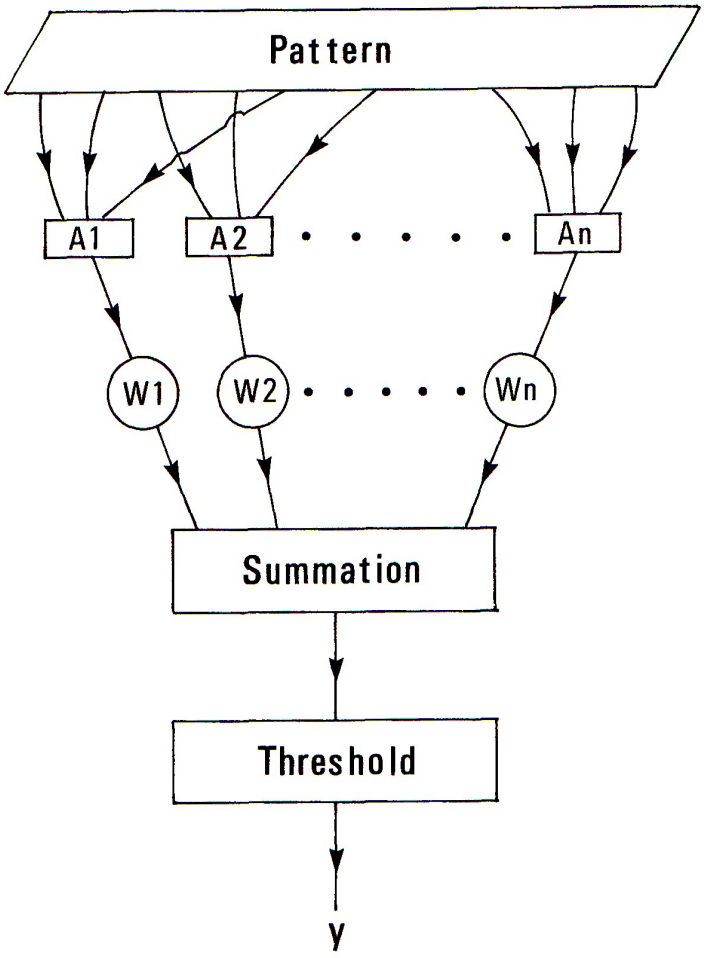

Figure 15.3: A perceptron, introduced by Rosenblatt in 1962. The perceptron is similar to a TLU, but its input is preprocessed by a set of association units (A-units). The A-units are not trained, but are assigned a fixed Boolean functionality.

Unit of McCulloch and Pitts. Like the neuron, the TLU has many input channels. To each of the N channels there is assigned a weight, $w_1, w_2, ..., w_N$. The weights can be changed; and the set of weights gives the TLU its memory and learning capabilities. Modification of weights in the TLU is analogous to the modification of synapses in a neuron, depending on their history. In the most simple type of TLU, the input signals are either 0 or 1. These signals, multiplied by their appropriate weights, are summed, and if the sum exceeds a threshold value, θ the TLU "fires", i.e. a pulse of voltage is transmitted through the output channel to the next TLU in the artificial neural network.

Let us imagine that the input signals, $x_1, x_2, ..., x_N$ can take on the values 0 or 1. The weighted sum of the input signals will then be given by

$$a = \sum_{j=1}^{N} w_j x_j \tag{15.1}$$

The quantity a, is called the *activation*. If the activation exceeds the threshold 9, the unit "fires", i.e. it produces an output y given by

$$y = \begin{cases} 1 & \text{if} \quad a \geq \theta \\ \\ 0 & \text{if} \quad a < \theta \end{cases} \tag{15.2}$$

The decisions taken by a TLU can be given a geometrical interpretation: The input signals can be thought of as forming the components of a vector, $x = x_1, x_2, ..., X_N$, in an N-dimensional space called pattern space. The weights also form a vector, $w = w_1, w_2, ..., w_N$, in the same space. If we write an equation setting the scalar product of these two vectors equal to some constant,

$$\mathbf{w} \cdot \mathbf{x} \equiv \sum_{j=1}^{N} w_j x_j = \theta \tag{15.3}$$

then this equation defines a hyperplane in pattern space, called the *decision hyperplane*. The decision hyperplane divides pattern space into two parts - (1) input pulse patterns which will produce firing of the TLU, and (2) patterns which will not cause firing.

The position and orientation of the decision hyperplane can be changed by altering the weight vector w and/or the threshold θ. Therefore it is convenient to put the threshold and the weights on the same footing by introducing an augmented weight vector,

$$\mathbf{W} = w_1, w_2, ..., w_N, \theta \tag{15.4}$$

and an augmented input pattern vector,

$$\mathbf{X} = x_1, x_2, ..., x_N, -1 \tag{15.5}$$

In the N+l-dimensional augmented pattern space, the decision hyperplane now passes through the origin, and equation (8.3) can be rewritten in the form

$$\mathbf{W} \cdot \mathbf{X} \equiv \sum_{j=1}^{N+1} W_j X_j = 0 \tag{15.6}$$

Those input patterns for which the scalar product $\mathbf{W} \cdot \mathbf{X}$ is positive or zero will cause the unit to fire, but if the scalar product is negative, there will be no response.

If we wish to "teach" a TLU to fire when presented with a particular pattern vector $\mathbf{X}$, we can evaluate its scalar product with the current augmented weight vector $\mathbf{W}$. If this scalar product is negative, the TLU will not fire, and therefore we know that the weight vector needs to be changed. If we replace the weight vector by

$$\mathbf{W}' = \mathbf{W} + \gamma\mathbf{X} \tag{15.7}$$

where γ is a small positive number, then the new augmented weight vector $\mathbf{W}'$ will point in a direction more nearly the same as the direction of $\mathbf{X}$. This change will be a small step in the direction of making the scalar product positive, i.e. a small step in the right direction.

Why not take a large step instead of a small one? A small step is best because there may be a whole class of input patterns to which we would like the TLU to respond by firing. If we make a large change in weights to help a particular input pattern, it may undo previous learning with respect to other patterns.

It is also possible to teach a TLU to remain silent when presented with a particular input pattern vector. To do so we evaluate the augmented scalar product $\mathbf{W} \cdot \mathbf{X}$ as before, but now, when we desire silence rather than firing, we wish the scalar product to be negative, and if it is positive, we know that the weight vector must be changed. In changing the weight vector, we can again make use of equation (8.7), but now γ must be a small negative number rather than a small positive one.

Two sets of input patterns, A and B, are said to be linearly separable if they can be separated by some decision hyperplane in pattern space. Now suppose that the four sets, A, B, C, and D, can be separated by two decision hyperplanes. We can then construct a two-layer network which will identify the class of an input signal belonging to any one of the sets, as is illustrated in Figure 8.2.

The first layer consists of two TLU's. The first TLU in this layer is taught to fire if the input pattern belongs to A or B, and to be silent if the input belongs to C or D. The second TLU is taught to fire if the input pattern belongs to A or D, and to be silent if it belongs to B or C. The second layer of the network consists of four output units which are not taught, but which are assigned a fixed Boolean functionality. The first output unit fires if the signals from the first layer are given by the vector $\mathbf{y} = \{0, 0\}$ (class A); the second fires if $\mathbf{y} = \{0, 1\}$ (class B), the third if $\mathbf{y} = \{1, 0\}$ (class C), and the fourth if $\mathbf{y} = \{1, 1\}$ (class D). Thus the simple two-layer network shown in Figure 8.2 functions as a *classifier*. The output units in the second layer are analogous to the "grandmother's face cells" whose existence in the visual cortex is postulated by neurophysiologists. These cells will fire if and only if the retina is stimulated with a particular class of patterns.

This very brief glance at artificial neural networks does not do justice to the high degree of sophistication which network architecture and training algorithms have achieved during the last two decades. However, the suggestions for further reading at the end of this chapter may help to give the reader an impression of the wide range of problems to which these networks are now being applied.

Besides being useful for computations requiring pattern recognition, learning, generalization, intuition, and robustness in the face of noisy data, artificial neural networks are important because of the light which they throw on the mechanism of brain function. For example, one can compare the classifier network shown in Figure 8.2 with the discoveries of Kuffler, Hubel and Wessel concerning pattern abstraction in the mammalian retina and visual cortex (Chapter 5).

Genetic algorithms

Genetic algorithms represent a second approach to machine learning and to computational problems involving optimization. Like neural network computation, this alternative approach has been inspired by biology, and it has also been inspired by the Darwinian concept of natural selection. In a genetic algorithm, the hardware is that of a conventional computer; but the software creates a population and allows it to evolve in a manner closely analogous to biological evolution.

One of the most important pioneers of genetic algorithms was John Henry Holland (1929-). After attending MIT, where he was influenced by Norbert Wiener, Holland worked for IBM, helping to develop the 701. He then continued his studies at the University of Michigan, obtaining the first Ph.D. in computer science ever granted in America. Between 1962 and 1965, Holland taught a graduate course at Michigan called "Theory of Adaptive Systems". His pioneering course became almost a cult, and together with his enthusiastic students he applied the genetic algorithm approach to a great variety of computational problems. One of Holland's students, David Goldberg, even applied a genetic algorithm program to the problem of allocating natural gas resources.

The programs developed by Holland and his students were modelled after the natural biological processes of reproduction, mutation, selection and evolution. In biology, the information passed between generations is contained in chromosomes - long strands of DNA where the genetic message is written in a four-letter language, the letters being adenine, thymine, guanine and cytosine. Analogously, in a genetic algorithm, the information is coded in a long string, but instead of a four-letter language, the code is binary: The chromosome-analogue is a long string of 0's and 1's, i.e., a long binary string. One starts with a population that has sufficient diversity so that natural selection can act.

The genotypes are then translated into phenotypes. In other words, the information contained in the long binary string (analogous to the genotype of each individual) corresponds to an entity, the phenotype, whose fitness for survival can be evaluated. The mapping from genotype to phenotype must be such that very small changes in the binary string will not produce radically different phenotypes. Prom the initial population, the most promising individuals are selected to be the parents of the next generation, and of these, the fittest are allowed produce the largest number of offspring. Before reproduction takes place, however, random mutations and chromosome crossing can occur. For example, in chromosome crossing, the chromosomes of two individuals are broken after the nth binary digit, and two new chromosomes are formed, one with the head of the first old chromosome and the tail of the second, and another with the head of the second and the tail of

the first. This process is analogous to the biological crossings which allowed Thomas Hunt Morgan and his "fly squad" to map the positions of genes on the chromosomes of fruit flies, while the mutations are analogous to those studied by Hugo de Vries and Hermann J. Muller.

After the new generation has been produced, the genetic algorithm advances the time parameter by a step, and the whole process is repeated: The phenotypes of the new generation are evaluated and the fittest selected to be parents of the next generation; mutation and crossings occur; and then fitness-proportional reproduction. Like neural networks, genetic algorithms are the subject of intensive research, and evolutionary computation is a rapidly growing field.

Evolutionary methods have been applied not only to software, but also to hardware. Some of the circuits designed in this way defy analysis using conventional techniques - and yet they work astonishingly well.

Artificial life

As Aristotle pointed out, it is difficult to define the precise border between life and nonlife. It is equally difficult to give a precise definition of artificial life. Of course the term means "life produced by humans rather than by nature", but what is life? Is self-replication the only criterion? The phrase "produced by humans" also presents difficulties. Humans have played a role in creating domestic species of animals and plants. Can cows, dogs, and high-yield wheat varieties be called "artificial life" ? In one sense, they can. These species and varieties certainly would not have existed without human intervention.

We come nearer to what most people might call "artificial life" when we take parts of existing organisms and recombine them in novel ways, using the techniques of biotechnology. For example, Steen Willadsen[9], working at the Animal Research Station, Cambridge England, was able to construct chimeras by operating under a microscope on embryos at the eight-cell stage. The zona pelucida is a transparent shell that surrounds the cells of the embryo. Willadsen was able to cut open the zona pelucida, to remove the cells inside, and to insert a cell from a sheep embryo together with one from a goat embryo. The chimeras which he made in this way were able to grow to be adults, and when examined, their cells proved to be a mosaic, some cells carrying the sheep genome while others carried the genome of a goat. By the way, Willadsen did not create his chimeras in order to produce better animals for agriculture. He was interested in the scientifically exciting problem of morphogenesis: How is the information of the genome translated into the morphology of the growing embryo?

Human genes are now routinely introduced into embryos of farm animals, such as pigs or sheep. The genes are introduced into regulatory sequences which cause expression in mammary tissues, and the adult animals produce milk containing human proteins. Many medically valuable proteins are made in this way. Examples include human blood-clotting

[9] Willadsen is famous for having made the first verified and reproducible clone of a mammal. In 1984 he made two genetically identical lambs from early sheep embryo cells.

factors, interleukin-2 (a protein which stimulates T-lymphocytes), collagen and fibrinogen (used to treat burns), human fertility hormones, human hemoglobin, and human serum albumin.

Transgenic plants and animals in which the genes of two or more species are inherited in a stable Mendelian way have become commonplace in modern laboratory environments, and, for better or for worse, they are also becoming increasingly common in the external global environment. These new species might, with some justification, be called "artificial life".

In discussing the origin of life in Chapter 3, we mentioned that a long period of molecular evolution probably preceded the evolution of cells. In the early 1970's, S. Spiegelman performed a series of experiments in which he demonstrated that artificial molecular evolution can be made to take place in vitro. Spiegelman prepared a large number of test tubes in which RNA replication could take place. The aqueous solution in each of the test tubes consisted of RNA replicase, ATP, UTP (uracil triphosphate), GTP (guanine triphosphate), CTP (cytosine triphosphate) and buffer. He then introduced RNA from a bacteriophage into the first test tube. After a predetermined interval of time, during which replication took place, Spiegelman transferred a drop of solution from the first test tube to a new tube, uncontaminated with RNA. Once again, replication began and after an interval a drop was transferred to a third test tube. Spiegelman repeated this procedure several hundred times, and at the end he was able to demonstrate that the RNA in the final tube differed from the initial sample, and that it replicated faster than the initial sample. The RNA had evolved by the classical Darwinian mechanisms of mutation and natural selection. Mistakes in copying had produced mutant RNA strands which competed for the supply of energy-rich precursor molecules (ATP, UTP, GTP and CTP). The most rapidly-reproducing mutants survived. Was Spiegelman's experiment merely a simulation of an early stage of biological evolution? Or was evolution of an extremely primitive life-form actually taking place in his test tubes?

G.F. Joyce, D.P. Bartel and others have performed experiments in which strands of RNA with specific catalytic activity (ribozymes) have been made to evolve artificially from randomly coded starting populations of RNA. In these experiments, starting populations of 1013 to 1015 randomly coded RNA molecules are tested for the desired catalytic activity, and the most successful molecules are then chosen as parents for the next generation. The selected molecules are replicated many times, but errors (mutations) sometimes occur in the replication. The new population is once again tested for catalytic activity, and the process is repeated. The fact that artificial evolution of ribozymes is possible can perhaps be interpreted as supporting the "RNA world" hypothesis, i.e. the hypothesis that RNA preceded DNA and proteins in the early history of terrestrial life.

In Chapter 4 we mentioned that John von Neumann speculated on the possibility of constructing artificial self-reproducing automata. In the early 1940's, a period when there was much discussion of the Universal Turing Machine, he became interested in constructing a mathematical model of the requirements for self-reproduction. Besides the Turing machine, another source of his inspiration was the paper by Warren McCulloch and Walter Pitts entitled *A logical calculus of the ideas immanent in nervous activity*, which von Neu-

mann read in 1943. In his first attempt (the kinematic model), he imagined an extremely large and complex automaton, floating on a lake which contained its component parts.

Von Neumann's imaginary self-reproducing automaton consisted of four units, A, B, C and D. Unit A was a sort of factory, which gathered component parts from the surrounding lake and assembled them according to instructions which it received from other units. Unit B was a copying unit, which reproduced sets of instructions. Unit C was a control apparatus, similar to a computer. Finally D was a long string of instructions, analogous to the "tape" in the Turing machine described in Chapter 7. In von Neumann's kinematic automaton, the instructions were coded as a long binary number. The presence of what he called a "girder" at a given position corresponded to 1, while its absence corresponded to 0. In von Neumann's model, the automaton completed the assembly of its offspring by injecting its progeny with the duplicated instruction tape, thus making the new automaton both functional and fertile.

In presenting his kinematic model at the Hixton Symposium (organized by Linus Pauling in the late 1940's), von Neumann remarked that "...it is clear that the instruction [tape] is roughly effecting the function of a gene. It is also clear that the copying mechanism B performs the fundamental act of reproduction, the duplication of the genetic material, which is clearly the fundamental operation in the multiplication of living cells. It is also easy to see how arbitrary alterations of the system...can exhibit certain traits which appear in connection with mutation, lethality as a rule, but with a possibility of continuing reproduction with a modification of traits."

It is very much to von Neumann's credit that his kinematic model (which he invented several years before Crick and Watson published their DNA structure) was organized in much the same way that we now know the reproductive apparatus of a cell to be organized. Nevertheless he was dissatisfied with the model because his automaton contained too many "black boxes". There were too many parts which were supposed to have certain functions, but for which it seemed very difficult to propose detailed mechanisms by which the functions could be carried out. His kinematic model seemed very far from anything which could actually be built[10].

Von Neumann discussed these problems with his close friend, the Polish-American mathematician Stanislaw Ulam, who had for a long time been interested in the concept of self-replicating automata. When presented with the black box difficulty, Ulam suggested that the whole picture of an automaton floating on a lake containing its parts should be discarded. He proposed instead a model which later came to be known as the Cellular Automaton Model. In Ulam's model, the self-reproducing automaton lives in a very special space. For example, the space might resemble an infinite checkerboard, each square would constitute a multi-state cell. The state of each cell in a particular time interval is governed

[10] Von Neumann's kinematic automaton was taken seriously by the Mission IV Group, part of a ten-week program sponsored by NASA in 1980 to study the possible use of advanced automation and robotic devices in space exploration. The group, headed by Richard Laing, proposed plans for self-reproducing factories, designed to function on the surface of the moon or the surfaces of other planets. Like von Neumann's kinetic automaton, to which they owed much, these plans seemed very far from anything that could actually be constructed.

by the states of its near neighbors in the preceding time interval according to relatively simple laws. The automaton would then consist of a special configuration of cell states, and its reproduction would correspond to production of a similar configuration of cell states in a neighboring region of the cell lattice.

Von Neumann liked Ulam's idea, and he began to work in that direction. However, he wished his self-replicating automaton to be able to function as a universal Turing machine, and therefore the plans which he produced were excessively complicated. In fact, von Neumann believed complexity to be a necessary requirement for self-reproduction. In his model, the cells in the lattice were able to have 29 different states, and the automaton consisted of a configuration involving hundreds of thousands of cells. Von Neumann's manuscript on the subject became longer and longer, and he did not complete it before his early death from prostate cancer in 1957. The name "cellular automaton" was coined by Arthur Burks, who edited von Neumann's posthumous papers on the theory of automata.

Arthur Burks had written a Ph.D. thesis in philosophy on the work of the nineteenth century thinker Charles Sanders Pierce, who is today considered to be one of the founders of semiotics[11]. He then studied electrical engineering at the Moore School in Philadelphia, where he participated in the construction of ENIAC, one of the first general purpose electronic digital computers, and where he also met John von Neumann. He worked with von Neumann on the construction of a new computer, and later Burks became the leader of the Logic of Computers Group at the University of Michigan. One of Burks' students at Michigan was John Holland, the pioneer of genetic algorithms. Another student of Burks, E.F. Codd, was able to design a self-replicating automaton of the von Neumann type using a cellular automaton system with only 8 states (as compared with von Neumann's 29). For many years, enthusiastic graduate students at the Michigan group continued to do important research on the relationships between information, logic, complexity and biology.

Meanwhile, in 1968, the mathematician John Horton Conway, working in England at Cambridge University, invented a simple game which greatly increased the popularity of the cellular automaton concept. Conway's game, which he called "Life", was played on an infinite checker-board-like lattice of cells, each cell having only two states, "alive" or "dead". The rules which Conway proposed are as follows: "If a cell on the checkerboard is alive, it will survive in the next time step (generation) if there are either two or three neighbors also alive. It will die of overcrowding if there are more than three live neighbors, and it will die of exposure if there are fewer than two. If a cell on the checkerboard is dead, it will remain dead in the next generation unless exactly three of its eight neighbors is alive. In that case, the cell will be 'born' in the next generation".

Originally Conway's Life game was played by himself and by his colleagues at Cambridge University's mathematics department in their common room: At first the game was played on table tops at tea time. Later it spilled over from the tables to the floor, and tea time began to extend: far into the afternoons. Finally, wishing to convert a wider audience to his game, Conway submitted it to Martin Gardner, who wrote a popular column on

[11] Semiotics is defined as the study of signs (see Appendix 2).

"Mathematical Games" for the Scientific American. In this way Life spread to MIT's Artificial Intelligence Laboratory, where it created such interest that the MIT group designed a small computer specifically dedicated to rapidly implementing Life's rules.

The reason for the excitement about Conway's Life game was that it seemed capable of generating extremely complex patterns, starting from relatively simple configurations and using only its simple rules. Ed Fredkin, the director of MIT's Artificial Intelligence Laboratory, became enthusiastic about cellular automata because they seemed to offer a model for the way in which complex phenomena can emerge from the laws of nature, which are after all very simple. In 1982, Fredkin (who was independently wealthy because of a successful computer company which he had founded) organized a conference on cellular automata on his private island in the Caribbean. The conference is notable because one of the participants was a young mathematical genius named Stephen Wolfram, who was destined to refine the concept of cellular automata and to become one of the leading theoreticians in the field[12].

One of Wolfram's important contributions was to explore exhaustively the possibilities of 1-dimensional cellular automata. No one before him had looked at 1-dimensional CA's, but in fact they had two great advantages: The first of these advantages was simplicity, which allowed Wolfram to explore and classify the possible rule sets. Wolfram classified the rule sets into 4 categories, according to the degree of complexity which they generated. The second advantage was that the configurations of the system in successive generations could be placed under one another to form an easily-surveyed 2-dimensional visual display. Some of the patterns generated in this way were strongly similar to the patterns of pigmentation on the shells of certain molluscs. The strong resemblance seemed to suggest that Wolfram's 1-dimensional cellular automata might yield insights into the mechanism by which the pigment patterns are generated.

In general, cellular automata seemed to be promising models for gaining insight into the fascinating and highly important biological problem of morphogenesis: How does the fertilized egg translate the information on the genome into the morphology of the growing embryo, ending finally with the enormously complex morphology of a fully developed and fully differentiated multicellular animal? Our understanding of this amazing process is as yet very limited, but there is evidence that as the embryo of a multicellular animal develops, cells change their state in response to the states of neighboring cells. In the growing embryo, the "state" of a cell means the way in which it is differentiated, i.e., which genes are turned on and which off - which information on the genome is available for reading, and which segments are blocked. Neighboring cells signal to each other by means of chemical messengers[13]. Clearly there is a close analogy between the way complex patterns develop in a cellular automaton, as neighboring cells influence each other and change their states according to relatively simple rules, and the way in which the complex morphology of a multicellular animal develops in the growing embryo.

[12] As many readers probably know, Stephen Wolfram was also destined to become a millionaire by inventing the elegant symbol-manipulating program system, Mathematica.

[13] We can recall the case of slime mold cells which signal to each other by means of the chemical messenger, cyclic AMP (Chapter 3).

Conway's Life game attracted another very important worker to the field of cellular automata: In 1971, Christopher Langton was working as a computer programmer in the Stanley Cobb Laboratory for Psychiatric Research at Massachusetts General Hospital. When colleagues from MIT brought to the laboratory a program for executing Life, Langton was immediately interested. He recalls "It was the first hint that there was a distinction between the hardware and the behavior which it would support... You had the feeling that there was something very deep here in this little artificial universe and its evolution through time. [At the lab] we had a lot of discussions about whether the program could be open ended - could you have a universe in which life could evolve?"

Later, at the University of Arizona, Langton read a book describing von Neumann's theoretical work on automata. He contacted Arthur Burks, von Neumann's editor, who told him that no self-replicating automaton had actually been implemented, although E.F. Codd had proposed a simplified plan with only 8 states instead of 29. Burks suggested to Langton that he should start by reading Codd's book.

When Langton studied Codd's work, he realized that part of the problem was that both von Neumann and Codd had demanded that the self-reproducing automaton should be able to function as a universal Turing machine, i.e., as a universal computer. When Langton dropped this demand (which he considered to be more related to mathematics than to biology) he was able to construct a relatively simple self-reproducing configuration in an 8-state 2-dimensional lattice of CA cells. As they reproduced themselves, Langton's loop-like cellular automata filled the lattice of cells in a manner reminiscent of a growing coral reef, with actively reproducing loops on the surface of the filled area, and "dead" (nonreproducing) loops in the center.

Langton continued to work with cellular automata as a graduate student at Arthur Burks' Logic of Computers Group at Michigan. His second important contribution to the field was an extension of Wolfram's classification of rule sets for cellular automata. Langton introduced a parameter A to characterize various sets of rules according to the type of behavior which they generated. Rule sets with a value near to the optimum ($\lambda = 0.273$) generated complexity similar to that found in biological systems. This value of Langton's λ parameter corresponded to a borderline region between periodicity and chaos.

After obtaining a Ph.D. from Burks' Michigan group, Christopher Langton moved to the Center for Nonlinear Studies at Los Alamos, New Mexico, where in 1987 he organized an "Interdisciplinary Workshop on the Synthesis and Simulation of Living Systems" - the first conference on artificial life ever held. Among the participants were Richard Dawkins, Astrid Lindenmayer, John Holland, and Richard Laing. The noted Oxford biologist and author Richard Dawkins was interested in the field because he had written a computer program for simulating and teaching evolution. Astrid Lindenmayer and her coworkers in Holland had written programs capable of simulating the morphogenesis of plants in an astonishingly realistic way. As was mentioned above, John Holland pioneered the development of genetic algorithms, while Richard Laing was the leader of Nasals study to determine whether self-reproducing factories might be feasible.

Langton's announcement for the conference, which appeared in the Scientific American, stated that "Artificial life is the study of artificial systems that exhibit behavior charac-

teristic of natural living systems...The ultimate goal is to extract the logical form of living systems. Microelectronic technology and genetic engineering will soon give us the capability to create new life *in silico* as well as *in vitro*. This capacity will present humanity with the most far-reaching technical, theoretical, and ethical challenges it has ever confronted. The time seems appropriate for a gathering of those involved in attempts to simulate or synthesize aspects of living systems."

In the 1987 workshop on artificial life, a set of ideas which had gradually emerged during the previous decades of work on automata and simulations of living systems became formalized and crystallized: All of the participants agreed that something more than reductionism was needed to understand the phenomenon of life. This belief was not a revival of vitalism; it was instead a conviction that the abstractions of molecular biology are not in themselves sufficient. The type of abstraction found in Darwin's theory of natural selection was felt to be nearer to what was needed. The viewpoints of thermodynamics and statistical mechanics were also helpful. What was needed, it was felt, were insights into the flow of information in complex systems; and computer simulations could give us this insight. The fact that the simulations might take place in silico did not detract from their validity. The logic and laws governing complex systems and living systems were felt to be independent of the medium.

As Langton put it, "The ultimate goal of artificial life would be to create 'life' in some other medium, ideally a virtual medium where the essence of life has been abstracted from the details of its implementation in any particular model. We would like to build models that are so lifelike that they cease to become models of life and become examples of life themselves."

Most of the participants at the first conference on artificial life had until then been working independently, not aware that many other researchers shared their viewpoint. Their conviction that the logic of a system is largely independent of the medium echoes the viewpoint of the Macy Conferences on cybernetics in the 1940's, where the logic of feedback loops and control systems was studied in a wide variety of contexts, ranging from biology and anthropology to computer systems. A similar viewpoint can also be found in biosemiotics (Appendix 2), where, in the words of the Danish biologist Jesper Hoffmeyer, "the sign, rather than the molecule" is considered to be the starting point for studying life. In other words, the essential ingredient of life is information; and information can be expressed in many ways. The medium is less important than the message.

The conferences on artificial life have been repeated each year since 1987, and European conferences devoted to the new and rapidly growing field have also been organized. Langton himself moved to the Santa Fe Institute, where he became director of the institute's artificial life program and editor of a new journal, *Artificial Life*. The first three issues of the journal have been published as a book by the MIT Press, and the book presents an excellent introduction to the field.

Among the scientists who were attracted to the artificial life conferences was the biologist Thomas Ray, a graduate of Florida State University and Harvard, and an expert in the ecology of tropical rain forests. In the late 1970's, while he was working on his Harvard Ph.D., Ray happened to have a conversation with a computer expert from the MIT

Artificial Intelligence Lab, who mentioned to him that computer programs can replicate. To Ray's question "How?", the AI man answered "Oh, it's trivial."

Ray continued to study tropical ecologies, but the chance conversation from his Cambridge days stuck in his mind. By 1989 he had acquired an academic post at the University of Delaware, and by that time he had also become proficient in computer programming. He had followed with interest the history of computer viruses. Were these malicious creations in some sense alive? Could it be possible to make self-replicating computer programs which underwent evolution by natural selection? Ray considered John Holland's genetic algorithms to be analogous to the type of selection imposed by plant and animal breeders in agriculture. He wanted to see what would happen to populations of digital organisms that found their own criteria for natural selection - not humanly imposed goals, but self-generated and open-ended criteria growing naturally out of the requirements for survival.

Although he had a grant to study tropical ecologies, Ray neglected the project and used most of his time at the computer, hoping to generate populations of computer organisms that would evolve in an open-ended and uncontrolled way. Luckily, before starting his work in earnest, Thomas Ray consulted Christopher Langton and his colleague James Farmer at the Center for Nonlinear Studies in New Mexico. Langton and Farmer realized that Ray's project could be a very dangerous one, capable of producing computer viruses or worms far more malignant and difficult to eradicate than any the world had yet seen. They advised Ray to make use of Turing's concept of a virtual computer. Digital organisms created in such a virtual computer would be unable to live outside it. Ray adopted this plan, and began to program a virtual world in which his freely evolving digital organisms could live. He later named the system "Tierra".

Ray's Tierra was not the first computer system to aim at open-ended evolution. Steen Rasmussen, working at the Danish Technical University, had previously produced a system called "VENUS" (Virtual Evolution in a Nonstochastic Universe Simulator) which simulated the very early stages of the evolution of life on earth. However, Ray's aim was not to understand the origin of life, but instead to produce digitally something analogous to the evolutionary explosion of diversity that occurred on earth at the start of the Cambrian era. He programmed an 80-byte self-reproducing digital organism which he called "Ancestor", and placed it in Tierra, his virtual Garden of Eden.

Ray had programmed a mechanism for mutation into his system, but he doubted that he would be able to achieve an evolving population with his first attempt. As it turned out, Ray never had to program another organism. His 80-byte Ancestor reproduced and populated his virtual earth, changing under the action of mutation and natural selection in a way that astonished and delighted him.

In his freely evolving virtual zoo, Ray found parasites, and even hyperparasites, but he also found instances of altruism and symbiosis. Most astonishingly of all, when he turned off the mutations in his Eden, his organisms invented sex (using mechanisms which Ray had introduced to allow for parasitism). They had never been told about sex by their creator, but they seemed to find their own way to the Tree of Knowledge.

Thomas Ray expresses the aims of his artificial life research as follows:[14] "Everything we know about life is based on one example: Life on Earth. Everything we know about intelligence is based on one example: Human intelligence. This limited experience burdens us with preconceptions, and limits our imaginations... How can we go beyond our conceptual limits, find the natural form of intelligent processes in the digital medium, and work with the medium to bring it to its full potential, rather than just imposing the world we know upon it by forcing it to run a simulation of our physics, chemistry and biology?..."

"In the carbon medium it was evolution that explored the possibilities inherent in the medium, and created the human mind. Evolution listens to the medium it is embedded in. It has the advantage of being mindless, and therefore devoid of preconceptions, and not limited by imagination." "I propose the creation of a digital nature - a system of wildlife reserves in cyberspace in the interstices between human colonizations, feeding off unused CPU-cycles and permitted a share of our bandwidth. This would be a place where evolution can spontaneously generate complex information processes, free from the demands of human engineers and market analysts telling it what the target applications are - a place for a digital Cambrian explosion of diversity and complexity..."

"It is possible that out of this digital nature, there might emerge a digital intelligence, truly rooted in the nature of the medium, rather than brutishly copied from organic nature. It would be a fundamentally alien intelligence, but one that would complement rather than duplicate our talents and abilities."

Have Thomas Ray and other "a-lifers"[15] created artificial living organisms? Or have they only produced simulations that mimic certain aspects of life? Obviously the answer to this question depends on the definition of life, and there is no commonly agreed-upon definition. Does life have to involve carbon chemistry? The a-lifers call such an assertion "carbon chauvinism". They point out that elsewhere in the universe there may exist forms of life based on other media, and their program is to find medium-independent characteristics which all forms of life must have.

In the present book, especially in Chapter 4, we have looked at the phenomenon of life from the standpoint of thermodynamics, statistical mechanics and information theory. Seen from this viewpoint, a living organism is a complex system produced by an input of thermodynamic information in the form of Gibbs free energy. This incoming information keeps the system very far away from thermodynamic equilibrium, and allows it to achieve a statistically unlikely and complex configuration. The information content of any complex (living) system is a measure of how unlikely it would be to arise by chance. With the passage of time, the entropy of the universe increases, and the almost unimaginably improbable initial configuration of the universe is converted into complex free-energy-using systems that could never have arisen by pure chance. Life maintains itself and evolves by feeding on Gibbs free energy, that is to say, by feeding on the enormous improbability of the initial conditions of the universe.

All of the forms of artificial life that we have discussed derive their complexity from the

[14] T. Ray, http://www.hip.atr.co.jp/ ray/pubs/pubs.html

[15] In this terminology, ordinary biologists are "b-lifers".

consumption of free energy. For example, Spiegelman's evolving RNA molecules feed on the Gibbs free energy of the phosphate bonds of their precursors, ATP, GTP, UTP, and CTP. This free energy is the driving force behind artificial evolution which Spiegelman observed. In his experiment, thermodynamic information in the form of high-energy phosphate bonds is converted into cybernetic information.

Similarly, in the polymerase chain reaction, discussed in Chapter 3, the Gibbs free energy of the phosphate bonds in the precursor molecules ATP, TTP, GTP and CTP drives the reaction. With the aid of the enzyme DNA polymerase, the soup of precursors is converted into a highly improbable configuration consisting of identical copies of the original sequence. Despite the high improbability of the resulting configuration, the entropy of the universe has increased in the copying process. The improbability of the set of copies is less than the improbability of the high energy phosphate bonds of the precursors.

The polymerase chain reaction reflects on a small scale, what happens on a much larger scale in all living organisms. Their complexity is such that they never could have originated by chance, but although their improbability is extremely great, it is less than the still greater improbability of the configurations of matter and energy from which they arose. As complex systems are produced, the entropy of the universe continually increases, i.e., the universe moves from a less probable configuration to a more probable one.

In Thomas Ray's experiments, the source of thermodynamic information is the electrical power needed to run the computer. In an important sense one might say that the digital organisms in Ray's Tierra system are living. This type of experimentation is in its infancy, but since it combines the great power of computers with the even greater power of natural selection, it is hard to see where it might end.

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