UČEBNÍ TEXTY
UNIVERZITY KARLOVY

OUTLINES OF EMBRYOLOGY

Jaroslav Slípka
Zbyněk Tonar

KAROLINUM

Outlines of Embryology

Jaroslav Slípka
Zbyněk Tonar

Reviewed by:
doc. MUDr. Jitka Kočová, CSc.
Depatrment of Histology and Embryology, Faculty of Medicine in Pilsen,
Charles University, Czech Republic
doc. RNDr. Viera Pospíšilová, CSc.
Institute of Histology and Embryology, Faculty of Medicine,
Comenius University, Bratislava, Slovakia

English proofreading and editing:
Sarah Leupen, Ph.D., Department of Biological Sciences,
University of Maryland Baltimore County, USA

Published by Charles University
Karolinum Press
as a teaching text for the Faculty of Medicine in Pilsen
Typesed by DTP Karolinum Press
Third, revised edition

ISBN 978-80-246-4181-2
ISBN 978-80-246-4198-0 (online : pdf)

Charles University
Karolinum Press 2019

www.karolinum.cz
ebooks@karolinum.cz

Contents

Preface to the first edition

There exists nothing in the world without history – and we cannot understand the present status quo of anything not knowing its history. From this it appears that one cannot understand also the living organisms, their body organization and function without knowing their history. Biological history represents a process of development of the organ structures, i.e.an ontogenetic process, started by meeting of two parenteral germ cells, passing through the prenatal and postnatal period and ended by the death of an inividual.

Human biological history has, of course, two faces – an ontogenetic and a phylogenetic, which are both in mutual correspondence and influence each other. We should not forget that also the process started by our animal predecessors, which evolved to the modern human, is mirrored in our individual development. For this development, i.e. human ontogeny, the term embryology became common, even though it covers not only the fate of an embryo, but the whole prenatal developmental process. To understand embryology, we should be informed at least about the main stages of its developmental history.

The whole text has been divided in seven chapters. The first one is devoted to the history of embryology, the topic which was until now mostly neglected in the textbooks of embryology, but according to our opinion it can provide essential help to students in orienting within the explosive scientific development of the whole subject, esp. in the way of understanding cancerogenesis or modern efforts to improve the human fertility.

The developmental topics start in a chapter of general embryology, named here as progenesis. We have used that term, even though we know, that it has also been used to describe a shortening of the developmental processes, and can play an important role in evolution of some species. But we understand under that term the earliest developmental period, leading from fertilization to the formation of all three germ layers and neurulation.

The whole text cannot completely follow the time sequence of the too-complex processes of development and even though we know that also other systems in successive chapters are not fully appropriate, we have divided the organogenetic part according the anatomical systems to enable an easy orientation to the whole topic.

After the preembryomic period, which takes the first four weeks of development, the main body organs are laid down, followed by the real embryonic period in which the main anatomical systems are established. Starting from the 9^{th} week during the fetal period, growth and maturation bring the fetus to the birth of a newborn in the 40^{th} week, at which time the intrauterine development ends and the postnatal period of the new individual's development begins.

These "Outlines" are not intended to replace comprehensive textbooks of Embryology, but they should serve as a short summary of the knowledge which has been given by the teacher in the lecture hall. They are written in simplified English and they should help the international students at the time of their preparations for the examination of embryology in the second year of the pregraduate medicine curriculum. They can also serve as a first orientation to developmental problems for the colleagues of other branches of medicine or general biology.

The author would like to express many thanks to all those who helped him to prepare these "Outlines". They include first of all his friends – colleagues from the Department of Histology and Embryology, Faculty of Medicine, Charles University in Pilsen, but also his students, who have listened to him, judged him, taught him and made his engagement meaningful.

Prof. Jaroslav Slípka, Dr. med., DSc.
Pilsen, 2010

Preface to the second edition

Embryology affects every person in the world. We all once were fertilized oocytes, zygotes, morulae and blastocysts. All of us managed to implant into the uterine mucosa while undergoing gastrulation. We all went through organogenesis. If you are interested in which processes are bedding these words, this book is for you. Just keep on reading.

In pregraduate course on medical embryology, the students are supposed to understand the basics of developmental processes that happen during the pregnancy. This starts with a sperm cell fertilizing an oocyte, and, if everything goes well, it leads to the birth of a newborn. Although we are not able to understand or explain all (or even the majority of) the processes during the prenatal development, our aim is to provide you with the most relevant information you should be aware of as healthcare professionals. Understanding embryology explains a lot on anatomical structure of the human body, including the variability of anatomical structures and organs found in every individual. Although this small book covers mostly the normal prenatal development, it may help you to understand also some developmental defects.

This textbook outlines the courses in embryology, taught to the international students of general and dental medicine in the second year of their pregraduate studies at the Charles University, Faculty of Medicine in Pilsen. The first edition was prepared in 2010 and modified in 2012 by Prof. Dr. Jaroslav Slípka, DSc (1926–2013), who was an enthusiastic and inspiring researcher and teacher at the Department of Histology and Embryology. The second edition was updated in 2018 to reflect some of the advances in teaching of embryology. Nevertheless, the illustrations and the concise concept of the book designed originally by prof. Slípka were kept. We recommend using this textbook for revising and summarizing the essential knowledge. For full color textbooks and atlases that are necessary for understanding the basics of human prenatal development, see the literature recommended. As the development of the human body reflects a number of more general principles common for various zoologicla taxons (placental mammals, amniotes, vertebrates, chordates, triblastics etc.), students interested in a more deeper understanding of the underlying processes are referred to literature on evolutionary biology.

We wish all our students might enjoy the insight into the prenatal development of human body. Welcome to the world of Embryology!

Zbyněk Tonar
Pilsen, 2018

I. History of embryology

We suppose that the first basic facts of human prenatal development were known already to the ancient Egyptians, who came in contact with various stages of fetuses and even embryos during the embalming of pregnant women. There exists also a whole list of observed malformations of human miscarriages, which was used for prediction of the future by Babylonian priests in ancient Mesopotamia 5,000 years ago.

The ancient humans tried to explain the accidental findings mostly as a result of the activity of supernatural powers and in that way, like most of the anthropological information, human development was mostly included as a part of the religious category.

The sacred scriptures of the Hindu religion in the second millennium B.C. describe their ideas on the developmental processes during pregnancy, which they considered to be a result of a junction of mother's blood and father's semen, but the Old-Indian priests also already had simple experience in heredity, and they provided instructions on choosing a wife to prevent heritable illnesses. The ancient Greeks also respected the importance of the environment during pregnancy and they recommended that the pregnant woman should be surrounded by beauty only, and during the wedding day the newly married couple should not drink wine.

These ideas were taken over in the ancient Greek science and the "father of medicine" *Hippocrates* (460–377 B.C.) had already compared human development with the development of the chick. But the first serious information on developmental processes was collected by *Aristotle* (384–322 B.C.) who proclaimed relatively correctly, that a human embryo originates from the material of both the mother and father. The mother provides only the raw material (postmenstrual discharge) and the man through his semen the organizing principle. Various organs are at first fashioned in a simplified way before becoming structurally and functionally complex. His epigenetic view on successive development of organs (e.g. the heart appears sooner than lungs) has influenced his successors and in several features corresponds to our contemporary knowledge.

An exceptional position among the ancient Greek scholars was occupied by *Galen* (130–201 B.C.), who in his anatomical studies also described the nourishment of the embryo, and his humoral theory influenced medicine throughout the whole Middle Ages.

The Romans added only a little to the basic theories of the Greeks and they adopted more or less the Greek views, like *Gaius Plinius Secundus (Pliny the Elder)* (23–79 A.D.), who in his large series of 37 books "Natural History" covered in an encyclopedic way the entire knowledge of nature at that time, also including medical information.

There was a big stagnation of scientific progress in Europe after the fall of the Roman Empire. But in Arab countries a new cultural power appeared in connection with the development of new religion formed by Mohammed in the 7th century. Even in the holy script of Islam – the Holy Qur'an – are mentions of the stages of the human, which starts from a small drop in the mother's womb into a form of a leech or "suspended thing" (embryo?) and finally in a "chewed substance" (somites?). It is interesting that *Mohammed* in his "sayings" (hadeeth) indicates relatively accurately the embryonic period, when he narrates that the body components should be shaped after 42 days and the "hearing, vision, skin, flesh and bones are created."

When Mohammed died (632 A.D.?) Islam had already spread throughout the whole of Arabia, Persia, Syria, Egypt, North Africa, and Spain. The Arabs grasped the cultural element of the conquered nations very quickly, and they built their own advanced culture which can be characterized as a perfect synthesis of Old-Indian, Persian, Greek and Roman science which they completed by their own contribution of experimental methods.

In that way, there emerged at the time of European cultural darkness, in the Islamic countries a sort of Arab Enlightenment Era, considered as the golden age of Arab culture within the 9th–13th centuries. Among the Persian and Arab scholars of that Era of Reason who paid attention to medicine and problems of human development *was Ibn Rhazes* (850–923) and *Ibn Sina – Avicenna* (980–1037), who based his work on Aristoteles and Galen and won by his "Canon of Medicine" fame at that time in the whole world. Another scholar was *Ibn Heitham* (965–1038), who denied the old view of the function of eye and proved that sight depends on the passage of light-rays through the eye. The next great man of science and esp. of medicine was *Ibn Rushd – Averroes* (1126–1198) whose principal work was in the form of commentaries on Aristotle's writings, which he further developed.

Among Arab scholars were also authors who believed in a sort of an evolutionary development of the living organisms and even put humans on the top of their evolutionary ladder of animals (*Al Masudi*) so that it can be spoken of as a sort of "chain of being" or even about "Darwinists" of the 10^{th} century. The Persian and Arab science influenced the whole of Europe and no wonder, that at the Universities, which were founded at the same time in Europe, the students were asked to study from the Latin translations of those Persian and Arab authors.

It was not until the European renaissance that the theories of Aristotle and Arab authors were further elaborated. A great personality of the early renaissance was *Leonardo da Vinci* (1452–1519), who among others continued the investigation of human body and its development. In Bologna *Volcher Coiter* (1534–1576) and *Ulisse Aldrovandi* (1552–1605) studied the development of chick from the beginning of incubation to hatching. They have been considered as the real founders of embryology.

The founder of scientific anatomy was *Andreas Vesalius* (1514–1564), the author of the first modern illustrated textbook of anatomy "De humani corporis fabrica libri septem". His successors in Padua were *Fallopius* (1514–1562), who described the female genital organs and placenta, and his pupil *Fabricius* (1537–1619) who examined the development of some animals and compared them with human embryos and fetuses.

He influenced one of his students in Padua, an Englishman *William Harvey* (1568–1657) who is more known as a discoverer of blood circulatory system, but he was also interested in problems of development and contrary to Aristotle was persuaded, that it is the egg only from which all life originates to produce more eggs. His motto was: "Omne vivum ex ovo".

Harvey was in contact with a much younger Czech scholar *Marcus Marci* (1595–1667) who as a distinguished scientist of the Prague University by his discoveries in physics. Through his work applying his research in optics to the study of developing embryos, he anticipated the much later theory of morphogenetic fields.

The primary importance of the egg for beginning of development was strongly proclaimed by *Malpighi* (1628–1694) who thought that he observed preexisting parts of the fetuses in the unincubated hen's egg. He was a representative of *preformation theory* in its "ovist" form. This "ovistic" theory assumes that the egg gives the starting material for the development and the male semen is only a trigger of the developmental process.

Other preformationists propagated an opposite source of embryonic primordium, namely the semen – i.e. the male is the bearer of the whole development. This "animalculist" theory arose on the basis of invention of the microscope in the 17th century. *Antony van Leeuwenhoek* (1632–1723) from Leiden and his student *Ham* were the first who, using a primitive microscope, could observe the human spermatozoon. They thought to see in the head of sperm a preformed individual – a so called *homunculus*.

In Italy, *Lazzaro Spallanzani* (1729–1799) performed experiments in regeneration of some organs of amphibians, and even experiments with fertilization, adding sperm to the eggs of various animals, but he never left his ovistic conviction of a preformed individual in the egg.

These primitive preformation views were corrected only during the 18th century by *Caspar Friedrich Wolff* (1734–1794) who in his "*Theoria generationis*" (1759) claims that development starts from living homogenous substance and proceeds by gradual, i.e. *epigenetic* differentiation of tissues and organs – as a result of "vis essentialis".

At that time *Jean Baptiste Lamarck* (1744–1829) presented his theory of evolution on the basis of adaptation of organisms to the environment and on formation of organs according their function, and on heredity of acquired features.Another distinguished medical celebrity at that time was *Jiří Prochaska* (1749–1820), who was a defender of the epigenetic idea and criticized Spallanzani's preformation. His main scientific contribution was his modern conception of the nervous reflex, but he was also interested in extrauterine nidation of embryo and described some human malformations.

A uniform creative plan of living structures was defended by *Johann Wolfgang Goethe* (1749–1832), a famous German poet and romantic natural philosopher who supported the idea of organ homology, such as that of the incisive bone in various mammals. Another example was his explanation of skull segmentation as a result of a conversion of cervical vertebrae.

The same uniform plane was considered by *Etienne Geoffroy Saint-Hilaire* (1772–1844). He studied birth defects, considered them as deviations of ontogeny and created the term *teratology*. He was persuaded that current organisms had developed from the extinct ones and contributed to the creation of a modern view of natural evolution – mainly in discussions with *George Cuvier* (1769–1832), who was an advocate of the "catastrophism" theory and a well-known founder of comparative anatomy.

One of his followers was *Johann Friedrich Meckel* (1781–1833) , who like his father and grandfather introduced in Germany the studies of comparative anatomy. He made considerable contribution to understanding of the development of the nervous and intestinal systems.

The real founder of a modern embryology was the successor of Wolff – *Karl Ernst von Baer* (1792–1876) who described the human egg (1828) and who studied the embryos of various animals. He described similarities in developmental processes in all studied vertebrates and postulated that the embryo passes from general patterns to the specific form of the species. He noticed also the similarities of embryos of higher forms with the adults of evolutionary lower animals.

At the same time his contemporary *H. Ch. Pander* (1794–1865) performed pioneering work on the development of chick embryo and demonstrated the existence of germ layers, which were denominated later by *Robert Remak* (1815–1869) as ectoderm, endoderm, and mesoderm. Another distinguished student of von Baer was *M. H. Rathke* (1793–1860) who described gill arches in the embryos of birds and mammals.

All these findings prepared a path to the revolutionary discoveries of the 19^{th} century, which involved and stimulated all branches of biology, including embryology. Among these, the most important landmarks were first the proclamation of the cellular theory, which has always been called Schwann-Schleiden theory. *Matthias Jakob Schleiden* (1804–1881) studied plant embryo cells and described the formation of a cell from its nucleus (1838). One year after *Theodor Schwann* (1810–1882) using the discoveries of Schleiden's plant-cell formation theory, observed within the embryonic cartilage of tadpoles cells, similarities to plant cells and published 1839 his famous work of structural identity of animal and plant bodies.

There were, of course some earlier indications of cellular structures of animal and plant bodies. Already in 17^{th} century was *Robert Hook,* who in 1665 spoke about plant cells. At the beginning of the 19^{th} century were several authors who spoke about cellular tissue (*Blainville, Lamarck*), but nearest to the official cell declaration was a Czech *Jan Evangelista Purkyně* (1787–1869), who has been considered as one of the great geniuses in the field of biological discoveries. He discovered the germinal vesicle in the chicken egg (1825), described the cilia in the oviduct, the large cells in the cerebellum, the conducting fibers in the myocardium, etc. *Purkyně* described the structure of nervous tissue, composed from "granules" (i.e. cells) at a medical meeting in Prague in 1837 and already his assistant *Gabriel Valentin* (1810–1883) described the identity of animal and plant cellular tissue in 1835, i.e. four years before Schwann.

The well-known propagator of cell theory was *Rudolf Wirchow* (1821–1902) who studied the pathological changes of the cells and proclaimed that "omne cellula e cellula" i.e. that cells cannot originate from other forms but only from other cells.

His younger contemporary *Wilhelm His sen*. (1831–1904) was the first causal embryologist who believed that ontogenetical events are the mechanical results of differential growth in cells. In his effort to construct a system of human developmental stages, he reconstructed and described the developmental series of early human embryos. He published a detailed work on chick development in which he was the first to describe the neural crest.

The conception of the cell as a general unit of life was immediately accepted and without this conception the next revolutionary discoveries of that era would hardly have been possible.

It was particularly the formulation of the evolutionary theory of *Charles Darwin* (1809–1882) who in his work "*On the origin of species by means of natural selection*" (1859) explained the evolution of adaptation of organisms to the environmental conditions by the way of natural selection – i.e. contrary to Lamarck who suggested the adaptation occurred through active changes by individual organisms.

In that book as well as in his next book "*The descent of Man and selection in relation to sex*" (1862) in which he argues that Man has evolved through natural selection from a series of animal forms, he used a lot of anatomical and embryological arguments, borrowed from his predecessors. He spoke also about significance of the heredity of species variations, which can evolve only into varieties and finally into new species. But he could not know the principles of genetics.

The new evolutionary theory created, of course, at first a number of opponents from the rank of creationists, but soon there also started to appear Darwin's supporters. In addition to *Alfred Russel Wallace* (1825–1913), who at the same time formulated a selection theory, the most known was *Thomas Henry Huxley* (1825–1895) a highly gifted English scientist, a comparative anatomist and creator of a general theory of germinal layers.

In Germany it was *Ernst Haeckel* (1834–1919), who contributed much of the science of Darwinism. He brought many proofs to the rightness of evolutionary theory mostly by emphasizing the developmental processes and on their basis he formulated his version of "Biogenetical principle", which in its simplified form claims that ontogeny recapitulates phylogeny. His book "Anthropogenie" published in 1874 was the first scientific textbook of human embryology.

The laws of genetics were not formulated until *Johann Gregor Mendel* (1822–1884), who published 1866 in Brno his main work "*Versuche über Pflanzen-Hybriden*" on the inheritance of traits in the garden pea. He postulated that the phenotype is controlled by factors now known as genes. These factors exist in pairs and undergo separation during formation of gametes so that each mature gamete receives one member of the gene pair. When Mendel performed his experiments, the situation in chromosomes in mitosis and meiosis had not been described.

The significance of Mendel's work went unrecognized until 1900 when it was independently discovered by *Hugo deVries* (1848–1935), Carl *Correns* (1864–1933) and *Erich Tschermak* (1871–1962). The "*Mendelism*" in the first decades of the 20th century was rapidly developed and Mendel's laws were included in cytology. The term "*genetics*" for the science of heredity was first used by *William Bateson* (1861–1926).

Even at the beginning of the 20th century most studies concluded that humans had a diploid number 48 and haploid number of 24 chromosomes. However in 1956 *J.H.Tijo* and *A Levan* reported that humans have 46 chromosomes, and 1959 Lejeune described the first genetic disorder, caused by an abnormal number of chromosomes – the *Down syndrome*. In 1941 G. Gregg described the relation of the German measles to the malformations in newborns, the results of the atomic bomb in Japan (1945) and the Thalidomide (Contergan) catastrophe in Germany (1959–1962) revived deeper studies in teratology. In 1971 the International Teratology Society was formed.

The first followers of G. Mendel regarded the genetic theory of spontaneous mutation as the real explanation of the evolutionary processes and as a substitution of Darwin's theory. But even since the early decades of the 20th century, postmendelian discoveries of genetic mechanisms, population-genetic and an explosion of general biological and paleontological knowledge contributed to the combination of Darwin's theory of evolution by natural selection with Mendelian heredity. These combined ideas, expressed in the works of *T. H. Huxley, T. Dobzhansky, B. Reench, E. Mayr* and others represented a new united view, named "Neo-Darwinism". This "modern synthesis" has helped to improve the explanation of the origin and maintenance of variations within the population and problems of species origin.

Together with the accumulation of new achievements of embryology, developmental biology turned its interest to the relation between ontogeny and phylogeny. A new view on that relation brought the theory of phylembryogenesis, created by *Alexei Nikolajevič Severcov* (1866–1936), who showed that evolutionary changes can affect any developmental stage, and that the changes in earlier stages are much more evolutionarily significant then the changes in the later stages. His student *Ivan Ivanovič Schmalgauzen* (1884–1963), stated that the genetic information is preserved in ontogeny on the molecular level in the individual phenotype which is under the influence of selection.

These views led to the creation of a special discipline, so called "Evo-Devo", which became a paradigm of "postmodern" synthesis and a research program in which developmental biology is combined with genetic and evolutionary theory.

At the end of 19th century biologists made many cytological discoveries, so that at the turn of the century scientists identified the chromosomes (*Wilhelm Waldeyer*, 1836–1921) as the cellular components that carry genes, and *Thomas Hunt Morgan* (1866–1945) brought a proof that the genetic factors were physical, located on the individual chromosomes and combined genetics with embryology – the era of molecular biology started. In 1944 *Oswald Theodor Avery* demonstrated that the genetic material was the DNA. 1953 *James Dewey Watson* and *Francis Harry Crick* constructed a model of DNA structure, that is the primary carrier of genetic information.

Genetic information, encoded in genes, determines the proteins in the cell, and has to be transferred from DNA by the help of RNA, which realizes the translation into protein molecules. This *central dogma of molecular biology,* although corrected later on, represented a milestone in the development of all branches of biology, including embryology.

The geneticists distinguished between a genotype, being the sum of the genetic information encoded in the individual's DNA, directing the development of a phenotype. We have to keep in mind that the classical preformists spoke about preformed structures, but the modern conception accentuates the coded genetic instructions which in a way can replace the "vis essentialis" of the vitalistic biologists. The genetic instructions, according which development proceeds are preformed, but their realization is epigenetic, and acting upon embryonic cells from outside – as *Peter Brian Medawar* (1915–1987) said: "genetics proposes, epigenetics disposes". Therefore the process of development – the unfolding of a phenotype – is epigenetic, but development is also preformed because the zygote contains an inherited genetic program, that largely determines the phenotype. The zygote represents a primary totipotent stem cell and proceeds during differentiation in a cascade of stem cells of gradually limited potency.

The technology of the in vitro fertilization (IVF) was successfully developed by *Robert Edwards* and *Patrick Steptoe* in 1970s, so that on 28.7.1978 the world's first "test tube baby" was born (Louise Brown).

Progress in molecular genetics has led to explosive development of gene engineering which was at first realized mostly in veterinary medicine (e.g. Dolly the sheep). A number of diagnostic methods became part of human assisted reproduction techniques (ART).

The cellular theory and the discovery of the principles of evolution and genetics rank with the greatest achievements in the history of natural science. The ideas of Darwin and Mendel form the basis of modern biological science in general and embryology in particular.

After the main activity of embryologists to search for the phylogenetic relationship of structures, influenced by Haeckelian theory of recapitulation, the efforts began to explain the development through mechanical forces (*His)* and then the interest was shifted to the introduction of experimental embryology and explanation of cellular differentiation.

Wilhelm Roux (1880–1924) and *Hans Driesch* (1867–1941) performed experiments on the developmental potency of blastomeres and *Hans Spemann* (1869–1941) studied the mutual reactions of tissues during development; he formulated together with *Hilde Mangold* (1898–1924) the concept of induction, which was further studied by Julian Huxley (1887–1975). The chemical nature of the organizers was studied by *Joseph Needham* (1900–1995) and *Conrad Waddington* (1905–1977) known for their biochemical approaches to embryology.

The very significant concept of induction postulates the existence of an organizer, which represents part of an embryo, capable of exerting a morphogenetic stimulus on another part. This conception evolved gradually toward a view that development represents a chain of successive inductions which start initially in the single field set up by the primary organizer. This "morphogenetic field" concept recalls the old ideas of Marcus Marci and further His' concept of "organ forming areas". The fact that the inductions are associated with direct cell movements led to the formulation of a concept of "positional information", elaborated by *Lewis Wolpert* (*1929) who supposed that the cells have their position specified and interpret their positional value by differentiating in a particular way.

To the great personalities of the 19th century biology should be added at least also the French scientist *Claude Bernard* (1813–1878), who worked in experimental physiology and contributed fundamentally to understanding of the importance of equilibrium between the inner and outer environment – of the so called homeostasis. To secure the maintenance of homeostatic physiological balance requires, of course, a system of regulatory mechanisms. Gradually it became clear that they are two organ systems which share in steering and regulating the life functions of the organism – i.e., the nervous and endocrine humoral systems.

The attention of morphologists to neuroanatomy has a long history which typically parallels the development of the whole field of morphology which started as a pure descriptive science and has gradually changed from classical anatomy to the modern morphology, which has to complete the morphometric view with the view of function and development. The knowledge of the nervous system went from the description of Leonardo across the functional conception of Jiří Procháska and I.P.Pavlov to the discoveries of neuroendocrine substances like endorphins and encephalin, which join the neuroanatomy with humoral endocrinology.

The humoral system has been gradually divided into an endocrine and immune system, so that we can now speak about a regulatory neuro-endocrine-immune system. From the conception of Claude Bernard of the secretion of some organ products into blood, endocrinology has come a long way from isolating and studying the simple hormones to the neuroendocrine substances and to studies of membrane and cytosol receptors.

The immune system has been split off of endocrinology as a third regulatory system, even though it forms a unified humoral system along with the endocrine system. Even though the first foundations of immunity were laid by *Edward Jenner* (1749–1823) at the end of the 18th century, the explosive development of immunity started in the 1960s when several laboratories described the difference between T and B lymphocytes.

A real revolution in cytology was the invention of electron microscopic techniques just before the 2nd World War. It has allowed us to examine the submicroscopic structures up to the size of a large molecule. From that time the morphology became closer to physiology and biochemistry it has thus allowed us to follow various biochemical substances within the tissues. The explosive development of histochemical and cytochemical methods