

PHARMACEUTICALS AND BIOTECHNOLOGY IN THE CZECH REPUBLIC

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Introduction

Principles of heredity were discovered in the 19th century by Gregor Mendel, the friar in abbey of St. Thomas in Brno, who studied the heritability of flower color in the pea. His work was forgotten and was rediscovered only at the beginning of the 20th century. Nevertheless, this shows that genetics has a long tradition in the Czech Republic. Besides, traditional biotechnologies as brewery, winery or distillery are abundant in the Czech Republic. Also important are the fields of organic and macromolecular synthesis. Although the Czech Republic does not belong to the pioneers of the molecular biology, some of the Czech laboratories are European-class, showing very good results in the field of modern biotechnology. For example, the laboratory of A. Holý has synthesized nucleotide derivatives that are very effective inhibitors of reverse transcriptase. These drugs are widely used in the therapy of serious viral diseases such as AIDS, hepatitis B and cytomegalic retinitis.

Research and development

The main centres of the biotechnology, molecular biology and pharmaceutical research and development in the Czech Republic are located in Praha and other large cities with universities: Brno, Olomouc, Hradec Králové, Plzeň and České Budějovice. The majority of the research facilities belong either to the Academy of Sciences of the Czech Republic (AS CR), to the universities or to the Ministry of Health.

Commonly used techniques in these laboratories include DNA sequencing, gene cloning in heterologous hosts, production of recombinant proteins and their purification, production of poly- and monoclonal antibodies and cell and tissue cultures. Several laboratories have mastered the technique of preparation of transgenic animals and plants as well as breeding of germ-free animals.

One of the main areas of biotechnology research in the Czech Republic is the structure of the genome and regulation of its expression. The recently founded Centre of Integrated Genomics has the leading role in this field. The centre consists of selected laboratories of the Institute of Molecular Genetics and Institute of Physiology AS CR, Charles University and the College of Chemical Technology in Praha. The Centre is sequencing and mapping the genome, and studying gene expression and function, regulation of the cell cycle and cell differentiation. Other institutions are also studying genomes from various species:

- The human genome at the Institute of Molecular Genetics
- The mouse and rat genome at the Institute of Molecular Genetics, the Biophysical Institute AS CR and in the Science Faculty of Charles University in Praha
- Pig and other farm animal genomes are studied at the Institute of Animal Physiology and Genetics in Uhřetěves, the University of Veterinary and

Pharmaceutical Sciences Brno, the University of South Bohemia in České Budějovice and the Science Faculty of Masaryk University in Brno

- Wild game genome is studied at the Institute of Animal Physiology and Genetics AS CR in Uhřetěves
- Insect genomes at the Institute of Entomology AS CR in České Budějovice and the Institute of Experimental Botany AS CR in Praha
- Parasitic organism genomes at the Institute of Parasitology AS CR (where vaccines and antibodies for diagnosis and treatment of parasitic diseases are also prepared)
- Yeast and microbial genomes at the Institute of Microbiology AS CR in Praha

Three institutes of the AS CR are studying the structure and function of the nucleus and interaction of the nucleic acids and proteins: the Institute of Biophysics in Brno, and the Institute of Experimental Medicine and the Institute of Organic Chemistry and Biochemistry in Prague.

The Proteome Centre consists of the laboratories of the Military Academy of JE Purkyně, the Medical Faculty of Charles University in Hradec Králové, and the Masaryk Memorial Cancer Institute in Brno. The center studies mechanisms of intracellular parasitism of bacteria. The Biomolecular Centre, which includes laboratories at the Science Faculty of Masaryk University, the Veterinary Research Institute and the Institute of Biophysics AS CR in Brno, are studying molecular microbiology and NMR spectroscopy of nucleic acids. The Centre of Structure and Dynamics of Complex Molecular Systems and Biomolecules, consisting of laboratories at the Institute of Physical Chemistry of J. Heyrovský, Institute of Physics, Institute of Organic Chemistry and Biochemistry and Institute of Chemical Processes AS CR in Praha, are studying modeling and interactions of DNA and peptides.

The Institute of Molecular Genetics and the Institute of Organic Chemistry and Biochemistry AS CR is analyzing retroviral structure. Plant viruses and viroids are studied in the Institute of experimental Botany and the Institute of Molecular Biology of Plants AS CR, and their proteases in the Institute of Entomology. The excellent laboratory of A. Holý in the Institute of Organic Chemistry and Biochemistry synthesizes potent antiviral compounds derived from nucleotides and nucleosides. Three of the compounds are produced by the U.S. pharmaceutical company Gilead Sciences for treatment of AIDS (tenofovir, Viread), cytomegalic retinitis (cidofovir, Vistide) and hepatitis B (adefovir, Hepsera) and are commercially very successful.

Several institutes at the AS CR are studying molecular biology and genetic modifications of plants:

- The Institute of Entomology, Institute of Biophysics and Institute of Experimental Botany study agricultural plants (potato, tobacco and flax)
- Hop and ornamental flowers are studied in the Institute of Molecular Biology of Plants
- The genetics of resistance to various diseases in the plum is studied in the Research Institute of Crop Production
- Molecular mechanisms and biotechnology of photosynthesis are studied in the Centre of Mechanism, Ecophysiology and Biotechnology of Photosynthesis consisting of the laboratories of the University of South Bohemia, the Institute of Microbiology, the Institute of Molecular Biology of Plants and the Institute of Landscape Ecology

Immunology

Traditionally, molecular immunology and immunogenetics have high standards in the Czech Republic. The Centre of Molecular and Cell Biology, including laboratories at the Institute of Molecular Biology AS CR and the Science Faculty of Charles University, is studying antigen receptors and mechanisms of antigen presentation in dendrite cells. The Institute of Microbiology AS CR is assessing mechanisms activating cellular immunity, and researchers at the University of Veterinary and Pharmaceutical Sciences Brno are looking at the immune system of the horse. The mechanisms of transplant rejection are studied in the Institute of Molecular Biology AS CR, the Institute for Clinical and Experimental Medicine in Praha and the Masaryk Memorial Cancer Institute in Brno.

Molecular and cellular biology

The molecular biology of oogenesis, spermatogenesis, fertilization and early embryogenesis is under investigation at the Institute of Animal Physiology and Genetics and Institute of Molecular Biology AS CR. Research in molecular and cellular fundamentals of major diseases, and the search for new treatments, is under way at a number of laboratories – the majority of the effort is devoted to studies in cancer therapy. The joint effort of the laboratories of the Institute of Experimental Botanic and the Medical Faculty of Palacký University in Olomouc has led to the isolation from plants of a new group of cytokine derivatives that regulate the cell cycle. The first of the new compounds, olomoucine, emerged as a potent inhibitor of cyclin-dependent kinases and started a new generation of the anticancer drugs. Further compounds are in development, including roskovitin, bohemine and purvalanol, which are even more potent and specific. An attractive new approach - that of targeting drugs to cancer cells while leaving normal tissue unharmed – has been developed as part of a collaboration between laboratories in the Institute of Microbiology and the Institute of Macromolecular Chemistry AS CR. Researchers have conjugated cytostatic drugs and targeting molecules (eg monoclonal antibodies against tumor surface antigens) to water-soluble polymeric carriers. The macromolecular chemotherapeutics release the active drug only after binding to the target tissue and show considerably reduced side effects. Gene therapy of tumors and DNA vaccines are in development at the laboratories of the Institute of Haematology and Blood Transfusion, the Medical Faculty of Charles University, the Institute of Molecular Genetics and the Institute of Organic Chemistry and Biochemistry AS CR in Praha. Tumor therapy through activation of the immune system (NK killer cells) is under assessment at the Institute of Microbiology AS CR. The Medical Faculty of Masaryk University and the Masaryk Memorial Cancer Institute in Brno are also engaged in experimental oncology.

The Centre of Experimental Research on Cardiovascular Diseases is composed of selected laboratories at the Institute of Physiology AS CR, the Institute of Clinical and Experimental Medicine and the 3rd Medical Faculty of Charles University. The Centre is engaged in studying the molecular mechanisms of atherosclerosis and hypertension. The Institute of Physiology AS CR, the Institute of Experimental Medicine AS CR and the Medical Faculty of Charles University, are studying neurodegenerative disease, and genetic methods for potential treatment of obesity are in development at the Institute of Physiology. Treatment of inherited mitochondrial diseases is under assessment at the Institute of Physiology AS CR and the 2nd Medical Faculty of Charles University in Praha.

Preparation and modification of stem cells for replacement of the damaged cells in the central nervous system, heart and other organs is studied in the Centre of Cell Therapy and Tissue Replacement which includes laboratories at the 2nd Medical Faculty of Charles University, Institute of Clinical and Experimental Medicine and three institutes of AS CR: the Institute of Experimental Medicine, the Institute of Animal Physiology and the Genetics and Institute of Macromolecular Chemistry. The Centre's work has two main aims: to prepare human stem cell lines and to develop biocompatible polymers suitable as carriers for these cells. The center has recently claimed its first significant success: preparation of the first cell line of human stem cells. The center has number of collaborators, including the Mendel University of Agriculture and Forestry Brno, the Faculty Hospital Motol and others.

Fundamentals of modern biotechnology are being assessed at the Centre of Molecular and Gene Biotechnology, which includes laboratories at the Institute of Microbiology, Institute of Molecular Biology, Institute of Chemical Technology and five biotech and pharmaceutical enterprises: Biopharm, Envisan-Gem, Galena, Immunotech and Vidia. These groups are engaged in the biosynthesis and biotransformation of bioactive compounds, development of new recombinant proteins with immunosuppressive effects for therapeutic use and monoclonal antibodies for diagnostics. The researchers are also developing biotechnology protocols for the production of transgenic animals and for biodegradation of contaminated soil and sediments using a wide range of organisms.

The Institute of Macromolecular Chemistry AS CR and the Institute of Chemical Technology Praha are developing and studying biodegradable polymers and polymers for medical applications. This discipline has traditionally high standards in the Czech Republic. The focus is on the development of skin replacements and synthesis of macromolecular carriers for targeted applications and controlled release of active substances. Macromolecular membranes can also be used for encapsulation of transplanted nerve cells in order to protect them from the immune response of the host organism – this field is also being studied at the Centre of Cell Therapy and Tissue Replacement.

Czech pharmaceutical research is concentrated at the Research Centre of Structure and Mechanisms of Action of Potential Drugs, which includes laboratories at the Pharmaceutical Faculty of Charles University in Hradec Králové and two enterprises: Zentiva and Generi Biotech. The work at the Centre focuses on anti-inflammatory drugs, chiral compounds, gene therapy, and gerontology therapeutics isolated from plants. Development of a vaccine against boreliosis is underway at Bioveta (Ivanovice Na Hané) and new drug dosage forms are in progress in the Galenic Lab (Ostrava). Chondroprotectives and alimentary supplements based on collagen hydrolysis for protection and regeneration of the locomotor system are in development at Orling (Ústí nad Orlicí).

Production

Biotechnology enterprises

The most common items produced by Czech biotech companies are monoclonal antibodies and antibody-based diagnostic kits. The biggest producer of immunoanalytic tools is **Immunotech** (Praha). It offers immunodiagnostic reagents and kits for oncology, diabetology, cardiology, rheumatology, clinical and experimental immunology, haematology and veterinary medicine. It also has its own research department. **BioVendor** (Brno) produces monoclonal and polyclonal antibodies and RIA and ELISA diagnostic kits. **Clonestar** (Brno) produces monoclonal and polyclonal antibodies and offers custom synthesis of various peptides. **Exbio Olomouc** produces and distributes immunologic diagnostic tools for haematology and haemocoagulatory analyses. **Exbio Praha** produces monoclonal antibodies and immunologic kits. **Hena** (Miličín) produces chicken antibodies.

The Czech company **Vidia**, located at Praha suburb Jesenice, produces diagnostic kits for detection of herpes viruses, auto-antibodies and occult bleeding. Besides, they offer determination of full spectrum of viruses using molecular biology tools. **Itest plus** (Hradec Králové) focuses on production of in vitro diagnostic tools for microbiology labs. Its products are aimed primarily at serological identification, and exploitation of enzymatic properties of microorganisms. Key products include diagnostic discs and preparations for medical mycology. **Test-Line** (Brno) is engaged in development, production and distribution of laboratory diagnostics for human and veterinary medicine including immunoenzymatic ELISA kits, Western blots, antigens, and bacterial diagnostics. **O.K. Servis BioPro** (Praha) is established on the market for laboratory instruments, food ingredients and laboratory diagnostics. It offers immunological reagents and antibodies, kits and reagents for serological tests, monoclonal antibodies for veterinary applications, PCR kits for determining pathogens, and mass screening tests for BSE and scrapie-prionic check.

Geneage Technologies (Praha) produce recombinant proteins. They offer DNA chips and sequencing, and other services for molecular biology and genomics. **Generi Biotech** (Hradec Králové) offers a molecular genetic service, produces biotechnology components for molecular biology (oligonucleotide synthesis), and develops diagnostic tools based on molecular biology. Its research department focuses towards potential gene therapeutics. **Top-Bio** (Praha) specializes in production of high quality reagents for polymerase chain reaction (PCR), RNA isolation from cells and tissues and reverse transcription into cDNA. **Biopharm** (formerly the Research Institute of Biopharmacy and Veterinary Therapeutics) located in the Praha suburb Jílové offers synthesis of antigens and peptides and preparation of transgenic animals and pluripotent stem cells. The company also produces a vaccine against coccidiosis and conducts its own research in the area of veterinary therapeutics and herbicides for plant protection. **Genomac International** (Praha) offers screening of DNA mutations, sequencing and paternity tests. Its own research is oriented towards prevention and diagnosis of tumours of the large intestine, prostate and pancreas. **Lonza Biotec** (Kouřim) is part of the international company Lonza Group and is the largest biotechnology enterprise in the Czech Republic. It produces L-carnitin for human and veterinary use. Recently founded company **Apronex** (Praha) manufactures biologically active proteins by biotech methods. One of its products is recombinant protein TRIAL regulating apoptosis. Company **AppGenics** constructs transgenic animals producing various compounds for use in diagnostics and therapy of diseases.

Several biotechnology companies in the Czech Republic offer services in environmental protection. **Envisan-Gem** (České Budějovice) specializes in waste management, decontamination and use of specialized applied microbiology. Its microbial preparation GEM100 breaks down petroleum hydrocarbons, phenols and other organic compounds to carbon dioxide and water. **Aquatest** (Praha) is engaged in monitoring and evaluation of the environment and carries out sanitation and re-cultivation of dumps and devastated land. The company also offers water budget studies and tests potable and mineral water.

Dekonta (Praha) specializes in biodegradation of waste and bioremediation of polluted soil. At present, the company processes large amounts of waste and deals with hundreds of ecological contracts and projects, and has an annual turnover of about 200 million CZK. **G-servis** (Praha) provides consultations and services for waste removal. The company uses its in-house technology, Bio-servis, to dispose of petroleum products and polycyclic aromatic hydrocarbons in a wide spectrum of contaminated materials. The company applies biological sanitation technology to matrices of various type (soil, solid waste, waste water etc.) using either *in situ* or *ex situ* biodegradation process according to the actual needs.

Earth Tech CZ (formerly KAP, Praha) specializes in ecological services in the area of environmental protection. The company uses modern technologies such as biodegradation, venting, steaming, airsparging, stripping and solidification to remove petroleum products, polyaromatic hydrocarbons, polychlorinated biphenyls, chlorinated hydrocarbons, heavy metals and other dangerous substances. **MEGA** (Praha) offers biotechnological decontamination of water wastes using aerobic and anaerobic bacterial cultures. The company uses technology of immobilised cells and enzymes “LentiKats”.

Pharmaceutical firms

There are about 10 large and 15 smaller pharmaceutical companies in the Czech Republic. Among the biggest pharmaceutical producers belong Farmak, Ivax Pharmaceuticals (formerly Galena), Zentiva and Spofa, which mostly produce active pharmaceutical ingredients (API) and generics. Some of the generics are version of in-house drugs with expired patent protection. Some of these large producers have much fermentation experience, and have large capacity fermenters.

Zentiva (Praha-Měcholupy) has the leading position on the Czech drug market. Its majority owner is the US investment fund Warburg Pincus. A is typical generic company, Zentiva produces about 130 various drugs to treat diseases of the digestive tract, blood, heart, central nervous system and respiratory system. Furthermore, it also produces analgesics, anti-rheumatics, hormones, antibiotics, muscle relaxants and preparations for dermatology. Daughter company of Zentiva Group, **VÚFB**, is responsible for the research activities, e.g. research in new anti-inflammatory drugs aimed at inhibiting the biosynthesis of arachidonic acid and its metabolites etc.

Ivax Pharmaceuticals (Opava) is the second largest pharmaceutical company in the Czech Republic. It produces a number of substances by extraction from natural sources as medicinal herbs and ergot (ergot and morphine alkaloids) or by fermentation of fungi. It also manufactures therapeutic Cedetrin based on monoclonal antibody. It also produces generic version of its own in-house drugs where the patent protection has expired, including Anacid, Novo-Passit and Sanorin. **Spofa** (Praha) produces about 20 different preparations, including hormones, neuroleptics, spasmolytics, anaesthetics and drugs for vasodilatation and fibrinolysis. The company

also extracts active compounds for drug production from animal tissues (e.g. Insulin, Aprotinin, Heparin, and Foliculotropin). **Farmak** (Olomouc) produces pharmaceutical ingredients and a number of generic drugs for treatment of hypertension, depression, psychosis and sleep disorders. Farmak also manufactures sedatives, anti-coagulating agents and drugs for vasodilatation.

Pliva - Lachema (Brno) produces diagnostics, cytostatics, and drugs for treatment of central nervous system and for supportive therapy. **Bioveta** (Ivanovice na Hané) makes a wide selection of veterinary drugs (antibiotics, chemotherapeutics, vaccines, vitamins, diagnostics and hormones - altogether about 200 different drugs), and is also developing a vaccine against Borreliosis for human use. **Radiopharmaceuticals Division of the Nuclear Research Institute Řež** manufactures number of radioactive drugs with annual turnover around 80 mil. CZK. One of their products, ¹⁸F-fluorodeoxyglucose is used for PET. **Sevapharma** (formerly ÚSOL Praha) produces immunological diagnostics (viral, bacterial, immunochemical) and allergens for diagnostic and hypo-sensitization. Sevapharma also manufactures vaccines and human transfer factor (Immodin) for normalization of damaged cellular immunity. **Infusia** (Hořátev) makes infusion solutions.

The recently opened Czech branch of the international pharmaceutical company **Baxter** manufactures new recombinant vaccine against influenza, immunoglobulins and other biopharmaceuticals for blood coagulation, haemodialysis, vaccination and oncology at its plant located in Bohumile (formerly SEVAC). Ferring has opened new branch, **Ferring-Léčiva**, in the Praha suburb Jesenice. Here the company develops and produces peptide preparations analogous to natural hormones (e.g. Adiuretin and Minirin for treatment of primary nocturnal enuresis, Oxytocin etc.). **Medicom International** (Brno) produces antidepressives, hormones (synthetic calcitonin and antiandrogens), expectorants, cytostatics and anti-inflammatory, antirheumatic and antimycotic drugs.

The Contipro group (Ústí nad Orlicí) comprises the companies **Contipro C**, **CPN** and **Bioconti**. **Contipro C** produces pharmaceutical substances, drugs and medical devices. Another member of the group, **CPN**, is engaged in research and development of pharmaceuticals products. **CPN** main product is biotechnologically manufactured hyaluronic acid which is distributed to leading pharmaceutical companies. **Orling** (Ústí nad Orlicí) produces chondroprotectives and food supplements based on collagen hydrolysate, which are used for protection and regeneration of locomotor system of humans and animals.

Among the smaller firms belong **Ardeapharma** (Ševětín) produces solutions for infusion and **Medites Pharma** (Rožnov pod Radhoštěm) produces concentrates for hemodialysis, cytostatics, antiviral drugs, antidotes and drugs for treatment of cardiovascular system. **Dyntec** (Terezín) produces vaccines for human and veterinary use. **Noventis** (formerly Intercaps, Zlín) makes soft and hard gelatinous capsules and dosage forms for vitamins E, D₂, and D₃, as well as producing Mono Mack (isosorbide-5-mononitrate) for the treatment of heart ischemia. **SVUS Pharma** (Hradec Králové) makes diclofenac, tramadol, captopril, vitamin E and metformin. Several small companies produce vitamins, beta-carotene, unsaturated fatty acids, dextromethorphan, retinoic acid, artificial sweeteners (**Urseta** - Doksy u Kladna and **Vítar** - Zlín), expectorants, mucolytics (**Biomedica** - Praha) and natural herbal preparations (**Areko** - Praha, **Bylinář** – Pardubice and **Dr. Popov** - Plzeň). **Uniregen** (Náchod) makes food supplement imuregen, regenerative cream and ocular gel.

Preclinical and clinical tests

In the Czech Republic, several firms conduct clinical testing of drugs. Company **I.Q.A.** (Praha) was founded in the year 2000 as a spin-off from the Research Institute of Pharmacy and Biochemistry. The company is engaged in the preclinical and clinical testing of drugs. Its employees have experience with testing of various drugs such as nifedipine, mexiletine, labetalol, pentoxifyline, cefazolin, cephalotin, and nitroglycerin. **Zak-Pharma Services** (Brno), **Cepha** (Praha), **Clinst** (Praha) and **Pharm Test** (Hradec Králové) also do all phases of clinical testing. The Czech branch of the international pharmaceutical company **AstraZeneca** is also engaged in clinical testing of drugs. **Biotest** (Pardubice) specializes in toxicological tests and **Biopharm** (Jílové) is engaged in toxicology and testing of veterinary drugs.

Conclusions

Biotechnology and pharmaceutical research in the Czech Republic is conducted to reasonable standards. Some laboratories have achievements comparable to leading European laboratories, with the most competitive results in immunology, rheumatology and medicinal chemistry. The latter research has led to the development of acyclic nucleotide derivatives, which are potent antiviral drugs, used for treatment of AIDS and hepatitis B. Phytohormone derivatives with inhibitory effects on cyclin-dependent kinases are in development for the treatment of cancer and are very promising. The research in macromolecular polymers and hydrogels led to the development of soft contact lenses and now continues in other successful applications eg targeted macromolecular chemotherapeutics etc. Quality research is underway also in neurosciences, oncology, diabetology and metabolic diseases. However, the major weakness in Czech research is getting the results to the stage where the international pharmaceutical and biotechnology industry becomes interested. The number of registered patents is very low, possibly because the motivation of researchers to apply for them is small. In the field of biomedicine, there were only eight patents and 23 certified technologies registered in the period between 1998 and 2002.

Czech pharmaceutical companies have little interest in developing new drugs, and are oriented towards generics. Thanks to the interest of international pharmaceutical companies, some of the new drugs discovered and developed in Czech laboratories have finally found the way to the market, and others have been patented and are in clinical testing. However, several other new and promising compounds are sitting in drawers and their developers have given up efforts for their commercialization or even have never tried it.

With few exceptions, biotechnology companies in the Czech Republic are rather small. Their most common products are monoclonal antibodies and immunologic diagnostic kits based on their use. A few small companies also produce vaccines and a few others are involved in biodegradation. Biotechnology has longer business cycles and is higher risk compared with traditional areas of the healthcare industry, and therefore these companies suffer from insufficient capitalization. The first hopeful sign of a change is the entry of venture capital into IQA.

The way to faster progress in biotechnology could be the formation of biotechnology parks and clusters, which provide a route for research laboratories to collaborate with pharmaceutical and biotechnology companies. The beginning of such a cluster emerged in Praha – Krč in 1994, where the campus of biological institutes of the AS CR included an incubator of high-tech companies. About 20 small companies went through it, including a number of biotechnology-oriented companies. However,

the incubator suffered from insufficient space and financing, and had to close in 2002. The first biotechnology cluster in the Czech Republic is being built in South Moravian Innovative Centre in Brno. The Centre was founded in 2002 by four corporate bodies: South Moravian Region, Technical University Brno, the Masaryk-University, and the statutory town Brno. In 2005 two new members were accepted: University of Veterinary and Pharmaceutical Sciences Brno, and Mendel University of Agriculture and Forestry Brno. The cluster will focus mainly on biotech companies engaged in environmental protection, decontamination of waste water and soil etc. Smaller biotechnology parks are starting, in Olomouc at the campus of the Institute of Experimental Botanic AS CR, and in Nové Hradky at the campus of the University of South Bohemia. However, unless there is an increase in interest from pharmaceutical companies in introducing these research results into their program, and in motivation of the researchers to move their compounds to a stage where they are commercially interesting, this situation will not improve.