Biological Research in Czechoslovakia

Prague. Scientists in Czechoslovakia have been eagerly expanding their contacts with colleagues in the West. The effort, marked among biologists, has been made in both directions, by invitations to large numbers of Westerners to attend conferences in Czechoslovakia and by travel abroad by Czech scientists, both for brief meetings and for longer periods of work in Western laboratories.

The policy of widening contacts benefits from a generally friendly attitude toward science in the Communist government of President Antonin Novotny. The Communist regime has enlarged the universities since it took power in 1948 and built a number of large new laboratories. It created the Czechoslovak Academy of Sciences in 1952 and allowed the academy and its Slovak offshoot to expand from a staff of 1000 a decade ago to about 110 institutes and 10,600 employees by the end of 1963.

The staff, in fact, almost equals the combined total of similar agencies in Great Britain, France, and Germany. But the Czech academy maintains institutes in more different fields than the Western agencies do: about a quarter of the academy units study aspects of literature, history, and politics. Other units consider problems of industrial or medical technology.

Some scientists feel that the staffs have swelled to the point of interfering with good work and that the most pressing issue now is to sort out the many institutes which are scattered in odd, crowded corners all over Prague. The government, too, apparently is distressed by the great growth of staff and has given notice of a tightening of budgets. Construction of even the first stage of a complex of biological laboratories in the suburb of Krč has been slowed. A second phase has been postponed for several years. Academician Otto Wichterle and his colleagues in the institute of macromolecular chemistry were able to enjoy their new building for only a short time before they received word that sections of other institutes would require space.

A major factor in government support of the Czechoslovak and Slovak Academies of Science has been that such scientists as František Šorm and Ivan Málek are active Communists. Both of these important members of the Czechoslovak Academy are in the biological sciences, and this fact, coupled with the discouragingly higher cost of research in fields like nuclear physics, has given biological science a strong place in the academies' budgets.

Šorm is the president of the Czechoslovak Academy and head of its institute of organic chemistry and biochemistry, where Josef Rudinger directs the laboratory of synthetic peptides and, until he recently joined UNESCO, Bořivoj Keil worked on the amino acid sequence of chymotrypsin.

Until 1960, Málek headed an encompassing institute of biology. In that year, the institute was split into smaller, more manageable institutes, and Málek became head of the institute of microbiology. Málek also is one of the four vice presidents of the Czechoslovak academy, a member of the Communist Party central committee, and a delegate in parliament.

Improving communications with Western scientists required active cooperation from the government. Allocations of scarce foreign exchange were needed to purchase the substantial quantities of western equipment to be found in Czech laboratories. Foreign exchange was needed also for foreign travel, especially since the government has sometimes forbidden scientists to accept Western fellowships.

The number of Czech scientists traveling to the West is large. For example, the printed list of 1800 delegates (275 of them Americans) to the 12th International Entomology Congress in London 8–16 July carried the names of 35 Czechs, of whom 22 gave papers. Although only 8 Czechs attended the first meeting of the Federation of European Biochemical Societies in London 23–25 March, about 40 attended the 6th International Congress of Biochemistry in New York 26 July–1 August. Even a relatively small meeting, such as the second colloquium of the European Group of Cancer Virologists at the abbey of Royaumont near Paris 25–28 June, attracted Czech scientists. At Royaumont, where only 58 authors were listed on the program, no less than 5 were Czechs, including three from the institute of experimental biology and genetics.

A technique for attracting scientists to Czechoslovakia is to hold symposiums in fields of interest to one or two institutes of the academy of sciences. The academy's own publishing house has issued full English texts of a number of these—for example a meeting on macromolecular chemistry and another on peptide synthesis in 1958 and meetings on mechanisms of antibody formation in 1959, membrane transport in 1960, and paper chromatography, durability of concrete, general topology, and mechanisms of immunological tolerance in 1961.

The 1959 meeting on antibody formation was so successful that it was repeated this year, 1–5 June. A full English text is to be issued. At the meeting there were 27 Czech and 26 American participants. Some of the participants in the 1959 meeting, including F. M. Burnet of Australia, Pierre Grabar of France, J. H. Humphrey of the United Kingdom, and G. J. Thorbecke and J. W. Uhr of the United States, returned for the 1964 meeting. Among the new participants, G. M. Edelman of the Rockefeller Institute discussed the formation of 7*S* antibody molecules by reassociation of light and

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New biological laboratories at Krč.

heavy polypeptide chains and S. J. Singer of the University of California at San Diego described recent studies of antibody combining sites by affinity labeling. Notable among the Czech contributors was Jaroslav Šterzl, head of the immunology branch of the microbiology institute and student of the inductive phase of antibody formation.

The same eagerness to expand Western contacts lay behind the successful attempt to secure a Pugwash meeting for the resort town of Karlovy Vary in September. Also involved in the drive for contacts is the institute of virology in Bratislava, headed by Dionýz Blaškovič, president of the Slovak Academy of Sciences. This institute has issued the complete series of its journal, *Acta Virologica*, in Russian and English since the journal was started in 1957. The institute has scheduled a symposium on nonspecific resistance to viruses for September. About 15 Americans are scheduled to attend, along with such European virologists as Andre Lwoff of the Institut Pasteur in Paris.

Western biologists could see this contact-building process at work recently during the 15th general assembly of the International Union of Biological Sciences in Prague 17–22 July and at the same time get a good look at the status of biology in Czechoslovakia.

In welcoming their guests, the Czech biologists stressed some of their large projects in applied biology. The delegates were taken on a tour of southern Bohemia, where they were shown a station where algae are cultivated experimentally in open-air pools and also under artificial light. The station is a branch of Málek's microbiology institute. The delegates were also shown a number of intensively cultivated fish ponds.

On the final two days of the IUBS assembly there were symposiums on recent advances in fundamental and applied biology, organized by C. H. Waddington and Málek. In discussing cultivation of algae, Málek said that the economics are not good at the moment, but that the potential yield of protein for animal fodder or direct human consumption is great enough to warrant considerable research effort: production of algae and other economically important microorganisms comes much closer to true industrialization of biological production than anything achieved with hand-held agricultural tools or mechanical cultivation and harvesting of higher plants and animals.

Jaroslav Hrbáček of the Czechoslovak academy's hydrobiological laboratory described experiments in raising fish in ponds and reservoirs. Hrbáček said that despite a tenfold increase in yields of carp over the past 30 years, achieved by intensive fertilization of ponds with lime and superphosphate, the yield had reached only 800 kilograms per hectare, against 2200 kilograms in some heavily cultivated tropical ponds. The yields were far below the potential of an alga farm. Hrbáček proposed that intensive feeding could double the central European fish yields and triple the tropical ones. He noted that artificial fertilizers brought into the ponds by runoff from fields increased the catch, as did cultivating ducks on some ponds.

In the symposium on fundamental biology, Milan Hašek described the work of the institute of experimental biology and genetics, which he heads. The institute studies immunological tolerance and autoimmunity. More than a decade ago, Hašek began to experiment with inducing tolerance by joining the eggs (embryos) of two Leghorn chickens, by analogy with P. B. Medawar's experiments with cattle twins. The fusion of these eggs produces a long-lived tolerance in each chicken for the cells of the other.

Often the tolerance leads to chimerism, that is, multiplication of the donor's cells in the recipient chicken along with the recipient's own cells. Thus the donor cells serve as a continuously renewed pool of antigen, apparently required for maintaining tolerance. In about 80 percent of the cases, the two chickens, as adults, tolerate skin grafts and do not form agglutinins after injection of erythrocytes labeled with chromium-51. To test a system with more genetic distance, however, Hašek has turned to fusions of 15-day-old embryos of Peking duck (*Anas platyrhincos*) and 17-day-old embryos of Muscovy duck (*Cairina moschata*). Tolerance is induced in about 25 percent of the cases.

With colleagues, including A. Puza (University of Kosice), M. Hašková, and J. Hort, Hašek also induced tolerance in adults by complete replacement of the recipient's blood with transfusions of blood previously subjected to 20,000 roentgens. They induced tolerance in newly hatched birds with repeated twice-weekly doses of blood, each dose amounting to 0.2 percent of the recipient's body weight.

These induced tolerances were shut off by stopping the supply of antigen, external in experiments with transfusions, or internal in the "parabiont" animals where chimerism had been induced. Hašek's group also experimented with reinducing tolerance after abolishing it. This process was easier in 2-year-old animals than in 3-month-old animals.

Hašek feels that these results fit generally with the tentative definitions of immunological tolerance set forth by Medawar at the 1961 immunology meeting in Czechoslovakia: "Tolerance is the consequence of a central failure of the mechanism of response; tolerance is essential nonreactivity. Tolerance is immunologically specific. . . . It

is antigen that induces tolerance; the stimulus that induces tolerance in the young animal is the same as that which would excite immunity in an older animal. . . . The inception of tolerance depends upon some quantitative or qualitative peculiarity of young animals."

Other scientists in Hašek's institute are working on immunity against and tolerance of tumors induced by Rous sarcoma virus. Jan Svoboda, head of the cytology laboratory of the institute, studies the interaction of Rous sarcoma virus with mammalian cells in vitro and in vivo. V. Klement examines the production of infectious Rous virus in hamster tumors induced by the virus. P. Koldovský experiments with immunity against tumors induced by the virus in mice. Earlier, Koldovský and Svoboda succeeded in rendering mice (strain A) tolerant to a benzopyrene-induced tumor by injecting irradiated and repeatedly frozen and thawed tumor cells into newborn mice. The mice could not be immunized against the tumors as adults, and all of them died of tumors after they had been injected with normal tumor cells. A group of control animals which had not been treated against tumors also died. Surviving was a group of mice left untreated until adulthood and then immunized with doses of killed tumor cells at 10 and 13 weeks. A later experiment showed that attempting immunization with higher doses of killed tumor cells had the opposite effect: the mice's resistance was broken in a kind of "immunological enhancement," and they died faster than the controls.

Recently, Koldovský has sought to increase the susceptibility of tumors to radiation therapy by a treatment beforehand with antiserums to tumor-specific antigens.

The genetics laboratory of the institute is directed by Alena Lengerová, who graduated from Karlova University in Prague in 1948 and joined the radiobiological laboratory of the old biology institute in 1950. She has been much interested in tolerance between different grafts introduced into mice which have received lethal doses (1000 rads or more) of radiation. The radiation suppresses the immune response.

In 1961, Lengerová visited the British Medical Research Council's radiobiological unit at Harwell to learn how to use the small altered mouse chromosome T6 as a marker in immunological experiments. This chromosome is found in all cells of a particular mouse strain produced by irradiation, and M. H. Lyon of Harwell has developed a strain containing two of them. The marker chromosomes can be used to determine whether a particular kind of cell graft is being rejected.

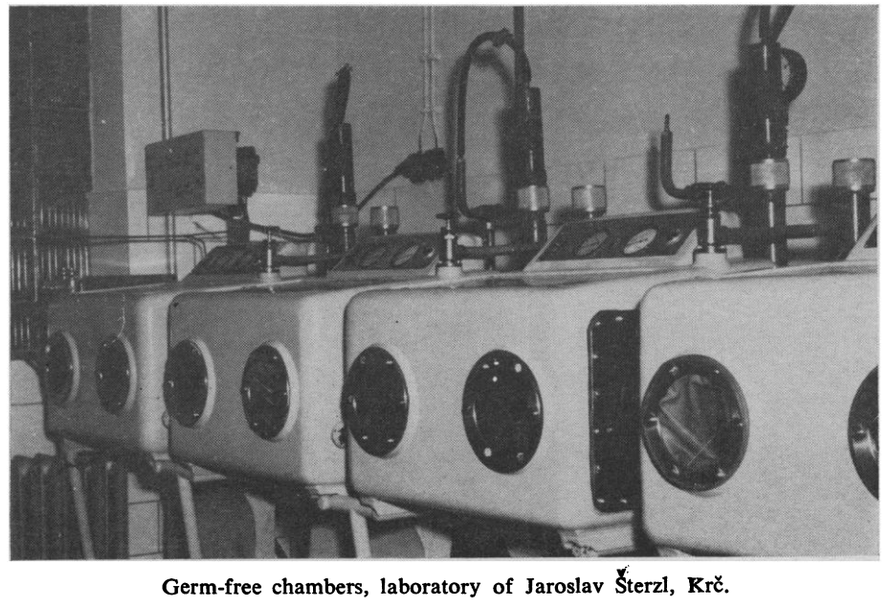
One example of Lengerová's use of this system is an experiment performed with Constance M. Clarke and H. S. Micklem of Harwell. Lethally irradiated mice received sensitizing injections of either bone marrow or fetal liver cells. Then the mice of each group were challenged with foreign fetal liver cells. The mice which had received bone marrow rejected the second graft, and those which had received fetal liver cells did not.

More recently, Lengerová has been studying the induction of tolerance to skin grafts in F2 hybrids of the A and CBA strains of laboratory mice. She reported to the 11th International Genetics Congress in The Hague in 1963 that preliminary experiments with this system, in which the number of antigens derived from the original inbred A and CBA strains would be widely different, showed a correlation between tolerance and coat color.

One of Lengerová's colleagues, Marta Vojtíšková, has been studying the cessation of spermatogenesis in guinea pigs caused by injections of testicular material. Vojtíšková and others found that guinea pigs could be protected against this effect of autoimmune castration by injection of a testicular extract soon after birth followed by other injections at 14-day intervals.

Another laboratory at Krč visited by scientists at the IUBS meeting was Jaroslav Šterzl's immunology section of the microbiology institute. Among his co-workers are Miroslav Holub, who cultures and studies lymphoid tissue types and serves as an editor of the academy's popular science magazine, *Vesmír* (Cosmos); František Franěk, whose chemical work on the isolation and characterization of gamma globulins in piglets points to the same need for combining light and heavy fragments for activity as that found by Singer and Edelman; I. Říha, who studies the time of appearance of 19*S* and 7*S* antibodies after immunization; and Ivo Miler, who concentrates on the influence of intestinal flora in the development of immune reactions in normal and germ-free animals.

To study induction of antibody formation, Šterzl uses lymph node and spleen cells isolated from newborn animals (most recently piglets reared in germ-free chambers and fed a mixture of amino acids and vitamins). Antibody action is exhibited on plaques according to a method worked out by N. K. Jerne. The experiments have led him to conclude that the idea of a distinct "inductive phase," during which immunologically competent cells differentiate into antibody-producing cells, has been confirmed. "The results show explicitly," Šterzl said during the recent symposium on antibody formation, "that antibody formation does not start spontaneously during individual development if the individual is protected from antigenic stimuli."



Germ-free chambers, laboratory of Jaroslav Šterzl, Krč.

In north Prague is another unit of the microbiology institute, the laboratory of cellular metabolism under the direction of Arnošt Kleinzeller.

During a visit by K. V. Thimann of Harvard University and Maurice Chevremont of the University of Liége, Arnošt Kotyk of Kleinzeller's laboratory described recent experiments with a red lipid-forming organism, the yeast *Rhodotorula gracilis*. This organism absorbs large concentrations of sugars which it either does not metabolize at all or uses only a small portion of. This can result in an internal concentration of D-xylose nearly 1000 times as great as that outside the yeast cell after 8 hours. Even under anaerobic conditions the internal concentration can still reach 300 times the outside concentration. Other sugars examined in this system so far have been D-arabinose, L-rhamnose, and D-glucose. The last sugar, which the yeast uses, reaches only a concentration 1.8 times that outside under aerobic conditions, and it is not absorbed at all under anaerobic conditions.

Kotyk spent 1947–50 in the United States, first in a high school in North Carolina and then at the University of California at Berkeley. Returning to Karlova University, he received the equivalent of a Ph.D. in 1957 and began work on transport across cell membranes in Kleinzeller's laboratory. In 1962–63 he worked in the laboratory of W. Wilbrandt in Bern on experiments which showed that two sugar molecules are bound to each molecule of the protein or lipoprotein "carrier" that is postulated by workers in membrane transport. Kotyk noted that if each carrier has two "binding sites," then a model of its action and structure must be complicated. Wilbrandt was working on human erythrocytes, which establish their final concentration of sugars in about 2 hours.

One of Kotyk's current interests is the inhibition of sugar transport in erythrocytes by such compounds as dinitrophenol, sodium iodoacetate, oligomycin, and phloridzin. In the presence of DNP and iodoacetate, erythrocytes having very large concentrations of D-xylose lose the D-xylose and come below or to equilibrium with the outside level.

The 1962 thesis topic of Jiřina Bosáčková of the cellular metabolism laboratory was the transport of inorganic ions and *p*-amino hippurate in isolated cells of rabbit kidney cortex. Bosáčková found that the individual cells excreted material as effectively as whole slices of tissue. The finding meant that each individual cell possessed some kind of contractile material, a possibility that has cropped up again in recent work Bosáčková is doing with Kleinzeller on the role of ouabain in inhibiting the excretion of sodium from cells.

When she worked at the Chicago Medical School laboratory of Robert K. Crane in 1962–63, Bosáčková studied the transport and accumulation of 6-deoxyglucose in hamster intestine cells. She observed a maximum concentration about 20 times greater than the outside concentration. Unlike the transport in red yeast, this active movement against a concentration gradient appears to involve sodium and potassium ions. Sodium stimulates and potassium inhibits transport.

Karel Janáček of the same laboratory has recently found that a drop in potential across frog skin, a technique for studying the transport of ions across the skin, takes place in two distinct steps that correspond to two morphologically distinct layers in the skin.

The entomology institute is organized in four departments scattered in eight places (including two converted apartments).

Vladimír Landa, who runs the institute's laboratory of insect development, is interested in chemosterilants. Other researchers like I. Hrdy and P. Masner, in cooperation with K. Sláma František Sehnal and V. Novák, head of the physiology laboratory, study the development of the reproductive organs of insects and the influence of hormones on their development.

Studies of the relationships of insects to sugar beet plants are made in the ecology laboratory of V. Skuhravý. With him are such researchers as K. Novák and J. Rusek. P. Starý assists in the hormone studies. The entomology institute gives much attention to the resistance of useful insects, such as predators (Colorado potato beetle) and parasites (aphids), to insecticides. The aim of such studies is to help determine the best day for administration of organophosphates—that is, the day on which the damage is greatest to the pest and least to the useful insect. In the laboratory of insect pathology under J. Weiser, researchers such as A. Samšiňáková are studying a fungus, *Beauveria bassiana*, which is very effective against the first and second instars of some beetles and might be used against the Colorado potato beetle. Also studied is *Bacillus thuringiensis*, which attacks lepidoptera.

Widely known in the West is the virology institute in Bratislava, headed by Dionyz Blaškovič, which now has 40 scientists and 140 other workers. Although the institute studies model systems of cell–virus relationships of interest to physicians, veterinarians, and plant breeders, the work has moved away from an early emphasis on applied studies and fieldwork. There are five main research groups: (i) Respiratory viruses, led by Ladislav Borecky. This group studies influenza and Newcastle disease viruses. In the work on Newcastle and other myxoviruses, the Borecky group has close contact with the scientists working with Werner Schafer of the Max Planck Institute for Virus Research in Tübingen, Germany. (ii) Neuroinfective viruses, led by Helena Libíková. Such arboviruses as tick-borne encephalitis viruses are this group's major concern. (iii) Veterinary virology, led by Ivan Brauner, a corresponding member of the Czechoslovak Academy of Sciences. (iv) Rickettsia, under Rudolf Brezina. This group has studied the multiplication of *Coxiella burnetii* in susceptible cells and confirmed that there is a stage in its reproductive cycle similar to one in larger viruses. (v) Plant viruses, led by Vlk Valenta. This group has studied many species of leafhopper insects as carriers of a wide range of aster yellow and stolbur viruses. The interference between various strains of these viruses was studied before the Valenta group turned to the basic properties of the viruses and their relationships to their hosts.

Dionýz Blaškovič studied in Prague in the 1930's before moving to the state institute of hygiene of Slovakia as a microbiologist when the Germans occupied Bohemia and Moravia in 1939. In 1946–47 he was a Rockefeller Foundation fellow at the University of Michigan laboratory of Thomas Francis. Blaškovič has concentrated on influenza viruses and recently he has studied cell metabolism during the acquisition of the "so-called antiviral compounds."

Among the other important researchers at the virology institute are biochemist F. Sokol, immunologist B. Styk, and J. Vilček, who studies interferon. They are among the hundreds of Czech scientists who are driving to open new contacts between East and West.—VICTOR K. MCELHENY