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Ivan Málek [1909–1994]: a tribute

CELEBRATING MÁLEK

The year 2009 marked the 100th birthday anniversary of Ivan Málek, academician, renowned and motivating scientist, inspiring scholar, and dedicated humanitarian. This landmark was celebrated by a conference in Prague held under the auspices of the Institute of Microbiology of the Academy of Sciences of the Czech Republic and the Masaryk Institute and which acknowledged the outstanding research achievements, and highlighted the deeply held social conscience and internationalism of this great 20th century man. This editorial tribute is based partly on conference papers and my personal reminiscences of Málek and comments on his legacy. Having met Ivan on a few occasions, knowing several of his collaborators, and being a fellow continuous culture enthusiast, the penning of this tribute came as a signal opportunity and honour.

Ivan Málek 1909–1994 (Copyright 1969 Springer. Reproduced with kind permission from Springer Science + Business Media).

MÁLEK'S SCIENCE

It is appropriate to start with some of the scientific accomplishments that chart Málek's career. The opening 'memories' essay[1] very appropriately by Arny Demain – a friend of Málek for nearly 30 years, and Jarda Spızek, sets the scene. They recall Málek's early interest in medical microbiology and his involvement with the first small scale production and trials of penicillin in Czechoslovakia during German occupation in WWII. Antibiotic production and therapy was a central objective at this time and after getting to know about penicillin production methods at the Connaught Laboratories in Canada Málek established in 1949 the first manufacturing plant in his country near Prague. University teaching also was a major activity and in 1948 he moved to Charles University at Hradec Králové and set up a microbiology group in new School of Medicine, the progenitor, as Martin Bilej.[2] points out, of the Department of Microbiology of the Central Institute of Biology and ultimately the prestigious Institute of Microbiology of the Czechoslovak Academy of Sciences of which Ivan Málek was founder (1962) and first director. During this period Málek had also served as chair of the Czechoslovak Society for Microbiology, director of the CIB department, helped found the journal *Folia Microbiologica*, and, most significantly, developed his innovative work on the continuous culture of microorganisms and cells. In the latter context an event of lasting consequence took place in 1958 namely Málek's convening in Prague of a symposium devoted to continuous cultivation of microorganisms – what was to be the first of a succession seminal meetings in Prague and the United Kingdom over the next 26 years, *''the contributions to which form the bedrock of a substantial part of twentieth century microbiology''*.[3] At this meeting Málek enunciated his concept of the physiological state of microorganisms, while other prominent contributors included Aaron Novick (USA) and Denis Herbert (UK).

The development of Málek's ''Prague School of Continuous Culture'' is charted by Pavel Kyslik and Ales Prokop[4] and in particular they review the industrial applications of the methodology in Czechoslovakia to produce fodder yeast, microalgae, ethanol and beer, and to treat waste water. Their paper also deals with the impact of continuous culture on reactor design and control strategies, mutant selection and the stability of recombinant organisms, and high-density fermentation. Málek surely would have been gratified by the authors' reference to the current renaissance of continuous culture, a few applications of which I highlight later. Other prominent research promoted at the Institute of Microbiology is discussed by Jan Nesvera.[5] He begins by reminding readers that the word 'genetics' was taboo in the Czechoslovakia of the early 1950s; nevertheless under Málek's guidance, programmes of research on pneumococcal transformation, transduction of *Salmonella*, and the genetics of the model mycobacterium *Mycobacterium phlei* were established. Following the ''Velvet Revolution'' molecular biology and microbial genetics flourished at the Institute when significant attention was given to applied microbiology (e.g. streptomycetes/antibiotics, corynebacteria/amino acids), yeast molecular biology, and pathogenic bacteria.

Málek had a keen interest in the development of antibiotics and his influence can be seen today in work by Spizek et al[6] that restates the magnitude and dynamic nature of infectious diseases and reviews the approaches that are available in the search for new compounds. Their emphatic support for natural products is timely and necessary and their message unambiguous: ''*the decision on the part of several pharma companies to get out of the*

natural products business is gross foolishness''. Reference also is made to the development of multistage continuous cultures for investigating antibiotic fermentations, the importance of which Málek had drawn attention to in his 1955 book on the reproduction and cultivation of microorganisms.[7]

Málek actively encouraged research and development into environmental pollution and its control, microalgae and microbial ecology. This legacy is reflected in the work of Paca *et al*.[8] on trickle bed and biofilm reactors designed to treat mixtures of paint solvents and in the development of microalgal biotechnology in Czechoslovakia and the Czech Republic.[9] The microalgae programme clearly demonstrates Ivan Málek's espousal of activities outside his immediate sphere of expertise but where his intellect could help progress the work of others. Málek's encouragement of the plant physiologist Ivan Setlık led to the formation of the Algalogical Laboratory within the CIB and the subsequent blossoming of laboratory and field algal production, notably the cascade cultivation system. The authors point out the changing priorities of algae-based biotechnology from the early focus on biomass to the search for bioactive compounds and more recently the quest for biofuels and carbon sequestration. During my student days at the University of Nottingham and my first academic post, at Bedford College (University of London), I was exposed to many aspects of soil microbiology. The paper by Jirı Gabriel[10] speaks to Málek's very evident influence on this field – ecosystems are open as opposed to closed, *ipso facto* continuous flow apparatus is likely to be a very promising laboratory analogue in the study of microbial ecology. These investigations were spear headed by Jirı Macura and one of the inspirations for the cultivation equipment came from the soil percolation unit designed about 15 years earlier by Leslie Audus my head of department at Bedford College.

MÁLEK'S LEGACY

Continuous culture in Málek's era was the method of choice for dissecting organism-environment interactions but later the interest in microbial growth physiology declined as molecular biology and more recently genomics asserted their influence on biology. However, renewed appreciation of continuous culture over the past decade has been triggered noticeably by the introduction of high-information-density (HID) technologies to investigate all aspects of microbial behaviour and the consequent necessity to collect data under rigorously defined and regulated conditions. Continuous culture increasingly is being adopted not only for continued studies of physiology, ecology and evolution, but also for systems biology-based approaches to a wide span of problems ranging from global regulation to a better understanding of microbial pathogens and their treatment. A few contemporary examples of Málek's legacy follow but interested readers will find comprehensive discussions in the recent reviews of Bull[3] and Ferenci.[11]

1. More sophisticated analytical techniques are showing that genotypic and phenotypic heterogeneity can develop very rapidly in chemostat populations and is determined to a large extent by the batch phase of growth prior to continuous cultivation, i.e. the genetic variation within the starter population; while investigations of element-sparing in yeast are revealing how natural selection might operate on the material costs of gene expression itself. Continuous cultures continue to be used for the directed evolution or strain improvement of microorganisms and recent focus has been on complex phenotypes selected for industrial processes and products that, in turn, have led to new reactor developments such as the cytostat.
2. Continuous cultures are good surrogate systems for analyzing many aspects of microbial ecology and recent applications have probed the effects of viruses on food webs; carbon turnover in transitional states such as riverine-estuary-ocean systems; phenotypic plasticity exemplified by the complementary chromatic adaptation of microalgae; and further analyses of predator-prey interactions taking into account the concept of ecological stoichiometry to understand feeding dynamics. Ecophysiological studies in general will impact our capacity to design and operate improved waste treatment and environmental management systems and, perhaps optimistically, to predict and prepare for local and global environmental perturbations.
3. The application of HID technologies in concert with continuous culture platforms is opening up fascinating new ways for penetrating pathogenesis. For example, a genome scale metabolic model of *Mycobacterium tuberculosis* has been published recently that throws light on the switch from the acute to the persistent stages of infection, and that can be correlated with high to low growth rate properties previously established in chemostats. A component of this switch appears to be the *mce1* gene locus that may modulate the inflammatory response and allow the pathogen to persist without being eliminated or eliciting disease conditions. Similarly chemostat techniques are being effective in probing the development of peridontal diseases; for example, limiting the pathogen's growth by heme induces a major virulence response. Of course, caution must be exercised when extrapolating from chemostat experiments to real ecosystems, the former only offer mirrors of reality.
4. As noted above, the promise of industrial scale continuous culture processes were strong during Málek's time but their direct influence has been less than anticipated. The impact has been incremental rather than innovative and largely referable to process and product development and optimization.

Two final aspects of continuous culture practice and theory should be noted. First, regarding theory, the term steady state in the context of chemostat experiments needs to be used with considerable circumspection (see Ferenci[11]), a point made decisively, for example, by recent transcriptome monitoring of *Trichoderma* cultures. Other extensions of chemostat theory have addressed a long sought relationship between K_s and K_m, the saturation constant and the affinity of a transporter for the growth-limiting substrate; and re-evaluated the quantification of maintenance energy requirements. Secondly we should note the developments in equipment that encompass such disparate goals as field operable systems for ecological research, *in situ* collection of metabolome data, and microfluidic continuous cultures.

MÁLEK THE HUMANITARIAN

In addition to acclaiming the scientific achievements of Ivan Málek part of the anniversary conference was devoted to his activities within the Academy of Sciences (and hence national science politics) and in the wider international arena. The science historian Robert Budd provided an assessment of Málek's international standing and empathy at the conference. Although brief reference to some of these activities is made by several of the contributors, it would be remiss not to acknowledge more fully here this

aspect of the man's life. In his lecture Budd emphasized the strong sense of responsibility Málek expressed in alleviating hunger, disease and pollution, particularly in the developing world, and in urging a new ethical code for scientists. The former concern led to his early commitment to the work of the ICRO-UNESCO-UNEP Panel on Applied Microbiology with which the eminent Swedish microbiologist Carl-Göran Hedén was also closely associated. These two pioneers subsequently joined forces in another international movement of scientists, Pugwash. The development of Pugwash ''*as an enterprise in which scientists have come together in support of efforts to mitigate deadly conflict*'' has been described in detail by Perry Robinson[12] but a few pointers here to Málek's commitment deserve attention. Together with Karel Raska, a former director of the Institute of Epidemiology and Microbiology in Prague, he presented a paper at the 11th Pugwash Conference in 1963 calling for ''*an agreement on international co-operation in protection against biological warfare, and disarmament in the field of biological weapons*''. The following year at the 14th Conference in Karlovy Vary, Czechoslovakia, a working group with Málek as its rapporteur and Hedén as a member was established that quickly evolved into the Study Group on Biological Warfare. Perry Robinson's article should be consulted for further information but four functions embodied in Pugwash that epitomize the Málek philosophy warrant restating: the importance of person-to-person contacts between those with well-informed things to contribute; continuity of such contacts; doing research that informs policy options; and communication on issues beyond the policy makers.

REMINISCENCES

My direct contact with Ivan Málek regrettably was limited to just a few meetings and the first of these occurred in London in 1967. At that time I was a lecturer in John Pirt's department at Queen Elizabeth College. John, a former Editor of this journal, was part of the select ex-Porton continuous culture community and through him I began to use the technique in my own research. During Málek's visit to QEC we met and spent a convivial afternoon together talking science and other matters of mutual interest. Towards the end of our conversation he suggested that I should spend some time at the Institute in Prague, and how about the following summer? This invitation was and remains one of the greatest pleasures of my career and, as I learnt later, was entirely typical of Málek's generous international perspective. However, I had already arranged to have sabbatical leave during 1968-69 in Martin Alexander's lab at Cornell (incidentally Martin was another stalwart member of the ICRO Panel thereby closing another connection with Málek and Héden). So we agreed to contact each other on my return from Cornell and fix dates for a visit to Prague. Alas this was not to be: the Soviet Union's invasion of Czechoslovakia in 1968 in the aftermath of Alexander Dubcek's reforms (the Prague Spring) led successively to Málek's house arrest and banning from the Institute and all research. Málek was a Dubcek supporter and following successive purges of ''*major progressives and opponents of the invasion*'' he and Dubcek were among those who *forfeited* their seats in the Central Committee of the CP.[13] It was not until the 1980s that we were to meet again first Porton at the 8th Continuous Culture Symposium and then in New Dehli at the VII International Biotechnology Symposium in 1984. I found Málek to have aged but still very alert and enthusiastic. Among the topics we discussed in India was biotechnology in the developing world and he was particularly pleased that I had been drafted onto the ICRO Panel (on Martin Alexander's recommendation) and pursuing one of the missions that was close to his heart. Although this was to be the final time we met, from then on his influence and legacy came via friendships made with other members of the Institute of Microbiology and reference to his and Fencl's indispensable continuous culture text.[14]

My first visit to Prague was after the Velvet Revolution of 1989 that restored democracy to Czechoslovakia and came at the invitation of Bohumil Sikyta to chair and speak at a session of the IUMS Congress in 1994. It was as at this meeting that I was elected to honorary membership of the Czechoslovak Society for Microbiology, an event that further cemented my relationships with Czech microbiologists. Bo and I became good friends and four years later I was a guest of the Society on the occasion of its 70th anniversary; this congress, held at Hradec Králové, also commemorated the 650th anniversary of the prestigious Charles University to where Sikyta had moved after his time at the Institute in Prague. This was an opportunity to see again Arny Demain, another old friend of Málek's, and Jarda Spizek a later successor as director of the Institute of Microbiology, and to enjoy the wonderful hospitality of Bo and his wife at their country home. At about this time Jiri Damborsky came to our lab in Canterbury as a research intern from Masaryk University in Brno thereby consolidating our Czech contacts. Jiri greatly enhanced our work on microbial dehalogenases[15,16] and once again reflected Málek's internationalism by being funded by a UNESCO biotechnology fellowship. My final point of reference with Ivan Málek came unexpectedly but was to resonate strongly with his Pugwash concerns. In 2000 a NATO Advanced Research Workshop was convened in Piestany, Slovakia to discuss maximizing security benefits from technical cooperation in microbiology and biotechnology. The Workshop was co-directed by Dr Cyril Klement of the Slovak State Institute of Public Health and structured to enable discussion of issues relating to international cooperation. Among those participating were Jarda Spizek, Perry Robinson, and Harry Smith – ex Porton and another friend of Málek – who had asked me to report the results of a long term UK-Indonesia collaboration in biotechnology that I had co-ordinated from the UK side, so interfacing once again the community of continuous culturists and ethos of the ICRO Panel.

EPITAPH

The Anniversary Conference made a fitting tribute to a great human being. Several of the conference papers are notable for their extensive bibliographies particularly of work that may not be familiar to contemporary microbiologists. Coincidentally my own memories of Málek, Czechoslovak-UK contacts and of a remarkable period for science and politics in the 20th century were rekindled as I was preparing a review of post-genomics continuous culture at the time (unbeknown to me!) that the anniversary conference was occurring.[3] Málek lived through times of massive upheaval and stress but his indomitable and generous spirit and indefatigable passion for science and his fellow humans overcame much. Robert Budd describes him as ''a prophet of a new age in science'', while Demain and Spizek concluded: ''Since his passing away in 1994, we have sorely missed Ivan Malek'', to which all of us who knew him will raise our hands in admiration.

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