FOUNDATIONS IN CANCER RESEARCH: THE TURNS OF LIFE AND SCIENCE

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(originally published in: *Adv. Cancer Res.* 2008; 99: 1-32**)**

To Write or Not to Write?

Since I received the invitation from George Klein to write my recollections, I have been postponing my final decision for twelve years. Several factors influenced my hesitation. I did not feel old enough to undertake such an enterprise, but unfortunately I had been learning about departures, either physical or mental, of my colleagues who were younger than I. The biological clock is ticking in everybody, and the longer the distance from past events, the worse their memory is kept.

In fact, I am going to write about a non-existent country – Czechoslovakia, which was dissolved at the beginning of 1993, in the absence of any referendum, and which gave rise to the Czech Republic and Slovakia. However, strong ties among intellectuals of both newly established twin countries (who, according to my knowledge, would have preferred to stay together) remained viable and even formalized into joint scientific societies.

Encountering Western colleagues, we often discussed the unpredictable turns of the fate of this country, which at first glance appeared to be fatal for its culture and science. However, this was finally modified and regulated by our national sense of humour, heresy and free thinking, traditions deeply rooted in our culture. I realized that despite the fact that some of the escapes from and solutions to absurd situations look strange to straightforward Anglo-American thinking, they bemused and sometimes even attracted the listeners. This aspect represents an additional excuse for writing further pages.

Boys and Resurrection of Czechoslovakia

Being a boy after the end of World War II was a great privilege for several reasons. At the age of about ten years I was not deeply marked by the Nazi occupation like my older mates, who were expelled from the universities and in many cases sent to forced labour. The first three years after the liberation of the country in 1945 were extraordinarily fruitful and inspiring. There was general enthusiasm and willingness to catch up again with the civilized world. There was a plurality of views, a richness of information from both the East and West and many opportunities to learn about and love nature – being a boy scout – or to try to understand human thinking by joining discussion clubs or the academic YMCA. To me it has been highly attractive to collect natural objects and to try to learn about their origin or function. I loved amphibians and to some degree also snakes (as viper) that I bred at home. These years formed my generation, which later had to live, for such long years, out of this crop of these seasons.

However, disaster was looming and fell in February 1948 as a communist coup d'état. Boys at the lyceum were not affected directly, but the freshly released high-school graduates who in spring tried to enter university often reappeared with pale faces, commenting on their non-admission simply: "*La politique*".

However, for younger boys the situation also became more difficult. Good teachers started to make far less comments and some of them invited selected students to their homes to discuss history and other topics freely. Having reached the last year of the lyceum, I learnt that I would not be recommended to enter any university. Being educated in the spirit of liberalism by my father and inclined to comment on what was going on I finally faced real trouble. Several factors had been involved, but the most peculiar one stemmed from my friendship with one of my classmates. He had become seriously ill, and I taught him what he missed at school, for free of course, just on a friendly basis. When he recovered, he converted to orthodox communism and became a leading person of the Party at school. Evaluating me he postulated that although I was a good human being, I was an enemy of the working class. Such is, sometimes, the end of an open mind.

Again, the opportunity to study fortunately came about in 1951, when the political pressure was decreasing. Finally, I got notice that there was a chance for me to enrol, not in the humanities or at the highly desirable medical faculty, but with two provisions. The first was to get excellent notes in all subjects, which was against my mind and nature, so that I had to strongly force myself to comply with this unpleasant goal. The second condition was easier and involved labour work at the so-called Constructions of Youth, which included just digging and digging, however with a beautiful view of the hills of central Slovakia.

University

Vždyť také je mnohem větší nebezpečenství při koupi nauk než při koupi jídel.
Platon: Protagoras, aneb o výchově a občanské zdatnosti, Laichterova filozofická knihovna, svazek 22, (J.B. Kozák, ed.), Jan Laichter, Praha 1939.
For there is far greater peril in buying knowledge than in buying meat and drink.
Plato: Protagoras, 1995 ILT Digital Classics (translated by Benjamin Jowett)

Avšak správná filosofie a věda žádá pro všecky obory, aby lidé myslili, aby nastřádali co nejrozsáhlejších zkušeností (indukce), aby pozorovali a srovnávali všecko, co dáno v přítomnosti a minulosti, a aby své výsudky ze zkušenosti ověřovali zkušeností další, aby se nedostali dedukováním z malé zkušenosti, dedukováním ukvapených, do říše fantastiky.
Masaryk, T.G.: Světová revoluce za války a ve válce 1914-1918, Orbis a Čin v Praze, 1925.
However, the true philosophy and science requires of all its branches to make people think, to let them accumulate all possible experience (induction), to make them observe and compare all of the present and the past, and to make them verify the conclusions drawn from their experience by the new one, not to let them get, due to deducing from too little or too hasty experience, into the world of fantasy.
Masaryk, T.G.: The World Revolution during and in the War, 1914-1918, Orbis and Čin in Prague, 1925.

Finally, I safely matriculated at the Faculty of Science of Charles University which offered my particular choices. In those days, biology was taken as a preferential subject thanks to Lysenkoism, which for ideological reasons was implemented as the leading genetic teaching. However, in contrast to the humanities and social sciences, professors who did not fit with the forced ideological views were not removed but silenced. They were – even by

their mere presence – a reminder that there was a wealth of genetic knowledge based on the Mendelian laws. I should add that in second-hand bookshops, one could still find books dealing in detail with classical genetics, and these became the source of solid information to any one wanting to pay attention to them.

From the beginning, I should have been interested in cytology, which, unfortunately, was viewed only as a morphological discipline. I got essential training in methods in plant anatomy, but in those days there was almost no way to grow and influence living plant cells. Therefore, I requested and succeeded to get volunteer training in animal tissue culture in the laboratory of cell metabolism created by Dr. Keilová at the Academy of Sciences. This laboratory was interested in the study of possibly differing metabolic requirements between tumour and normal cells. I easily fell in love with tissue culturing and acquired my first experimental experience with the characterization of the growth properties and morphology of tumour cells. A great stimulus for my activity was represented by the arrival of a highly motivated, but to some degree eccentric PhD student Mojmír Brada. He came with new, sometimes too demanding and almost unrealistic approaches, which spanned from single-cell biochemical analysis to Rous sarcoma virus (RSV) transformation of chick embryo explants. At this point I should mention that I was amazed by the rapidity of RSV-induced cell transformation, which heralded the fact that the virus harbours the gene(s) responsible for such transformation. That is how I became attached to this model. Mojmír required my help with tissue culture and I learnt from him a series of new experimental techniques, including microinjection and individual cell isolation, which later became important. We became friends and I was working with him usually from the afternoon until late at night. Once, leaving after midnight and being in a good mood, he asked me how Socrates departed life and if I would like to get his books. I still had to finish some work and therefore I did not pay much attention to these strange remarks, agreeing to borrow books from him. The next day I learnt that Mojmír Brada committed suicide using cyanic acid – faster than Socrates did. Learning about this news, I got the feeling that the world around me had collapsed, with no great chance to advance because of my position as an isolated student. Later, I was told that Mojmír attempted suicides in the past.

All this happened at the time when Milan Hašek was taking over our department. With his arrival, the scientific atmosphere at the department changed profoundly because he brought with him the essential topic, immunological tolerance, originally called by him immunological approximation. Fortunately, I fitted within this field because some recent data of Morten Simonsen (1955) and Bob Harris (1956) pointed to the possibility that introduction

of heterogeneous doses of chicken blood in newly hatched turkeys significantly increased their sensitivity to RSV. Together with Milan Hašek we confirmed these results (Svoboda and Hašek, 1956) and extended them to ducklings (Svoboda, 1970). Because tolerance to RSV was elicited in both foreign avian species, we proposed that RSV triggers synthesis of a chicken antigen and therefore tolerance to chicken tissue favorizes growth of tumour cells (Svoboda, 1961).

It should be remembered that RSV-induced tumorigenesis in ducks was established as the first model of retrovirus heterotransmission. Work on it started in the thirties and later was elaborated by Duran-Reynals. It produced the first hint that retroviruses can overcome species barriers, as was exemplified several decades later.

The role of immunological tolerance in retrovirus heterotransmission has not yet been clarified. However, the phenomenon is reproducible. Immunological tolerance in ducks lasts only for a fortnight after hatching and older birds are fully resistant to RSV is spite of the fact that duck cells carry the RSV receptor (tvc). What is responsible for such powerful resistance is currently unknown. Factors in play might be natural immunity and mobilization of cell factors blocking virus replication. We might learn a lot from this phenomenon about the way to establish efficient resistance to a retrovirus.

As a student, I was assigned to Věra Hašková laboratory. She was a very nice person, efficient and intelligent; we collaborated on the comparison of immunological tolerance and enhancement and in attempts to genetically modify animals using DNA (Svoboda and Hašková, 1959; Hašková and Svoboda, 1962). She also gave me space for RSV research.

Before proceeding further, I should deal with the year 1956, when anti-Stalinist revolts broke out in Eastern Europe. In our country, mainly students were involved in peaceful demonstrations as described by Jan Klein in this series (Klein, 1994). At the faculty, together with a few others, we conceived a petition for the state and party organs in which we demanded democratic changes such as release of single party domination, independent judiciary, and other demands. After various sudden changes of fortune, I was called before a special committee where, luckily, university professors also participated. I was accused of "anti-socialist activity", but I defended myself by saying that I had not violated the Constitution. In this way I survived the first storm, but I expected that the second one would follow at the Institute. However, Milan Hašek, after a few questions, let me go. One question was very tricky. Hašek asked me whether I was informed about the reforming movement in Poland (as we were referring to) from the Voice of America or from Polish resources. I, of course, answered that the Polish information service was the source of our knowledge, but

there were also other sources not recommended to be mentioned. The only punishment was a year's delay in my admission to PhD studies, which was not a big deal.

I wrote about Hašek's unique personality recently (Svoboda et al., 2005). He had been devoted to his research, not forcing ideological influences on the laboratory. In daily communication he behaved in a liberal way. Once we discussed Mendelian genetics, which he did not like. I put forward the argument that although it does not explain everything, Mendelian genetics still should be taught. Essentially, Milan did not object. I witnessed that he was sincerely interested in ways how to modify the genetic make-up of organisms and accentuated the somatic cell genetics and transplantation. He had always been broad-minded and supportive to his colleagues, defending them from external and even political pressure, and was on friendly terms with his collaborators. In his active period he surpassed everybody in biological sciences in our country.

Virogenic Cells and Provirus Integration

My involvement in the immunological tolerance to avian retroviruses in birds culminated in my first trip to Moscow on the occasion of the National Meeting of Transplantation. This event took place in 1959, in the period of the thaw. I still remember O.B. Lepeshinskaya, a pillar of Lysenkoist cytology, who had no idea about what was going on at the meeting. In fact, she asked the co-chairman a control question - "Immunology?" On the other hand, bright young scientists such as George Svet-Moldavsky and Yuriy Vassilev raised their voices in favour of genetics, loudly opposing Lysenko's monstrous misconceptions.

The crowning event to our trip was a meeting with Lev Alexandrovich Zilber and his collaborators, namely with Igor Abelev. They were devoted to the isolation and characterization of possible tumour-specific antigens in human malignancies using various available techniques. The problem of such studies was that they did not distinguish between tumour and normal histocompatibility antigens.

I was associated with Zilber and his collaborator Kryukova as well as to George Svet-Moldavsky through their original discoveries of RSV pathogenic action in rats. What I learned indirectly was that their original aim had been induction of immunological tolerance to this virus. In this way, there was a common denominator of our interests. Zilber was a magnificent personality, who pioneered modern virology and cancer research under incredibly oppressive conditions. Later, during his repeated visits in our country, he remembered his

ordeals in a close circle of friends; we listened silently. An excellent account of his life and work was given by Kisselev et al. (1992).

We tried unsuccessfully to establish the role of immunological tolerance in RSV-induced haemorrhagic disease in rats (Svoboda and Grozdanovič, 1960) but we were able to show that this disease was caused by the virus because it could be prevented by antiviral sera (Rychlíková and Svoboda, 1960). Of interest was the occurrence of late tumours in RSV-inoculated rats. This phenomenon remained enigmatic because no vestige of the presence of virus had been found in them. That was why Zilber interpreted these findings within the frame of his virogenetic theory, according to which a virus is responsible for initiation of tumour formation but tumour cells need not produce the virus (Zilber, 1961; Kisselev et al., 1992).

Overall, the question of RSV involvement in tumorigenesis in the mammalian host touched essential problems of oncology and attracted me fully. First I decided to monitor late tumours appearing after RSV infection for any viral activity. The simplest way turned out to be inoculation of chicks with minced tumour tissues. Using this approach I found, in the case of tumour XC (from Latin cage No. 90), that after inoculation in chicks it produced RSV-containing sarcomas. Logically it indicated that the XC tumour contained the virus (Svoboda, 1960, 1961). After closer inspection it became apparent that XC cells do not harbour any infectious virus. For its production, association of structurally intact XC cells with chicken fibroblasts was mandatory (Svoboda, 1962).

In the meantime, I was sent, against my will, by our Academy to Red China, because at the beginning of the "Cultural Revolution", the Chinese liked to accept people involved in science and not in politics. The break between the Soviet Union and China was reaching its height. Visiting and lecturing at different institutions, I learnt that the scientific approaches that were followed those days in China were outdated and did not reflect what was going on in the world. I myself felt under scrutiny and was warned secretly by my interpreter that my views and comments were evaluated every evening. Life in that country was poor and grim. An appropriate comment I received from a Latin-American revolutionary granted asylum in China, who stated plainly that such sad communism would never fit with Latin-American sentiments.

The most important part of my trip in China was my way back, which included the flight over the immense green plains of Siberia until reaching Moscow. In the hotel, I liked to feed Chinese hamsters, which I carried as a gift with me on my seat. Unfortunately, some of them escaped when I opened the box and it cost me quite an exercise to collect them and put them back into the box. This event delayed my calling Zilber, whom I was supposed to meet.

It was a warm meeting with Zilber's family, who also remedied my digestion problems caused by exotic meals with Armenian cognac. I then explained to Zilber what we knew about XC cells and virus rescue. He let me speak without interruption and finally concluded that this was the way to understand the interaction of an oncogenic virus with a cell and its transforming activity. Therefore, I returned from this big journey in a far better state of mind than I had before leaving. I then resumed work with enthusiasm. First, we verified the species origin of XC cells (Landa et al., 1962) and the permanent presence of the RSV genome in them. Furthermore, we extended our XC model to rat cells transformed by co-cultivation with chicken Rous sarcoma cells (Svoboda and Chýle, 1963). We had found that this co-cultivation was an efficient way to produce transformation of mammalian cells by RSV, which was employed later as a useful tool for transformation of other cells of different species origin. There was another, for us important finding that *in vitro* transformed rat cells behaved in the same way as XC and that the virus could be rescued from them only after association with chicken fibroblasts, which indicated that this phenomenon was of more general importance.

However, XC cells remained my principal interest. In order to extend this project I invited Dušan Šimkovič as an experienced person in tissue culture to collaborate on long-term cultivation of XC cells. The goal was to establish monocellular clones, which we then successfully isolated (Šimkovič et al., 1963). When individual XC clones were compared in their ability to rescue the virus by cell association, a comparable number of cells from different clones ranging from 10^5 to 10^6 cells per inoculum led to virus rescue. These numbers agreed with the cell number required for virus rescue from uncloned XC cell population, showing that the virus genetic information had been spread equally within the cell population. In separate experiments we confirmed that in several grams of XC tumour tissue there was no infectious virus and its absence was confirmed serologically. This gave the final picture showing that XC cells harbour the viral genome (they are therefore virogenic), which is non-infectious but rescuable. This viral genome is indefinitely inherited in tumour cells as additional genetic information and is therefore integrated in them as a provirus (Svoboda et al., 1963). To this interpretation we were, of course, inspired by André Lvoff's concepts and cell cloning strategy that he employed.

All these studies raised the interest of my colleagues. Especially, Bob Huebner highly valued these results as opening a new approach to tumour virology. In fact, he started his investigation using RSV-induced tumours in hamsters, where he detected viral-specific complement-fixing antigen. Howard Temin sent me a very enthusiastic letter mentioning that he was interested in provirus integration into XC cells and that he was going to publish

similar data. There had been a disagreement between him and me about the mechanism of virus rescue. Temin, based on his chicken cell experience, proposed that virus rescue resulted from superinfection with avian or mammalian retrovirus. Contrary to that, I favoured the interpretation that the virus is rescued by cell association between XC cells and chicken fibroblasts that makes possible fusion and complementation between both types of cells. As we shall see later, the latter explanation turned out to be right (relevant correspondence given in Svoboda, 2003).

Not until 1964 did I have a chance to meet a Western virologist. In that year I was invited to attend the International Conference on Avian Tumour Viruses sponsored by NIH. Originally, it should have taken place in New Orleans, but was then moved to Durham due to the fact that no hotel capable to arrange common accommodation of black and white persons was available in New Orleans.

The meeting was held in a positive spirit favouring an essential role of viruses in the genesis of tumours, and was attended by virtually all scientists working in this field. For the first time, I could speak to Harry Rubin about virus defectiveness, to Howard Temin on provirus integration, and to Ludwig Gross, Peter Vogt and many others whom I had known from the literature. What impressed me was a sense of co-operation in the absence of deadly competition. Possibly, this could have been associated with the fact that in those days only a handful of scientists were engaged in tumour virus research and that this research was not in the front line.

After the meeting, I lectured in various University and State institutions. Everywhere I noticed that laboratories were well equipped, including centrifuges, and were supplied with standard tissue culture media and plastic dishes. This had been a dream for us, but also gave us a warning that we could not work on a broad scale but must stick to our XC cells, which grew in the poor media available in our country. As my friend Bob Dougherty suggested to me, I visited plumbing stores, where I bought a pump for the CO_2 incubator. Therefore, we were able to construct in Czechoslovakia the first functional CO_2 incubator maintaining controlled pH. At this, my first visit of USA, I was struck by the flexibility of American organizations, the availability of resources and the high standard of living. I felt that we had been lagging in many respects for at least 15 years.

There were additional occasions to return to the USA. On one of them, I visited Howard Temin in Madison, where he lived with his wife Rayla in a student apartment. After a thorough discussion of my talk we went to Vaclav Szybalski's department and engaged in discussion with his phage geneticist collaborators. They did not question our results but

emphasized an important issue that virus rescue might be mediated by chicken cell inactivation of a repressor present in mammalian cells.

Virus Rescue

After returning home I felt strongly that the key problem to be solved was the mechanism of virus rescue. Assuming that the cell-to-cell contact allows spontaneous cell fusion and that around 10^5 to 10^6 virogenic cells are required for virus rescue mixed with chicken fibroblasts, an agent stimulating the fusion should augment the virus rescue. From papers by Okada it was apparent that UV light inactivated Sendai virus would be the best candidate for the fusogenic agent. After some problems with getting the right batch of virus we succeeded in proving that it significantly increased the efficiency of virus rescue (Svoboda et al., 1967). This, of course, opened the way for further studies.

Before these results had been accomplished, I went to the meeting celebrating the 70^{th} birthday of L.A. Zilber. The conference was held in Sukhumi on the Black Sea, in the building originally belonging to Beriya and surrounded by a large park full of beautiful trees. Almost all Russian scientists appeared at that meeting and I felt that they were unusually relaxed as a result of the de-Stalinization going on. There had also been an impressive attendance from the Western countries such as the Melnicks, the Kleins, H. Koprowski, R. Huebner, and others. Some of them, who stemmed from Slavonic regions, communicated well in Russian, which pleased our hosts and contributed to an open atmosphere. At this meeting I spoke about tumour-specific transplantation antigen (TSTA). This antigen in RSV-induced mouse tumours had been characterized *in extenso* by Bubeník et al. (1967) from our laboratory as producing rejection immunity against tumours of the same aetiology. As I had shown (Svoboda, 1965), TSTA was also present in RSV rat tumours – the immunity could have been transferred adaptively, by lymphoid cells, and there was clear antigenic cross-reactivity between rat and mouse tumours. This led to the speculation that TSTA appearance was related to the RSV provirus and its transformation activity, which, as we shall see later, was fully substantiated.

Surprisingly, there had been no comment on TSTA, but many questions about virus rescue, which I mentioned only peripherally. Repeated questions were raised whether in our experiments a helper virus might be involved. As I had shown, it was not the case, and I stated again that for various reasons it was not. In extending the discussion to the analogy of our virogenic cells to SV40-transformed hamster cells, from which viruses can be rescued by cell

association, I engaged in a confrontation between Hilary Koprowski and Albert Sabin; the latter stood in favour of virus rescue. There were obvious differences between RSV as an RNA virus and SV40 as a DNA virus, but I pointed out that theoretically "the question still remains whether the transforming part of RSV is DNA". However, as was shown several years later, the cytological basis of SV40 and RSV virus rescue was the same in both cases.

There were some adventures linked to the life in Sukhumi. One free afternoon we went, together with Pavel Koldovsky, for a public bus drive in the country. Everywhere, rhododendrons in bloom seemed unharmed by goats feeding on their leaves. On our way back, Pavel instructed me to be careful about my wallet. He felt safe because his pocket was buttoned. When we left the bus, he found out that his wallet, along with the cut-off button, was gone. We then reported the incident to the police. The "militia" men were not surprised and assured us that, according to local habits, the documents would be returned within 24 hours – except the money, of course. This scenario took place as predicted.

Of the meeting participants, I was most impressed by Bob Huebner. At the reception dinner, he gave a highly stimulating talk stressing the international collaboration in the cancer field. As I learnt later, he meant it seriously. In the next years, we met on various occasions. I keep in mind his arrival in Prague, joined with a dinner at a beer pub. We discussed in English when we were suddenly interrupted by a Czech soldier, who approached me and told me that it was scandalous to speak to our enemy. Bob noticed immediately that something went wrong and asked me to translate the soldier's comments to him, which I did. He then replied, reminding the soldier that during the war he himself served as a doctor on a battleship in the Pacific and felt as an ally to Russia. This convinced our brave soldier, who shifted his mood to friendship.

Back in Prague, we wanted to reconcile the variable cytogenetic data obtained with RSV-transformed rat cells. There was no other way out than to employ another, more suitable model. Fortunately, we bred Chinese hamsters that I had previously brought from China (Hložánek et al., 1966). They were easily adoptable for karyologic experiments because of the low chromosomal number. In tackling the question whether virus-induced cell transformation is accompanied by karyologic changes, we exposed primary cultures to the virus and evaluated the cells before, during, and after the transformation. It turned out that no noticeable karyologic anomalies occurred at the stage of cell transformation. However, they appeared gradually after repeated passages, indicating clearly that they were related to tumour progression. We thus abandoned karyology for a long time. It should be stressed that the issue of the role of karyologic changes in tumorigenesis is now re-emerging.

I had several chances to meet with French scientists and I highly valued G. Barski, who along with Soriel discovered somatic cell hybridization, and Philippe Vigier, internationally recognized "rousologist". Philippe was originally very critical of our findings, but gradually became interested in them. Together with him, we obtained additional important data showing that virus rescue from XC cells was independent of any helper virus (Vigier and Svoboda, 1965).

I was very pleased by an invitation from Bob Harris, who also had attended the Sukhumi meeting and was head of the Mill Hill station belonging to the Imperial Cancer Research Fund. Bob's offer was attractive to me because he gave me total independence, a technician, and the possibility to invite my colleagues for a short stay. I was convinced that I would come to a laboratory where tissue culture was running routinely. How disillusioned I felt when I learnt that chicken fibroblasts didn't grow! There was only one solution – to check every component of the culture medium. I made all the possible exchanges between individual batches of different provenance, but this did not lead to any conclusions. The last factor in play was distilled water prepared in an apparatus equipped with rusty iron electrodes. My next move was to ask for glass distillation. My request was turned down with the note that the present apparatus was working well – a typical English approach. Finally I persuaded my colleagues and bought a glass distiller. The newly prepared media made possible efficient growth of chicken fibroblasts, which opened the way to establishment of a quantitative assay detecting RSV cell transformation. I adjusted this assay for measurement of virus rescue from virogenic irradiated cells fused by Sendai virus with chicken fibroblasts. Under these conditions, the virus production was increased 100 times as compared to co-cultivation. The rescue was proportional to the number of virogenic cells and correlated with the heterokaryon formation (Svoboda and Dourmashkin, 1969). All these results of tedious work convinced me that in the case of virus rescue, we were dealing with a phenomenon where the virus genome in a non-permissive mammalian cell was not expressed fully until complemented by chicken cell machinery. Not many people believed it. Among the sceptics was Howard Temin, who engaged his pupil John Coffin in a virus rescue project, and John successfully confirmed our data (Coffin, 1972).

In the IRCF laboratory, I made friends with an excellent electron microscopist and cell morphologist Bob Dourmashkin, who helped me efficiently in these studies. I esteemed Bob Harris for his magnanimity and a very friendly approach to me. I should not forget the bright American Bob Bassin, who later went to John Moloney and with whom we discussed both science and politics. Also the external contacts were enjoyable and inspiring. They included

Warren Levinson, whom we visited at London University and who gave us important suggestions about performing RSV assays. After returning to the USA, he introduced the RSV model into Mike Bishop's laboratory.

I was impressed by Tony Epstein, by his critical but constructive approach to tumour viruses, and made friends with gifted PhD student Robin Weiss, who obtained the first data that in some chicken lines, genes encoding retroviral proteins are expressed, and opened the way to the definition of endogenous retroviral genomes. Very enjoyable was my visit in Glasgow at the Institute of Virology chaired by Michael Stoker. Retrovirology was represented there by Ian Macphearson, who discovered normally looking revertants in a population of RSV-transformed hamster cells. Because the virus was not rescuable from some of them, Ian interpreted this failure by loss of the provirus and proposed an episomal provirus state. Discussing this issue, I suggested increasing the number of virogenic cells used for virus rescue experiments, which worked, and the episomal hypothesis fell into oblivion. Ian had a great sense of humour, but he was serious when a note hit his Scottish heritage. For instance, he was ready to defend the existence of the Loch Ness monster until the last drop of his blood.

In England we lived together with my wife happily – like a pair of squirrels, which by the way were plentiful around our house. My contacts with our country were irregular, but reading the German journal Der Spiegel, I got a feeling that deep changes in the direction of democratization and party power limitation were on the way. Fresh news reached us from Milan Hašek, who visited us in London. He was fully in favour of reforms and democratization, being fed up, similarly as most of party intellectuals, with inefficient bureaucracy and party reglementation.

Two important meetings took place before my leaving England. The first one was organized at the Wistar Institute in the USA and was focused on virus induction by cell association. I tried to get together all the people who were contributing to this subject (Svoboda et al., 1968). In this respect the meeting was very successful. It enabled getting the information based on RSV in context with SV40 studies and showed that both were very close to each other. The greatest advancement was made by Mary Weiss, who studied the segregants of human-mouse (SV40-transformed) hybrids and demonstrated that the SV40 genome is preserved only in cells carrying certain mouse chromosomes. This indicated clearly that the SV40 viral genome is integrated at least at the chromosomal level. Unfortunately, when working with rat-chicken hybrids we were not in the position to analyse the hybrid progeny due to the non-viability of such hybrids. For me, the crowning event to our meeting

at the Wistar Institute was Okada, a modest person who opened the field of artificial cell hybridization.

The second meeting, convened by Michael Stoker, was devoted to the molecular biology of viruses and sponsored by the Society of General Microbiology. This was a unique session, which of course included traditional Franco-British confrontation. For our work, two talks were important. The first was given by Luc Montaignier, indicating the presence in the cell of double-stranded RNA that might be employed at certain stages of RSV replication. The existence of such RNA was criticized strongly by André Lwoff, but Luc stuck to his conclusions. The second inspiring lecture was presented by Werner Arber, who dealt with the known and unknown mechanisms of bacteriophage host cell modifications and predicted restriction enzymes. We had already tried to detect some signs of RSV modification in mammalian cells, however, with a negative outcome. In my talk I presented a classification of different types of RSV interactions with mammalian cells. Of special interest was non-productive (non-virogenic) interaction characterized by tumour cells from which no virus was rescuable and in which no virus component was detected. However, such cells harboured TSTA specific for RSV-transformed cells. I concluded saying that "only the part of the viral genome responsible for transformation is present in these cells" (Svoboda, 1968).

This RSV transformation part, later called "oncogene", remained in the centre of our interest also in later, more difficult years.

Prague Spring 1968

Under the Roman domination, the Greeks lost the self-confidence that belongs to political liberty, and in losing it acquired a paralysing respect for their predecessors.
Russell, B.: A History of Western Philosophy, Touchstone Book, Simon and Schuster, Inc., New York, 1972.

Činí, co chtějí, neboť jim nikdo nic říkati nesmí.
Dačický Mikuláš z Heslova: Paměti, Výbor (Emil Pražák, ed.) Slunovrat, edice české prózy a poezie, sv. 33, Československý spisovatel, Praha 1975.
They do as they wish, for nobody may contradict them.
Mikuláš Dačický z Heslova: Memories, Selection (Emil Pražák, ed.) Slunovrat, Edition of Czech prose and poetry, vol. 33, Československý spisovatel, Prague 1975.

I was eager to return to Prague and witness the unprecedented changes that were going on. However, I kept in mind that the freedom of information and criticism that I enjoyed so much in London could be far less in Prague and would eventually fade out. On one of my last evenings I felt the need to see Pasternak's Doctor Zhivago and prompted my wife to go with me. This extraordinarily made film gave me a lot of warning about the cataclysms that may happen in a communist-ruled country. Essentially everything was possible! But I still believed that the flow of events in our country was irreversible and would be accepted by our Big Brother – USSR.

In the summer, the first World Congress of Virology was organized in Helsinki, in an optimistic spirit, also resulting from establishment of an independent virology organization. I travelled with Slovakian virologists, who were "well equipped". Later, a Swedish custom officer pointed without hesitation to a small smart luggage. When opened, five bottles appeared. Being asked by the officer what it was my colleague answered simply: "Slivovitz". The officer did not ask more; in those days Scandinavians were very friendly to us, probably being better informed than we were.

At the meeting, virus rescue was one of the important topics and I joined in actively. After my talk I met Albert Sabin, who warned me that the Soviet Army was getting concentrated around our borderline, which was alarming. On our way back we landed in East Berlin and, contrary to others, we were not allowed to leave the plane. It looked as if we were carrying an infection.

On 21st August at 3 a.m. I was woken up by a call of my colleague, who stated simply: "They're here". I was shocked, but soon ran to the streets to check what was going on. Prague was packed with Russian armaments and soldiers looking hungry and thirsty, but nobody offered them food or drink. I approached one soldier and asked him: "Where do you think you are?" His answer was simple: "In Germany". The most explosive situation was near the radio broadcasting station, where a Soviet tank went aflame lit by the demonstrators, and the horrified soldiers started to shoot into the crowd from their machine guns. Most of the people, including me, escaped in the corridors of the broadcasting station, but several were killed. Later I took part in street demonstrations and we were also joined by some American students. Surprisingly, they knew Harry Rubin’s involvement in the civil right movement.

The invasion of the Warsaw Pact armies had disastrous consequences also for our work. Supplies were disorganized and our foreign contacts lost. I was especially waiting for Warren Levinson, with whom we planned a joint molecular hybridization study of our virogenic and non-virogenic cells. Under such conditions, the solution for me would have

been to emigrate. There was no obstacle to such a decision because I held a US visa and, in addition, an official invitation from Bob Huebner to join NIH as an independent scientist. However, shortly after the invasion, I received Milan Hašek's letter sent from Austria, in which he declared that he was not going to return until the Russians retreat and that for the time being he was handing over the directorship of the Institute to me. I was deeply shocked by this Danaian gift and for several days I felt torn apart by the question whether to stay or to go. Finally I decided to stay, assuming that Milan wrote the letter in the impulse of the moment and that he would return. Actually, he came back about a year later and I handed the directorship back to him. Otherwise, the general situation in the country until 1971 had not been seriously oppressive. The Institute was further supervised by the Academy, which was not significantly interfering with the Institute's matters. There was also a strong public feeling that emigration of creative people would help the Russians to devastate our country. Finally, I was wrong in estimating that the occupation could not last more than 5 years. Later, Milan's estimate was 20 years, which was almost exact.

Therefore, without problems I attended several international meetings dealing with virus rescue by cell association. At a French meeting I spent late hours discussing with Harry Rubin our experiments and general political and cultural problems. His insight was phenomenal and in answering the question about his preferences, he stated "Czech films and Kirkegaard". Fascinating was the Amsterdam meeting organized in honour of O. Mühlbock, who had retired. All scientists who had essentially contributed to retrovirus research and oncogenesis were given a chance to present their data and views. In my talk I provided an avian leukosis-sarcoma virus overview (Svoboda, 1972) and underlined data indicating the presence of viral specific DNA in infected cells. Furthermore, I discussed cell association and phenotypic mixing as approaches to virus rescue.

The atmosphere of this meeting was, for several moments, highly confrontational. I remember Bob Huebner shouting at Howard Temin "You don't understand virology!" and the reply was "You don't understand oncology!" Also, I did not understand why such a heated debate. Behind these strong arguments were serious discrepancies in interpreting the origin of the cancer process. Temin (1972) proposed an original idea, that a proto-virus, simple provirus, integrates by reverse transcription and once integrated in the vicinity of potentially oncogenic genes it produces their activation resulting in cancer conversion. Huebner and Gilden (1972), on the other hand, stressed the role of endogenous retroviruses in triggering such a process and emphasized the role of de-repression of viral oncogenes by other factors. Then, sitting in a bus I heard a discussion between two eminent scientists, indicating that the

future Nobel Prize was at stake, which usually is accompanied by scientific fireworks. The competition at such a level was beyond my mind, because I felt deeply disturbed by thinking about what was awaiting me back in Prague. An encouraging fact was that on my return to my country I was accompanied by Robin Weiss, the first and last international member of our Institute. A very positive event was that Milan Hašek had returned. I handed over the directorship to him. However, the worst was yet to come.

Dark Years

S řečí byli bychom ztratili i paměť, kdyby v naší moci bylo zapomínat tak jako mlčet
Tacitus, Z dějin císařského Říma, Antická knihovna sv. 31, Svoboda 1976, Praha
With the faculty of speech we would have lost our memory, if it were in our power to forget in the same way as to be silent
Tacitus, From the History of Caesarean Rome, Library of Antiquity Vol. 31, Svoboda 1976, Prague

At the beginning of 1972, Husák's puppet government tightly closed the border. Then, purges started. I was called to a committee where I was asked whether I agreed with the Soviet occupation. The answer was no and I emphasized my argument by mentioning that the President of our Republic also did not agree. This was something they did not like to hear and consequences followed. I lost my department and remained solitary with no direct help. The same happened to Milan Hašek. It meant that we had to proceed by a slower path. Something of a curiosity was the ban to publish, fortunately lifted after half a year thanks to the intervention of Viliam Turzo from Bratislava. I decided to visit Turzo in his Institute, which in those days was also deserted. He behaved in a relaxed way, but when I wanted to pay the dinner, he stopped me and gave me a Hungarian small gentry lesson, saying in Slovakian: "Pán sa neponáhlá, pán sa nečuduje, pán neplatí", which means: "A gentleman does not hurry, is never astonished, and does not pay".

Before all this happened, we undertook re-examinations of different types of RSV-mammalian cell interactions (Svoboda et al., 1971) and using all the present-day knowledge we eliminated any role of endogenous retroviruses in virus rescue, confirming the previous statement that chicken cell, not viral, factors were required for virus formation. Moreover, we characterized non-virogenic cells as lacking the viral replicative gene product, but still were transformed and contained TSTA. This was a basis for my future plans aimed at the

characterization of the virus-transforming part present in non-virogenic cells. There was a visible hiatus between the great progress in retroviral oncology as performed on animal models and the lack of similar findings in humans. Therefore, we assumed that some human tumours might harbour cryptic proviral sequences as in the case of non-virogenic cells. Such cryptic proviral oncogenic sequences could be occasionally transmitted by recombination with another virus. This hypothesis turned out not to be valid, but at least for a while helped sustain the interest in human retroviruses.

The field of retroviruses was moving fast as a result of the discovery of reverse transcriptase made by Temin and Mizutani (1970), and simultaneously, Baltimore (1970). Still, the question remained as to whether the whole retroviral genome becomes integrated into the cell DNA. Such a possibility might have been tested using transfection of chicken cells with DNA isolated from virogenic cells. We agreed upon collaborative transfection experiments with Miroslav Hill on the occasion of the Czechoslovak Biological Society session in Brno in 1966. However, our ways had diverged – he went to France and I to England. After the occupation he established himself at Gustave Roussy in Paris and together with his wife they published a report describing the positive outcome of XC DNA transfection of chicken fibroblasts (Hill and Hillova, 1971). We were working on the same project in parallel. However, in the first step I wanted to test whether the nuclei from XC cells could be transferred to chicken fibroblasts and there trigger virus rescue, essentially because we had already learned, using the enucleation procedure, that the nucleus is the seat of the provirus (Donner et al., 1974). In spite of treating XC cells with hypertonic solution during nuclei isolation, where no cell should have survived, when I seeded a suspension of nuclei in the culture flask, XC cell colonies later appeared. These unsuccessful experiments produced some delay in reaching the second step that involved DNA transfection. I should mention that I was strongly warned by our eminent virologist not to try such an approach as it was senseless, but I did not follow his advice. In independently designed experiments published about half a year after Hill we obtained positive transfection with a single exposure of chicken fibroblasts to DNA from two lines of mammalian virogenic cells (Svoboda et al., 1972). We also characterized the resulting viruses in the focus assay and assessed their efficient replication.

Things again turned in a wrong way, being even worse than the previous events. In the laboratory, there was a student who was taking drugs (as we learned later) and who deliberately destroyed most of tissue culture samples, trying to put the blame on somebody else. As the consequence of previous purges I was powerless to stop such crazy behaviour and I therefore called the police to clarify this case. In fact, the culprit was soon identified and I

supposed that the case was finished, as the police officer assured me. However, the secret police smelled an open door to our Institute and started to search through the documents and to interrogate people. Somebody informed them – and it was no secret – that in 1967 I was awarded an American Jane Coffin Child Memorial Grant. *Quelle delicatesse* for the secret police in an occupied country! I became suspect No. 1 because I was receiving American money without any obligation except for writing a progress report. The secret police officers came with a (for them) convenient story of my being involved in espionage. I then underwent long hours of interrogation, being asked stupid questions such as from where I knew Mrs. Ford, the grant administrator, and whether she was a relative of car builder Mr. Ford. After several months I became exhausted and depressed. The last trump they drew from their pocket was their indignation that I bought several scientific books with the grant money. Because I kept these books in my office, there could not be any doubt of the money being misused. After that, I was relieved of interrogation, but my prospects remained grim. The US President launched the Virus-Cancer programme, which should have joined the leading scientists in the field. Being invited, which I took as an honour, I asked the police whether I could take part at this highly important meeting. I was told that it was possible, but the final decision would be given at the airport. I thus prepared for the meeting and was waiting in the lounge. Close to the departure I was informed that I was not allowed to go. Similarly, James Watson invited me for a Cold Spring Harbor symposium, but again I was not allowed to go although Watson wrote a special letter to the then President of the Academy – there was no answer. Fortunately, the text of my talk was smuggled to the symposium by Jan Závada. We presented there the ultracentrifugation analysis of XC DNA fragments efficient in transfection and concluded that only the DNA molecules that are of the provirus size or larger are active in transfection experiments. Furthermore, we documented profound differences in sensitivity to transfection among different avian cell strains (Svoboda et al., 1975).

Of decisive importance was a "rescue" visit of British scientists led by Michael Stoker and based on the Academy agreement. They organized a scientific meeting with their Czech colleagues and strongly recommended our Academy to support research in virology and cancer. This was a turning point. In the dark years, additional visits of scientists from the West such as the repeated visits of Abraham Karpas helped us keep an optimistic spirit.

Partial Thawing and Molecular Biology

... freedom of thought in human history – not freedom in general, which has too many ambiguities, and may even be identified with the freedom of the strong to exploit the weak, but freedom to think and to speak.
Stone, I.F.: The Trial of Socrates, Anchor Books Doubleday, New York, Little, Brown and Company, 1988.

I am describing all these events because they forced me to accept the fact that I was not able to stay in the forefront of retrovirology any more, as well as to stay in contact with the dramatic development in this field. Retrovirology became technically demanding and dependent upon the availability of special biochemicals such as labelled nucleoside triphosphates, restriction enzymes, etc., which were out of reach for us. Our only resource, the American grant, was lost in the end. Looking for an open question within our reach, I became persuaded that of key importance were non-virogenic cell lines, where we postulated the presence of only the RSV transforming part. I still kept in touch by mail with Marcel Baluda and he agreed to perform a collaborative study aimed at provirus definition in one non-virogenic mouse cell line. Using liquid hybridization (labelled viral RNA annealed to cell DNA) it became apparent that in the mouse cell line employed, only about one third of proviral sequences are present (Svoboda et al., 1977). In the meantime, the molecular definition of RSV oncogene v-*src* was achieved by Stéhelin et al. (1976). The question remained whether it corresponded to the partial provirus in non-virogenic cells, which was likely, and how such a provirus might have arisen. Relying in those days on my own "working force", I decided to induce, by cloned RSV, a series of hamster tumours in order to obtain fresh defined material and to get an estimate of different virus-cell interactions. Very often, proviruses in hamster cells were defective, sometimes amplified, but the presence of inducible full proviruses was encountered regularly. One out of 24 tumours behaved as non-virogenic and thus we obtained a proper model for further studies. The situation at the Institute started to improve thanks to the new director, Josef Říman, who himself was a successful scientist and a clever and helpful person devoted to science. Under these new circumstances at the end of the seventies I was finally allowed to spend two months at Dominique Stéhelin's in Lille.

My visit in Lille had been totally concentrated on experimental work since the first day after my arrival. I brought with me representative samples of RSV-transformed cells and felt ready to become devoted to molecular hybridization procedures that for years had been escaping our technological possibilities. Dominique and his young enthusiastic collaborators were very helpful in introducing me to this fascinating field and I spent days and nights in the

laboratory in order to catch up with the time allocated to my visit. Soon I was able to analyse my cell lines. Shortly it became apparent that the non-virogenic line harboured a highly deleted provirus comprising about one third of the full provirus present in virogenic cells. Such a simplified provirus retained only the expressed oncogene v-*src* and viral regulatory sequences (LTRs). Thus finally non-virogenic tumour cells were characterized structurally (Svoboda et al., 1983). With good feeling, I was returning through Paris, where I was invited for a seminar at Collège de France and was pleased by a nice introduction given by Jean Dausset. To cross Germany by train, I was allowed a limit of 24 hours. As I was in phone contact with Fritz Deinhardt at the Pettenkoffer Institute in Munich, he made an arrangement with the German control at the entry borderline not to stamp my passport – in this way, my journey would be uncontrollable. The policeman at the border was informed and assured me that he was not going to give me the stamp, but unfortunately, by habit he had done so. For me this meant that after shaking hands with my friend in Munich I had to leave for Prague. Nothing dramatic happened, but this story illustrates some consequences of the divided Europe.

Returning home I faced a new task in establishing retroviral molecular biology. Graciously, Dominique supplied me with most of the necessary components required for starting molecular analysis, which I did, and I also attracted some students. However, there were important items such as enzymes and nitrocellulose, which came from the West with long delays and irregularly and which were not distributed according to scientific criteria.

Together with my former student Josef Geryk we attempted to rescue the highly simplified provirus consisting of LTR, v-*src*, LTR. After many fruitless attempts we succeeded in transmitting this provirus using cell fusion combined with very efficient superinfection with a helper virus (Geryk et al., 1986). Originally I assumed that rescued viruses would originate only by recombination between the provirus and the helper virus. The outcome of the experiments was more complex. In fact, we obtained clear evidence for the rescue and transmission of the LTR, v-*src*, LTR provirus without recombination (Svoboda et al., 1986), but also for its recombination with the helper virus. Using different markers we defined, together with Ram Guntaka, that recombination led to the acquisition of a part of the viral replication gene (*gag*), but the transforming gene v-*src* was kept intact (Svoboda et al., 1990). Of major importance was the LTR, v-*src*, LTR nucleotide structure. This required more sequencing, which was made possible by a kind gift from NIH colleagues Bob Gallo and Mika Popovic, who provided me with the sequenase kit that I decided to transport to Prague at any cost. I had to take with me two big boxes filled with dry ice and samples. After

reaching Frankfurt, I was first watching for the police, and when the patrol disappeared, I slipped to a narrow corridor leading to the lavatory and transferred dry ice from a reserve box to the sample box. Clouds of evaporated CO_2 were produced as if I were a carbonieri preparing an explosion. Fortunately, my unusual behaviour remained unnoticed and therefore I brought my treasures safely to my destination.

There appeared new problems related to provirus cloning that required phage packaging extracts. Finally, some had been home-made and some were kindly provided by Mariano Barbacid. We then obtained a clone that represented the first unique sequence cloned in our country and we established its primary structure. It became clear that the LTR, v-*src*, LTR provirus arose by regular reverse transcription of *src* mRNA, thus lacking any viral replicative gene sequence (Bodor and Svoboda, 1989). This finding provided confirmation of our original postulates and interpretations according to which the non-virogenic cells carried only the virus transforming part.

I was happy about this progress, but it became obvious that we could not rely forever on scientific charity and gifts. In one way we were in a preferential position, because we had access to defined breeds of chickens. We therefore focused part of our effort on the definition of avian leukosis virus (ALV) pathogenesis. Furthermore, we asked ourselves whether the proto-oncogene c-*src* could be captured by ALV lacking any *src* gene sequences. This happened with very low frequency and led to formation of a new avian sarcoma virus called PR2257 (Geryk et al., 1989). As revealed by sequencing, this virus acquired full c-*src* and, in addition, a long stretch of the c-*src* untranslated region. The virus became oncogenic by a single nucleotide insertion resulting in an altered reading frame at the c-*src* carboxy end. We prepared more interesting mutations (Yatsula et al., 1996), but the *src* field became so crowded that after a while we discontinued further work.

Deep changes triggered by Gorbatchov's Perestroika were on the move in the late eighties. This can be documented by the International Congress of Biochemistry held in 1988 in Prague, where my session on Retroviruses was attended by eminent scientists such as H. Temin, H. Hanafusa, S. Hughes, M. Lineal, R. Guntaka, and others. It became obvious that in certain aspects we were touching an especially simple provirus and its recombinations, not lagging behind Americans. There were excellent scientific discussions and open-minded comments on what was going on at our farm in Koleč and in my home. We succeeded in re-establishing confidence and collaboration with our colleagues, in which stayed especially Ramareddy Guntaka, Steve Hughes, and Howard Temin.

Silencing the Provirus and the Red Power

Chacun pour soi et Dieu pour tous.
Everyone for himself and God for all.
French proverb cited by F.M. Dostoyevsky in his Diaries.

Hrajte, hoši, komedii,
bouda je dost veliká;
však je v této vaší boudě
věru chasa všeliká.
Z básně "Centralistická", Jan Evangelista Purkyně: Opera omnia Tomus XI, Academia, Praha 1968.
Get down to your comedy
Guys, your hut is spacious;
With those you have to play inside –
Of all the kinds, good gracious!
From poem 'Centralistic', J.E. Purkynje, Opera Omnia Tomus XI, Academia, Prague 1968.

The year 1989 was very fruitful for me. From my past experience, especially from 1968, it indicated deep changes under way. This was heralded by disobedience to police orders, local demonstrations, finally culminating in a massive student demonstration. As usual, the police were ruthless, but in addition, horrifying news circulated that a student had been killed. Despite that it was untrue, it triggered an emotional reaction and enforced persuasion that the rotten regime of the Party secretaries, which was linked to Soviet occupation, should be overthrown and replaced by a democratic government. I felt deep satisfaction that the demand of 1968 would be finally revived and substantiated. That was why I took part in street demonstrations attended mainly by youngsters, while a mob of adults looked reluctantly at what was going on. I became persuaded that the end of the communist era was close when we reached the Old Town Square and rhythmical shouting was heard "Here it had started and here it all ends" (the communist coup d'état in 1948 was announced just at this same place). Then I moved to the Radio Broadcasting Station, where a group of people whom I joined were arguing with armed policemen and tried to persuade them that the end of their power had come. Later, I met by chance the wife of Ladislav Hejdánek, one of the main co-authors of Charta 77, and in their home I celebrated the end of the Red era. Ladislav

remarked pertinently that this end should have come earlier, but one cannot give orders to history.

The Academy soon became a boiling soup. The views on its future differed diametrically, because a body of respectable and honest representatives was lacking. Finally, an assembly of members of the Academy (Academicians) was called together, supplemented, however, with representatives of Academy Institutions selected by scientists. A new governing body called Committee for Directing the Institutes had been elected and O. Wichterle, a highly respectable person, was assigned in charge of it. I was also elected as a member of this body. Many tasks had to be solved, but as a result of incompetence and pseudo-radicalism of a part of the members, the meetings of the Committee were not very fruitful. However, some important points had been reached. The dissolution of the body of Academicians dominated the discussions, as well as reshaping the Academy as a Confederacy of Research Institutes and establishing elected scientific councils. All this was incorporated into a law, which the Parliament approved. I had a good feeling about it because the main accent was on science and the power of directors was limited by elected scientific councils. However, public recognition of science remained low because of some misleading concepts such as that the communism identified itself with scientific progress. In reality, it was just the contrary; the Soviet block was not capable to ensure the required tempo of science development in its non-military branches. I took advantage of Harold Varmus' stay in Prague, and thanks to the contacts of Ladislav Hejdánek I arranged his visit to President Havel. Harold had argued excellently in favour of science, but I don´t think he persuaded our president.

Another burden was put on me because I was elected director of our Institute. I was showing quite a lot of resistance to this honour because I remembered taking over the directorship in 1968 and the consequences, but I was almost forced to take this responsibility. At the Institute level, we tried hard to equilibrate the budget because the allocated money was less than we had expected. The situation improved later with founding of the Grant Agency and with the availability of foreign grants, for which I strongly recommended to apply.

Another problem arose with the opening of the border. Understandingly, students wanted to acquire experience abroad and suddenly started to emigrate. My group lost three of them at once. Unfortunately, there was no chance to attract some of the colleagues who had emigrated previously because the conditions we were able to offer them were not comparable with those in their current position. Furthermore, I had to replace those group leaders who had

signed collaboration with the secret police. Therefore, we had to reshape the scientific groups and finally succeeded in stabilizing the staff. In fact, people were allowed to go abroad for several years without losing their position in the Institute, which was the only attraction I was able to provide.

Being exposed to so many demanding tasks, only spare time remained for my scientific work. However, I still kept in close touch with my group. Of my continuous interest was the demonstration that the v-*src* oncogene induces specific tumour rejection immunity and contains the already mentioned TSTA. Based on my own experiments I supposed that the LTR, v-*src*, LTR provirus would be a suitable tool for determining the structure of TSTA, because its DNA produced fast growing sarcomas in chickens (Svoboda et al., 1992). Together with Jiří Plachý we made a series of experiments demonstrating that v-*src*-induced tumours tend to regress in one congenic line of chickens, but progress in another one. The difference between both chicken lines lay in the B (MHC) locus (Svoboda et al., 1996). Then using further approaches we demonstrated that in regressor chickens cell-mediated immunity is responsible for tumour rejection, that it is possible to elicit this immunity by DNA immunization (Plachý et al., 2001) and that a specific antigenic epitope is localized at the RSV v-*src* carboxy end. Thus v-*src*, as a result of a stretch of 12 new amino acid incorporations, acquires a specific rejection antigen. All this enterprise was made possible by breeds of our congenic chicken lines in the Koleč farm.

Another phenomenon was puzzling, namely the lack of reversion in our virogenic cell lines. In some lines such as XC cells, as we learnt from our experience, the proviruses were amplified and therefore the likelihood of getting them all silenced was very low. How was it with a hamster cell line carrying only one incomplete LTR, v-*src*, LTR provirus? We tackled this problem together with Jiří Hejnar and found that revertants in this cell line arose with a high frequency far exceeding spontaneous gene mutation (Hejnar et al., 1994). The provirus in revertants was highly and irreversibly methylated, as had been previously observed by John Wyke's group (Searle et al., 1984). However, when proviruses from revertants were demethylated, they re-acquired full oncogenicity, which proved that an epigenetic change was involved in the reversion. As suggested by some others' and our experiments, avian proviruses can be efficiently silenced in mammalian cells. Could such silencing be a consequence of provirus and especially RSV LTR methylation? A series of additional experiments agreed with this conclusion. Finally we demonstrated that RSV LTR protected by an antimethylation CpG island expresses reporter genes in mammalian cells far better than unprotected constructs. These studies are part of our long-term endeavour aimed at the definition of

chicken cell factors facilitating RSV production in mammalian cells. The quest for identification of cell factor(s) modifying the course of retrovirus infection became an important issue having a great impact on finding new therapeutic strategies, including HIV. Separately, I summarized seven so far known blocks of RSV replication in the mammalian cell (Svoboda, 1998). However, in the more intensively studied HIV even a higher number of cellular factors, either positively or negatively influencing virus replication, are in play. The long-term persistence provides retroviruses with a dangerous means to escape the defence activity of the organism. In pursuing the course of ALV infection in ducks lacking any endogenous viral chicken sequences we established that ALV had persisted in infected ducks for 5 years, which was the period of observation (Nehyba et al., 1990). This was corroborated by detecting proviral sequences in different organ tissues, especially during the early period after infection. Interestingly, we noticed periods of viraemia appearing and disappearing irregularly. This model of viraemia has been elaborated in detail (Trejbalová et al., 1999) and should be useful for further studies in this direction. By this I have exhausted our main interests in the last turbulent period.

Epilogue

Ať si bylo, jak si bylo, přece jaksi bylo, ještě nikdy nebylo, aby jaksi nebylo.
Hašek, J.: Osudy dobrého vojáka Švejka za světové války, Československý spisovatel, Praha, edice Slunovrat, 1987.
Good times or bad times, they always were times of a kind; no times have ever been without being of a kind.
Whatever the times, they always were liveable, no times were ever unliveable.
The Good Soldier Svejk and His Fortunes in the World War, Československý spisovatel, Prague, edition Slunovrat, 1987.

Dobré pivo, dívka hezká, to ti dává země česká!
Nápis v české hospodě.
Good beer and a pretty girl's cheer, that's what you get behind the Czech country frontier!
Inscription on a Czech pub wall

It is hard to imagine that I am supposed to write an epilogue because in science, there is no epilogue. What I want to do is to comment on some crucial issues mentioned in these

memoirs. People from the West might wonder how much space I dedicated to social life. Everybody who lived in Central Europe in the second half of the last century had to face the problem how to cope with the disturbing flow of events without losing moral consciousness. Not surprisingly, individuals behaved in various ways. I keep in my mind the shock when I learnt that almost half of the group leaders of our Institute had actively collaborated with the communist secret police. This I would never have imagined.

The mind of a scientist is generally obsessed by the vision that they are the "best and brightest" rulers in their field. In this respect, I would like to rememeber T.G. Masaryk, renowned thinker and first President of our Republic, who mentioned that he never liked to be the first but the second. Finally, he became the first not by pushing himself, but being pushed by others. Scientific success is the dream of every scientist, but it remains risky and unpredictable. As many of us know, such success requires clear, stubbornness ideas, and hard work. Of great importance is speed of research ensured by the required resources and by the availability of trained students. The great majority of people reach the level of good science. As Howard Temin, who belonged to the wisest men I have ever met, commented: "Don't sell good for the best". I have never been selling out good and critically respected the best.

We are now getting to the issue of scientific collaboration. Fortunately, I had been repeatedly supported by international agencies and by my Western colleagues. Nowadays, we have access to a series of American and European grants, which are still not used efficiently. However, with some nostalgia I remember the grounding period of retrovirology and cancer research in the sixties, when a handful of people involved were supportive to each other, knowing that the survival of these fields, which had been under heavy pressure from the side of classical disciplines, was in stake. At present, we have available international collaborative grants, which provide real stimulation to our laboratories and speed up the solution to problems by complementing technology and ideas. Recently, Mike Bishop raised his voice, favouring collaboration versus competition in science, and I agree with him (Bishop, 2003).

Coming from a small country, I would like to comment on this fact briefly. In the sixties Hašek put forward the idea that the Institute should create such a micromilieu that would protect it from external pressures. In that post-Stalinist era, the strategy provided a real shield. In spite of the fact that at present there is no need for such protection, a favourable co-operative and friendly atmosphere may contribute to attracting good people even from abroad. What kind of research can be performed in a small country? The answer is not simple, but preferentially, such research should be based upon outstandingly original observations and ideas.

I would also like to add a few words about loyalty. I was criticized by Howard Temin for not having left my country after the occupation. When I explained to him that the responsibility and probably the future of our Institute had been put on me, he agreed that mine was the right decision. I should underline that the Institute of Experimental Biology and Genetics represented a unique institution in our country and certainly was worth saving. Nowadays, the scientific institutes operate on the basis of selection of staff members from different resources and sometimes it is hard to distinguish among them. However, there remain places with a traditionally high level of original-minded and co-operative science such as at Cambridge University and McArdle in Madison, which hopefully will be preserved and will keep their traditions.

Finally, how does the situation stand and will probably develop in the directions I covered in this review? The oncogene v-*src* and others, as well as their normal counterparts, were defined as crucial members of cell signalling and gene regulatory pathways, which represents essential information. However, we are still lacking the full picture about how the oncogenes accomplish their tumorigenic activity. As a special example, I would take v-*src*, transforming rodent but not human cells.

Retroviruses have become functionally characterized at the level of single nucleotide stretches. However, a new frontier of retrovirus control has appeared, represented by cell factors that either stimulate or inhibit virus replication. We were confronted years ago with such factors in mammalian RSV-transformed cells, which for virus production required complementary chicken cells (Svoboda and Dourmashkin, 1969). Using transfection and cloning, more recently genes negatively regulating HIV entry (TRIM5α) and reverse transcription (APOBEC36) were described (Bieniasz, 2004). There is good hope that they will be utilized in the battle against HIV. Thus, the past is contributing to the present. I am going to finish with a very appropriate statement by Peyton Rous (1965):

'Perhaps to-morrow some cleaving discovery on the causation of tumours by viruses will demolish the inferences of to-day; yet what we now know would be worth little if none was made'.

Acknowledgements

I am deeply grateful to my parents for backing me in many hard situations. Similarly, my wife Ingrid helped me wholeheartedly to surpass many critical situations and turmoils. I am indebted to Ladislav Hejdánek for introducing me to critical thinking. I highly appreciate and value my mentor Milan Hašek, who had shown me new horizons in biology and stayed supportive and inspirational. Of the university professors I wish to thank F. Sládeček and F. Patočka for their patience with me and for the background to developmental biology and microbiology, respectively. At the Hašek´s department I made collaboration and friendship with Jan Grozdanovič, Pavel Koldovsky and Ivan Hilgert well as with Věra Hašková (see the text). Almost since my childhood I have been in friendly relationship with Jan Závada. Additional important virologists Ctirad John, Josef Říman, Dimitrij Slonin and Vladimir Vonka were always keen to consult and help. Among oncologists I was kept stimulated by a great enthusiast Jan Šula, Jaryna Jakoubková and by my Slovakian friends Villiam Thurzo, Čestmír Altaner and others.

I wish to thank cordially Š. Takáčová and H. Roubalová for editing this manuscript.

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