

An exploration of bioactive peptides: My collaboration with Ervin G. Erdős

DOI 10.1074/jbc.X118.003433

Rajko Igić^{‡§¶||1}

From the [‡]Department of Pharmacology, Medical School, University of Banja Luka, 78000 Banja Luka, the [§]Academy of Arts and Sciences, Republic of Srpska, 78000 Banja Luka, Bosnia and Herzegovina, the [¶]Department of Anesthesiology and Pain Management, Stroger Hospital of Cook County, Chicago, Illinois 60612, and the ^{||}Čitaonička 21, 25000 Sombor, Serbia

Edited by Herbert Tabor and the Reflections Committee

This paper provides a brief historical sketch of the science of biologically active peptides. It also offers the story of how Ervin G. Erdős, a pioneer in the study of metabolism of various peptides, influenced me through collaborations that span many years. I worked in Dr. Erdős's research laboratories in Oklahoma City, Dallas, and Chicago, and we shared research interests through visits across the Atlantic between the former Yugoslavia and the United States. Among other findings, we discovered angiotensin-converting enzyme in the retina, which opened up a new research direction for many scientists interested in serious ocular diseases. This tribute to my mentor paints a portrait of a man who, in addition to his dedication to science and his seminal discoveries about the metabolism of peptides, took the time to invest in training many young scientists. His fine personal qualities explain why all of those who worked with him hold him in such high regard.

In July 15th of 1969, I was in Basel, Switzerland for the Fourth International Congress on Pharmacology. I was a medical doctor who had become interested in scientific research, and was almost finished with the experiments for my dissertation, so the conference was a welcome break from the last few days in the laboratory. All the participants had a dinner together that night. I found my place at the table and slipped into my seat. Next to me sat a distinguished young gentleman with dark-rimmed glasses. I introduced myself, and he offered his hand and name, "Ervin Erdős from Oklahoma from the USA." I had certainly heard of him and had read his research papers on bioactive peptides.

Following an extended conversation, he mentioned that we would have a day off from the meeting for people to enjoy the sights in and around Basel, and he suggested that I join him to see Lake Lucerne. From that fortuitous meeting, I realized that Dr. Erdős was not one to sit idly by, especially if there were places to go. At the end of an enjoyable day together, he invited me to come to the USA as an exchange scientist to work with him on the metabolism of vasoactive peptides (*i.e.* peptides that affect the diameter of blood vessels and thus blood pressure). At the time, I never gave a thought to how that invitation could

change my career and my life. It would lead me to work and live on two continents, form a life-long collaboration with a wonderful mentor, and save me from participating in the Yugoslav war.

Back to the beginning

How did I find myself in Basel that day? I grew up in Sombor, currently part of Serbia. I attended the University of Belgrade and received my medical degree in 1963. Then I practiced medicine for two years as an employee of the Sombor General Hospital and as a general practitioner at Kucura, in Northern Serbia. However, I found myself wishing for something else. I decided to return to my studies, and got my Ph.D. degree in Pharmacology at the University of Sarajevo in 1970. My mentor for this advanced degree was Professor Pavao Stern, the program director and chairman of the Department of Pharmacology. His department was one of the best places for research in the former Yugoslavia, and he worked with a number of other prominent scientists.

Professor Stern was known for groundbreaking studies in several fields (1), making his laboratory an exciting place to work. For example, he had showed that substance P, a fine powder extracted from intestines that dilated blood vessels (*i.e.* that caused vasodilation) and effected contraction of smooth muscles, was a neurotransmitter of pain in the central nervous system (2). He also organized the First International Symposium on Substance P in 1961. The symposium was a rare occasion for scientists of the East and West to meet together, including some of the best pharmacologists and physiologists of that time, such as U. S. von Euler, Sir J. H. Gaddum, P. Stern, M. Vogt, B. Pernow, V. Varagić, F. Lembeck, and K. Lissak (Fig. 1). He is also credited as one of the discoverers of antihistamines (3). My own research with Professor Stern built from prior work showing that the effectiveness of anti-Parkinsonian agents was typically linked to their ability to block the activity of acetylcholine, another neurotransmitter. Thus we explored Parkinsonian tremor, the localization of acetylcholine in various parts of the brain, and the effects of cholinesterase inhibitors on rat behavior after application to specific regions of the brain.

Professor Stern's global interest in neurotransmitters and research related to Substance P, the peptidic sequence of which was determined the year after I graduated, meant that those of us in his lab were also familiar with other peptides being investigated at the same time that helped to regulate cardiovascular

The author declares that he has no conflicts of interest with the contents of this article.

¹ To whom correspondence should be addressed. E-mail: r.igic@excite.com or igicrajko@gmail.com.

This is an Open Access article under the [CC BY](#) license.





Figure 1. Participants of the First International Symposium on Substance P, Sarajevo, June 9–10, 1961, organized by my Ph.D. advisor Professor Stern. U. S. von Euler, one of the co-discoverers of Substance P, and Stern are standing as the third and sixth from the right, respectively.

and renal function. For example, the kinin polypeptides were known to be potent vasodilators, stimulating release of nitric oxide, bioactive lipids, and other mediators. On the other hand, the peptide hormone angiotensin II, a component of the renin–angiotensin system (RAS),² was known to cause vasoconstriction. At the time, research was focused on trying to understand the metabolism of these peptides to their active forms, the full scope of their bioactivity, and the mechanisms of inhibition. But how did the field build to this point?

Enter Ervin G. Erdős

The Fourth International Congress on Pharmacology in Basel, Switzerland, 1969, turned out to be another gathering of prominent scientists. When I met Dr. Erdős (Fig. 2), I thought that he was too young to be a full professor, because most professors and other gray-haired scientific grandees in our land were already in their sixties or seventies, whereas he was merely in his forties. However, Dr. Erdős was already an established researcher, having discovered three important enzymes and edited several valuable books (4–6). No wonder he was already considered by many to be “the leader of the field.”

Erdős was born in Budapest, Hungary, the country where his family had lived for centuries. He finished Gymnasium in Budapest just at the beginning of World War II. Because he was Jewish, he could not enter a university, so he spent several years working as a steel worker and later at forced manual labor (7). Close to the end of the war, he spent six months in the Sachsenhausen concentration camp in Berlin. After the war, Erdős entered medical school in Budapest. As a medical student in the late 1940s, he worked in the Department of Pathophysiology, where he did bioassays using strips of guinea pig intestine. This work resulted in his first publication (8) and influenced his future interest in pharmacological research.

Soon afterward, Erdős managed to get to Munich in the U. S. zone of Germany, where he took his final examinations and received his M.D. degree. In 1952, he joined the laboratory of Eugen Werle as a postdoctoral fellow. Werle and E. K. Frey had



Figure 2. Ervin G. Erdős in 2002.

discovered the serine protease kallikrein, which releases kinins such as bradykinin and kallidin from larger polypeptide precursors called kininogens (Fig. 3), establishing the kallikrein–kinin system (KKS); they described many of the basic mechanisms of this enzymatic cascade during the 1920s and 1930s. Dr. Erdős’ involvement in the kallikrein–kinin system research in Dr. Werle’s laboratory inspired his life-long interest in peptides and peptidases. Erdős left the Werle laboratory to emigrate to the USA in the middle of 1950s, where he worked in Pittsburgh; in 1963, he moved to the University of Oklahoma where he spent the next ten years.

Dr. Erdős’ initial discovery in Oklahoma was that an enzyme he named aminopeptidase removes the N-terminal Lys¹ of kallidin to form bradykinin, while a different aminopeptidase (aminopeptidase P) and a carboxypeptidase (kininase I or carboxypeptidase N) deactivate bradykinin by removing the N-terminal Arg¹ or the C-terminal Arg⁹, respectively (4) (Fig. 3). Later on, he discovered an enzyme in blood and tissues that inactivated bradykinin by removing the C-terminal Phe⁸–Arg⁹ dipeptide, which he named kininase II. Soon afterward, he and his colleagues showed that kininase II was identical to angiotensin-converting enzyme (ACE) (10), a finding I helped to confirm two years later (11). ACE was already known to convert the biologically inactive decapeptide angiotensin I to the very active octapeptide angiotensin II. Thus this new discovery linked KKS to the very complex RAS (Fig. 3), a hormone system that regulates blood pressure and fluid balance at both the systemic and tissue levels, and involves about 20 peptidases (including the namesake renin), almost the same number of angiotensin peptides, and six known receptors.

Erdős and colleagues also discovered other peptidases that act on these and related bioactive peptides. For example, prolyl-carboxypeptidase cleaves (and thus deactivates) angiotensin II and other peptides that contain a proline as the penultimate residue. Also, they showed new roles for known peptidases in degrading the kinins, such as neutral endopeptidase 24.11 (nepriylsin), carboxypeptidase M, and deamidase–cathepsin A. These discoveries further strengthen our understanding of these bioactive peptide systems (4, 12, 13).

In his early studies on peptides, Erdős also developed a concept that had important medical implications. Briefly, the concept was that the rapid enzymatic metabolism of kinins determines their transient effects. For this reason, bradykinin or

² The abbreviations used are: RAS, renin–angiotensin system; ACE, angiotensin converting enzyme; KKS, kallikrein–kinin system.

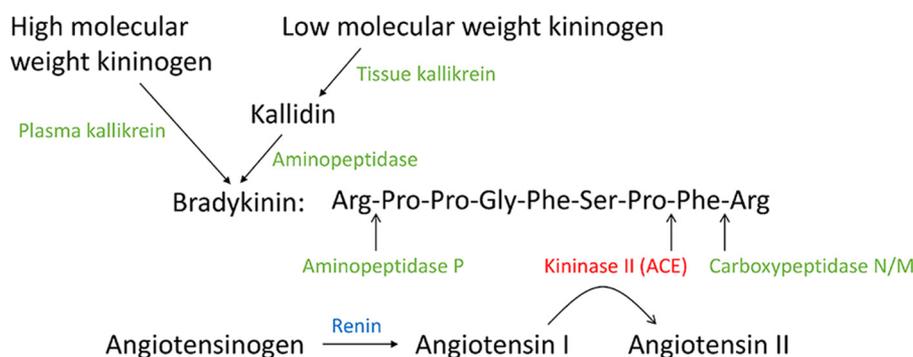


Figure 3. The peptides and peptidases in the KKS and a truncated portion of the RAS. The enzymes in the KKS are shown in *green*, and renin from the RAS is shown in *blue*; ACE, which acts in both systems, is shown in *red*.

kallidin would never be useful medications, but if they have a significant role in certain physiological or pathological conditions, agents that directly block their effects or inhibit their enzymatic degradation could be very important (14). This concept would lead to the development of ACE inhibitors, highly effective drugs for lowering blood pressure and decreasing oxygen demand by the heart.

In addition to the conceptual advances he made, Erdős also made important methodological advances that are lesser known. One such advance related to the kymograph, an instrument originally invented in the 1840s by a German physiologist, Carl Ludwig, to monitor blood pressure in animals, and used for more than half a century. How it worked was that the researcher attached a piece of thread to one end of an isolated tissue, with the other end of the tissue fixed in an oxygenated organ bath, and then connected the thread to a stylus that would scratch a smoked sheet of paper wrapped around the kymograph drum. The smoked drum would turn to record the contraction or relaxation of a lever connected to the smooth muscle organ. At the end of the experiment, recordings were preserved by immersing the paper in a liquid shellac fixative and drying them. It was incredibly laborious to use for a bioassay, although it was used for that purpose for many years and described as “the strongest tool in hands of a pharmacologist” (15). In Sarajevo, I frequently used a kymograph to record smooth muscle contractions of various tissues, so I can definitely vouch for it being a laborious process! Eventually, electronic recorders were substituted for the kymograph. The first such instrument was constructed in 1962 by Erdős and colleagues (16). Miniaturized electronic devices for registration of isotonic and isometric contractions soon became commercially available and widely used in many laboratories.

My adventure begins

It was with this backdrop that I took Erdős up on his offer for a scientific exchange. After I obtained my Ph.D. degree at the University of Sarajevo in 1970, I flew from Belgrade to the USA. I had always thought that many large Yugoslav cities were the height of development, but imagine my surprise when I stepped off the plane into a large and thoroughly modern airport! And there were such huge highways, certainly much wider and more extensive than the narrow roads throughout Sarajevo and elsewhere in Yugoslavia. When Dr. Erdős dropped me at the Faculty House, my temporary lodging, I had another surprise. This

facility with its Olympic swimming pool was far grander than any faculty quarters that I had ever seen in Europe. Oklahoma seemed to be a paradise indeed!

My surprise might have been due in part to the isolation we had been experiencing in Yugoslavia before 1948. At first some senior scientists were allowed to travel abroad, and speakers from outside Yugoslavia, and in other Eastern European countries, could be invited to the country, but eventually fellowships could be obtained for western countries. Initially, if local scientists traveled, their families had to stay at home (17). Whether due to decreased attention to regulations or my status as a Fulbright Fellow, I was delighted that my wife was allowed to join me.

My arrival at the University of Oklahoma was recorded in the “Roundup,” May 11, 1970, page 2:

“A FULBRIGHT FELLOW, Dr. Rajko Igić of Yugoslavia has arrived to spend a year working with Dr. Ervin G. Erdős, George Lynn Cross-research professor, in the Department of Pharmacology. He came here from the pharmacology department at the University of Sarajevo.”

The primary research focus of Dr. Erdős’ laboratory when I arrived was the metabolism of bradykinin and angiotensin. One mystery that followed from the initial discovery that kininase II and ACE are the same enzyme is that ACE has a higher affinity for bradykinin than for angiotensin I (18). This suggests that many cardioprotective benefits of ACE inhibition reflect increased bradykinin signaling rather than decreased angiotensin II signaling, especially with high dosages of ACE inhibitors. In particular, modulation of bradykinin receptors in the endothelium appears to be a major target of ACE inhibitors. This finding may lead to development of additional strategies that enhance bradykinin signaling. However, it is not entirely clear how modulation of bradykinin signaling contributes to the acute and chronic side effects of these drugs, so we had a lot yet to learn.

I plunged quickly into my work in the Erdős laboratory. My first larger project was to study the inhibition of ACEs from lung and plasma, using state-of-the-art techniques to purify the enzymes. When the final protein product was subjected to gel electrophoresis, ACE appeared as a single protein band that inactivated bradykinin and converted angiotensin I, confirming that ACE has both activities (11, 19, 20) The overlapping identity of ACE and kininase II was still controversial at that time

because many did not believe that the same enzyme could initiate vasoconstriction by two separate mechanisms (by activating angiotensin II and inactivating bradykinin) and because different sources of the enzyme required different chloride concentrations (21). However, these differences were resolved when it was discovered that ACE contains two active domains, each having different chloride requirements (22). Additional studies also confirmed that ACE is the main peptidase that cleaves bradykinin to an inactive metabolite (23), while other research showed that ACE cleaves a variety of biologically active peptides, including Substance P, opioid peptides (enkephalin, β -neuroendorphin, dynorphin1–8), and another peptide that causes vasodilation, neurotensin (24).

Early on, I depended upon bioassays to get results. I used strips of isolated rat colon incubated with an enzyme source to measure conversion of angiotensin I to angiotensin II. Strips of isolated rat uterus were used in a similar manner to show inactivation of bradykinin by enzymes. But now, instead of using the awkward kymograph, I had the original electronic transducer and recorder created by Dr. Erdös and his technicians at the Mellon Institute. The next step was measuring the hydrolysis of a simplified substrate, hippuryl-glycyl-glycine, in a Cary recording UV spectrophotometer, a new technique for me, because we did not have such a fine instrument in my department or any other research department in Sarajevo. I followed the hydrolysis of peptide bonds with TLC on ChromAR 500 sheets or silica gel-coated glass microfiber sheets. Six months later, with help of a colleague from the biochemistry laboratory, I developed a radioimmunoassay of angiotensin I using ^{125}I -angiotensin I and antiserum. This assay simplified some of our experiments and data collection immensely. Then I took my biochemical experiments to the whole animal.

Because the percentage of conversion of angiotensin I to II was still unknown, we designed an experiment to measure angiotensin I conversion after a single passage through the blood vessels of rat lungs. I perfused rat lungs *in situ* after the animal was anesthetized with sodium pentobarbital. The lungs were ventilated mechanically with a respirator. The right lung was perfused with Krebs solution at 37 °C, and perfusion pressure was recorded. Labeled angiotensin I was added to the perfusion fluid so that it passed through the lung, and the products were measured. The labeled peptide ($\text{Asp}^1\text{-Ile}^5\text{-[}^{14}\text{C]Leu}^{10}\text{-angiotensin I}$) and the split products were separated by high-voltage electrophoresis to determine the extent of conversion. During the single passage through the lung *in situ*, 45% of angiotensin I was converted to angiotensin II. The byproduct of ACE cleavage, the dipeptide His–Leu, was observed, but not the individual leucine and histidine amino acids that would have indicated processing by carboxypeptidases. Angiotensin II liberation was partially inhibited when a nonapeptide inhibitor (SQ 20881) was added to the perfusion fluid before the labeled peptide.

It was a moment of triumph to record such a definitive result in a whole animal, and simultaneously answer an outstanding question in the field. Yet a greater triumph was to come. Dr. Edward Frohlich's lab was in the building next to ours at the University of Oklahoma. He, too, was studying pulmonary circulation in rats. My skills with biochemical methods had

quickly improved during my work in Dr. Erdös' lab, but I had also impressed him with my surgical skills on laboratory animals. When Dr. Frohlich expressed frustration with laboratory preparations, Dr. Erdös told them, "I have a man who can cannulate a mosquito." They anesthetized a rat and invited us to come. In front of three men, I successfully performed the cannulation, making everyone happy, especially myself, and teaching their technician how to do it at the same time. I was glad that my reputation did not suffer, and Dr. Erdös was perhaps the happiest of all, because his claim of "mosquito cannulation" was no more than simple bragging.

The mentor emerges

Professor Erdös's laboratory was consistently populated with two or three young researchers from different countries. These individuals, like I did, came to spend a year or two in his laboratory to do research in biochemical pharmacology. It is important to emphasize the educational approach Dr. Erdös used with me and other young researchers. When I started to work in Dr. Erdös's laboratory, he frequently came from his office to observe my work, how I could pipette, how I could use a balance or microbalance, or how I sacrificed experimental animals for collection of tissues. I was not comfortable with his scrutiny at the beginning, but then I realized that he also observed all newcomers to his lab in this manner. He even carefully monitored older associates whenever they used a new method or performed an important experiment. In addition to his attention to technical details, and his help during the performing of the experiments, Dr. Erdös frequently supplied reprints of important papers that we read and discussed at our laboratory meetings. After I spent a year in his laboratory, he sometimes solicited my opinion about manuscripts that he had been asked to review. When we prepared a manuscript for publication, I always wrote a first draft of the methods and results section, and he followed with the rest of manuscript. In addition, whenever there was a visitor to the lab, he would invite me to sit in on the meetings. These inclusive moments were the best lessons for a young researcher. My second year in Dr. Erdös' laboratory was the most productive (11, 19, 20).

The young researchers who came from Japan were typically quite successful and published three or four research papers during their visit. I entered into an unexpected collaboration with one of these fellows, Shigetoshi Chiba, which brought together his expertise in blood delivery through the heart's pacemaker cells with my interests in cardiac effects of tremorine and oxotremorine, molecules that induce tremors in animals (25). This was actually a logical continuation of our common research interests, because I had studied these substances earlier during my Parkinson's research (26) and Dr. Chiba had published several papers using this method in Japan, but it had long-lasting consequences. When Dr. Chiba returned to Japan, he had a brilliant academic career. He became Chairman of a Department of Pharmacology, published numerous papers in peer-reviewed journals, and was appointed Dean of the Medical School at Shinshu University in Matsumoto. When I became a department chairman myself in Tuzla, I invited Dr. Chiba to give a seminar for our medical and graduate students. Then we formulated a collaborative arrangement between the Univer-

sity of Tuzla and Shinshu University in Matsumoto that was signed by the rectors of the universities. This brief but important alliance allowed two medical doctors from Tuzla and Banja Luka (Dr. Osman Sinanović and Dr. Ranko Škrbić) to train for two years in Japan.

American vignettes

During my two-year stay in America, I learned a lot, both scientifically and otherwise. Realizing that I wanted to remember many details, I bought a large notebook for recording my observations, impressions, and ideas. Among many other entries, I recorded my impressions of urban architecture and the structure of roads. For example, in America, dead end streets have a place at the very end that is wide enough for cars to turn around, which is not the case in Yugoslavia. I also noted that in Yugoslavia, builders rarely make entrances to public buildings, trains, or buses designed for handicapped persons, while handicap access is a regular (and now required) feature in public facilities in America. This enabled one victim of paralytic polio to complete his medical studies and ultimately to practice medicine in Chicago even when confined to a wheelchair. In contrast, the university administration in Tuzla refused to accept a medical school candidate who lacked one finger.

These differences in administrative procedures took up quite a large section in my notebook. Details related to differences in the laboratories of the two countries were even longer. For example, Erdős often reminded us to reorder chemicals, supplies, or experimental animals in a timely manner. This procedure was very simple. We just told his secretary what was needed. We reported any new substances or pieces of equipment that were urgently needed to Dr. Erdős; these would come by priority mail. What a contrast with ordering for our laboratories in Sarajevo! Professor Stern had to go through endless procedures, from the Dean's office, through the supplier to the ministry of Bosnia and Herzegovina. So it usually took months for approval to purchase a few milligrams of a chemical substance, and that would happen only if one was lucky and got approval. Professor Stern often wrote several letters a month to his colleagues abroad (mainly in the USA or Germany) begging them to send him the needed substances.

The greatest problem for me was to master spoken English. At my age, people only rarely acquired perfect command of English as a second language. Joseph Conrad, a Polish-English writer, was the exception that I can think of. He did not speak English fluently in his late twenties, but when he was exactly at my age (thirty-three), he became a captain of a steamer at a Belgian trading company. After three years, from 1890 to 1893, his spoken English became supreme, and his writing style is considered today as one of the best in English literature. One incident in particular pushed me to enroll in an evening class at a high school in Oklahoma City to improve my English. Early one morning, I got a call from the police department that my car was stolen. I misunderstood the idiom "Your car was found dead," and, in a panic, I quickly went to the lab and told Dr. Erdős that my car was stolen, with a dead man probably inside. When I got to the police station to retrieve my car, I learned the meaning of an English slang idiom "a dead car." Unfortunately, my attendance at night classes did not improve my English very

much. I concluded that to learn how to speak English, one should be a sailor rather than a scientist. Scientists usually do not speak much during the day; they mainly work individually or read.

Return to Sarajevo

Close to the end of my stay in Oklahoma, our first son, Petar Igić, was born. By being born in Oklahoma City, he immediately became an American citizen. I was also offered a position as a professor of physiology in Texas. However, although I had begun to feel quite at home in Oklahoma City, I thought it was time to return to Sarajevo. Professor Erdős donated various supplies for my laboratory back home, including some expensive peptides, so that we could continue our collaborative work. I also bought a number of textbooks and some small laboratory appliances, which I packed into boxes and a large chest. All in all, I returned to Sarajevo with gifts far more important than souvenirs.

When we returned to Yugoslavia, however, I realized I might have made a mistake. We had a problem finding an apartment for my family, which was only resolved when my schoolmate, Dr. Žanka Ilić, generously offered us her late parents' flat in Babića Bašta, the most beautiful part of Old Sarajevo. We were on a waiting list to buy a car and to enroll our baby in a nursery. Professionally, I was also frustrated by major resentment from some of my former colleagues. Furthermore, although I had received a research grant from the Republic Community for Science in Bosnia and Herzegovina that would help support my research, it was not always possible to use this money to buy certain pieces of laboratory equipment, and the administration of my department often rejected even simple purchases. I spent a lot of time convincing them that equipment, such as a modern balance for weighing laboratory animals or a freezer to store dead animals, would facilitate the function of our laboratory. Fortunately, I was able to buy the recorders, transducers, and a spectrophotometer needed for my studies.

After presenting a paper at the Sixth International Congress of Pharmacology in Helsinki, Finland, in 1975 (27), I was invited to a symposium on "Central Actions of Angiotensin and Related Hormones" in Houston, Texas. A member of the pharmacology department objected to the use of my own grant money for travel abroad and started the rumor that there was a secret political agenda involved. Fortunately, I was able to attend the meeting in Houston and presented my paper reporting the discovery of ACE in the retina. It was later published along with the discussions that followed each presentation (28). That was the first study on the RAS and KKS in ophthalmic literature (29, 30).

Yugoslavian vignettes

After my situation in the university quieted down, and I was feeling more confident that I was in the right place, I invited Professor Erdős to Sarajevo for three weeks as a Fulbright Exchange visitor. He would be our consultant and lecturer at the departments of pharmacology and nephrology in Sarajevo and also in some other Yugoslav cities. Professor Erdős stayed in Sarajevo for most of his visit. We also made arrangements for him to visit Split, Sombor, and Belgrade. During these side trips,

he had many interesting encounters. In fact, the stories of his experiences could fill an entire book, so I will give only one example. Professor Erdős and I embarked upon a sightseeing tour that would take us to Split, a large city along the Adriatic coast. We drove toward Split with one other colleague and did some fine sightseeing in Dalmatia, including Trogir and other sites of interest. An unexpected problem occurred when Professor Erdős was detained by the Split Police Department (in Serbo-Croatian, SUP). It started when the reception desk clerk of our hotel noticed that the professor's passport lacked a seal with the date of his entry into the country. A summons to the police department followed. I immediately called my colleague from Split, Dr. Zvonko Rumboldt, to come, and we accompanied the professor to the SUP. The head of the city police read aloud, in an authoritative voice, an extract from the Law about foreigners. He then stated that, according to the Law, the professor had entered the country illegally and that he must leave it within 24 hours.

"But I arrived in Zagreb on a plane that was practically empty," Professor Erdős said. "And the passengers passed through passport control without anyone noticing, because all the employees at that time were watching the Yugoslav national team compete in a soccer match." This information was digested by the head of the SUP with little effect.

Dr. Rumboldt then spoke up. "Look here, this is a distinguished scientist with a worldwide reputation. Professor Erdős is a man of integrity, and I, as a citizen of Split, would guarantee for him." Again, this plea fell upon deaf ears. Dr. Rumboldt argued passionately, but the head of the SUP was adamant. I could see that we were at an impasse—there seemed to be no hope of a positive resolution. Then I had an idea.

Choosing my words very carefully, I remarked, "After visiting Split, Professor Erdős is invited to Sombor to stay for two days in Tito's villa. He will lecture to a select audience of doctors and scientists from Vojvodina province." As I had hoped, my reference to the Yugoslavian president finally made an impact! The SUP head soon started to apologize to the professor for having kept him for so long, and offered us drinks all around. Although we politely declined the drinks, we were much amused by that sudden burst of hospitality. The truth was that we had arranged for Professor Erdős to stay in a beautiful villa owned by the municipality—this property originally belonged to Tito's deputy president Aleksandar Ranković, and when Ranković lost his position, the villa was available for our most distinguished guests. However, it is true that Dr. Erdős spoke to a group of local doctors and professors from the medical school in Novi Sad. There was much animated discussion following his lecture.

After our stay in Sombor, we stopped in Ravno Selo to visit my relatives and to show our guest a typical home in the village of Vojvodina. Professor Erdős was amazed not only by their tidy house with its large, well-ordered garden, a tractor, and other farm equipment, but also by the fact that they had a telephone in the house. His reaction was not surprising, because even as late as 30 years ago, a telephone was a rarity in many parts of the world, including parts of Europe.

Detours in Dallas

Erdős moved his laboratory to Southwestern Medical School in Dallas, Texas in 1973 and became a professor of pharmacology there. I would later spend three summer vacations, a period of two months each, in Dallas, working once again in his laboratory. The fact that I had such a position for the summer shows how the American medical education system differs from that in Yugoslavia. For one thing, professors in America work in their labs year round with only infrequent holidays. We in Yugoslavia have at least two months off in the summer in addition to two weeks of winter holidays. I usually stayed in the residents' dormitory, known as Residence Hall. This building was just across the street from the emergency clinic of Parkland Memorial Hospital where President John F. Kennedy died.

These summer sojourns at Southwestern Medical School gave me the opportunity to learn new techniques and to do the kind of research I could not possibly accomplish in Tuzla during the school year. For example, in Dallas, there were better facilities to measure enzyme activity than in my laboratories in Sarajevo or in Tuzla, and I enjoyed working with new collaborators. There was also an unexpected windfall due to the cooperation of the forensic medicine staff, which made it possible to study ACE in human tissues. Specifically, there were a large number of accidents and murders, particularly on weekends, and unclaimed bodies autopsied by the forensic medicine group could be used for research. I used the opportunity to continue some work begun in Sarajevo, of studying the activities of ACE and the RAS peptidase renin in the retina. As it turned out, ACE was far more active in the retina (31, 32) and retinal microcapillaries (33) than in plasma.

In Dallas, I met many other interesting researchers from various countries who had come to work with Dr. Erdős. There were postdoctoral fellows and colleagues from Japan, England, Israel, France, and Australia. Together, we were truly an international group. A visitor from France, Dr. François Alhenc-Gelas, collaborated with Dr. Erdős using immunochemistry to examine the distribution of ACE in human tissues. Even after he returned to Paris, he used to come back to Dallas for vacations. Some people from the lab could not understand why he preferred Dallas to Paris, and their disbelief was understandable.

While spending the summer in Dallas, I also noted the successful effect of a national campaign against smoking, especially among physicians and medical students. Their zealous approach to smoking cessation prompted me to start a similar campaign in Tuzla that could extend to the whole country. During my flight home across the Atlantic, I prepared a plan and sketched a poster (Fig. 4). When I returned to Tuzla and Banja Luka, I invited medical students there to participate in this national campaign. They printed 10,000 copies of the poster every year and distributed it all over Yugoslavia. Their campaign lasted for many years. Now, January 31st is the National Non-Smoking Day in Serbia and Bosnia & Herzegovina (34).

The collaboration continues

After I returned from Dallas to Yugoslavia, I moved my lab from Sarajevo to Tuzla where I became a head of the depart-



Figure 4. Antismoking poster. The following words are printed in Serbian (Cyrillic script), Croatian (Latin script), Macedonian, Slovenian, and English languages: We invite all smokers not to smoke on January 31st/Medical Students/We Care for Your Health.

ment. Without fail, Dr. Erdős came to visit my new place at the medical school in Tuzla and provide encouragement. Dr. Erdős gave several lectures to our graduate students and doctors from both Tuzla and Sarajevo.

In addition to my activities in research (35) and obligations for medical student lectures, I eventually became a program director of postgraduate studies (“poslediplomske studije,” in Serbo-Croatian) (36). I had an idea to improve the quality of science in Tuzla, and started by focusing on the lectures for this program. I invited many experts from various Yugoslav and foreign universities. Many of my former colleagues and profes-

sors were among them, including von Euler from Sweden, a former fellow student Kafait U. Malik, Shigetoshi Chiba, and of course Erdős.

Professor von Euler wanted to support my plan to build an international research center in Tuzla, where young scientists from Third World countries could work with guest faculty from developed countries. Visiting faculty members would stay in Tuzla for several months to initiate the common projects. Unfortunately, Professor von Euler died within a year after our discussion, and the Bosnian people were preparing for war rather than thinking about medical research.

When the war in Bosnia started, I fled Tuzla with my wife for my mother's home in Sombor, Serbia. I worked at the University of Novi Sad – Faculty of Medicine for several months, then emigrated to the USA. Professor Erdős, who had moved to the University of Illinois in Chicago by that time, helped me find a position at Cook County Hospital, in Chicago, and assisted me with adjusting to new surroundings. I worked in Chicago for twelve years as a Senior Scientist at the Department of Anesthesiology and Pain Management with Dr. Sara Rabito, an anesthesiologist, before health problems forced me to retire. During that time, I collaborated with Dr. Erdős and his group, who worked just across the street from me, and we published several additional papers together (37–39).

Final thoughts

Long ago, when I finished primary school in the village of St. Ivan (now Despotovo, Serbia), my teacher called my father to tell him that because I was the best pupil in the class, obviously I should continue my education. This teacher was thus responsible for my academic success. She sent me off to explore the unknown, to learn, to teach and to serve as a scientist, and to help mankind. It was not an easy path, but I have had so many wonderful teachers along the way, not the least of which was my mentor, Professor Erdős. I am very glad I met him, and I am grateful for the chance to work with him on various scientific projects. I especially appreciate his help and understanding when I became a Bosnian refugee. He helped me not only as a friend, but as a true brother. Thanks to Dr. Erdős, my acceptance in America during the Balkan war allowed me, my wife, and two sons to resume a normal life in the USA. I was able to continue research and other scientific activities, and avoid the war during the bloody disintegration of Yugoslavia. I've recently had the chance to reflect on four decades of research on both the KKS and RAS systems in the eye (40) and on the trauma of the disintegration of my country (9), and I cannot imagine what my scientific career and life overall would have been like without Erdős' influence.

Erdős' contributions to the science of peptides were a driving force in contemporary research in the field. He has received many distinguished awards for his discoveries and served as a mentor to numerous scientists who continue research in this field. In the middle of October 2017, Erdős celebrated his 95th birthday. About forty people, including his wife, sons, grandchildren, collaborators, and friends, came to help him celebrate a long and successful life. Most importantly, he joked around as usual and was very happy. It was an opportune moment to reflect on his many seminal scientific contributions that have had such importance for modern medicine, as in the ACE inhibitors for hypertension and other cardiovascular disease now taken by millions of patients. Certainly, the long and extraordinary career of Ervin Erdős elevates his name to the very top of the scientific elite.

Acknowledgments—I thank Dr. Alice R. Johnson-Zeiger (Gansevoort, NY) for reading the manuscript, and Professors Kafait U. Malik (Memphis, TN) and William B. Campbell (Milwaukee, WI) for suggestions.

References

1. Igić, R. (2003) Can outstanding research be done under less than ideal conditions? *Einstein J. Biol. Med.* **20**, 23–27
2. von Euler, U. S. (1977) Historical notes. In: *Substance P* (von Euler, U. S., Pernow, B., eds), pp. 1–3, Raven Press, New York
3. Kuschinsky, G., Lüllmann, H., and Mohr, K. (1993) *Kurzes Lehrbuch der Pharmakologie und Toxicologie*, Georg Thieme Verlag, Stuttgart
4. Erdős, E. G. (1966) Hypotensive peptides. *Science* **152**, 1284–1285 [CrossRef Medline](#)
5. Erdős, E. G., Back, N., and Sicuteri, F. (eds) (1966) Hypotensive peptides. Springer-Verlag, New York
6. Erdős, E. G. (ed) (1970) Bradykinin, kallidin, and kallikrein. In *Handbook of Experimental Pharmacology*, Vol. 25, Springer-Verlag, Heidelberg
7. Erdős, E. G. (2004) Peptides and the enzymes that release or inactivate them: A short history of my life and work entwined. In: *Selected Topics in the History of Biochemistry: Personal Recollections VIII* (Semenza, G., and Turner, A. J., eds) *Comprehensive Biochemistry*, Vol. 43, pp. 279–354, Elsevier B.V., New York
8. Erdős, E. G., and Csefko, I. (1950) Effect of calcium and atropine on the histamine- and acetylcholine-induced contraction of unstriated muscle. *Nature* **165**, 116 [CrossRef Medline](#)
9. Igić, R. (2017) *To Laza Kostić from Chicago*, Besjeda, Banja Luka (In Serbian)
10. Yang, H. Y. T., Erdős, E. G., and Levin, Y. (1970) A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. *Biochim. Biophys. Acta* **214**, 374–376 [CrossRef Medline](#)
11. Igić, R., Sorrells, K., Nakajima, T., and Erdős, E. G. (1972) Identity of kininase II with an angiotensin I converting enzyme. In: *Vasopeptides*, (Back, N., Sicuteri, F., eds) pp. 149–153, Plenum Press, New York
12. Ody, C. E., Marinkovic, D. V., Hammon, K. J., Stewart, T. A., and Erdős, E. G. (1978) Purification and properties of prolylcarboxypeptidase (angiotensinase C) from human kidney. *J. Biol. Chem.* **253**, 5927–5931 [Medline](#)
13. Gafford, J. T., Skidgel, R. A., Erdős, E. G., and Hersh, L. B. (1983) Human kidney “enkephalinase”, a neutral metallopeptidase that cleaves active peptides. *Biochemistry* **22**, 3265–3271 [CrossRef Medline](#)
14. Erdős, E. G. (1966) Hypotensive peptides: bradykinin, kallidin, and eledoisin. In: *Advances in Pharmacology*, (Garattini, S., and Shore, P. A., eds) Vol. 4, pp. 1–90, Academic Press, New York
15. Gaddum, J. H. (1964) An improved microbath. *Brit. J. Pharmacol.* **23**, 613–619 [Medline](#)
16. Erdős, E. G., Jackman, V., and Barnes, W. C. (1962) Instrument for recording isotonic contractions of smooth muscles. *J. Appl. Physiol.* **17**, 367–368 [CrossRef Medline](#)
17. Szekeres, L. (2003) Autobiography note with a message to young colleagues. *Pharmaca Iugoslavica* **41**, 33–37 (In Serbian)
18. Taddei, S., and Bortolotto, L. (2016) Unraveling the pivotal role of bradykinin in ACE inhibitor activity. *Am. J. Cardiovasc. Drugs* **16**, 309–321 [CrossRef Medline](#)
19. Igić, R., Yeh, H. S. J., Sorrells, K., and Erdős, E. G. (1972) Cleavage of active peptides by a lung enzyme. *Experientia* **28**, 135–136 [CrossRef Medline](#)
20. Igić, R., Erdős, E. G., Yeh, H. S., Sorrells, K., and Nakajima, T. (1972) Angiotensin I converting enzyme of the lung. *Circ. Res.* **31**, Suppl. 9, 51–61 [Medline](#)
21. Dorer, F. E., Kahn, J. R., Lentz, K. E., Levine, M., and Skeggs, L. T. (1974) Hydrolysis of bradykinin by angiotensin-converting enzyme. *Circ. Res.* **34**, 824–827 [CrossRef Medline](#)
22. Soubrier, F., Alhenc-Gelas, F., Hubert, C., Allegrini, J., John, M., Tregear, G., and Corvol, P. (1988) Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9386–9390 [CrossRef Medline](#)
23. Cyr, M., Lepage, Y., Blais, C., Gervais, N., Cugno, M., Rouleau, J.-L., and Adam, A. (2001) Bradykinin and des-Arg⁹-bradykinin metabolic pathways and kinetics of activation of human plasma. *Am. J. Physiol. Heart Circ. Physiol.* **281**, H275–83 [CrossRef Medline](#)
24. Skidgel, R. A., and Erdős, E. G. (1993) Biochemistry of angiotensin converting enzyme. In: *The Renin Angiotensin System* (Robertson, J. I. S., and

- Nichols, M. G., eds), pp. 10.1–10.10, Vol. 1, Gower Medical Publishing, London
25. Chiba, S., Igić, R., and Nakajima, T. (1972) Effect of tremorine and oxotremorine on the S-A node of the dog heart *in vivo*. *Tohoku J. Exp. Med.* **107**, 381–385 [CrossRef Medline](#)
 26. Igić, R. (1971) Effect of oxotremorine on the acetylcholine content of whole brain and various brain regions in the pigeon. *Br. J. Pharmacol.* **42**, 303–304 [CrossRef Medline](#)
 27. Igić, R., Robinson, C. J. G., and Erdős, E. G. (1975) Angiotensin I converting enzyme in the choroid plexus and in the retina. *Sixth International Congress of Pharmacology, Book of Abstracts*, Abstract No. 408, p. 176, Pergamon Press Ltd., Oxford
 28. Igić, R., Robinson, C. J. G., and Erdős E. G. (1977) Angiotensin I converting enzyme activity in the choroid plexus and in the retina. In: *Central Actions of Angiotensin and Related Hormones* (Buckley, J. P., and Ferrario, C. M., eds), pp. 23–27, New York, Pergamon Press [CrossRef](#)
 29. Van Haeringen, N. J. (1996) The renin-angiotensin system in the human eye. *Br. J. Ophthalmol.* **80**, 99–100 [CrossRef Medline](#)
 30. Choudhary, R., Singh, M., Kapoor, M. S., Singh, A., and Bodakhe, S. H. (2017) Therapeutic targets of renin-angiotensin system in ocular disorders. *J. Curr. Ophthalmol.* **29**, 7–16 [CrossRef Medline](#)
 31. Igić, R., Robinson, C. J. G., Milošević, Ž., Wilson, C. M., and Erdős, E. G. (1977) Activity of renin and angiotensin converting enzyme in retina and ciliary body. *Liječ. Vjesn.* **99**, 482–484 (In Serbo-Croatian) [Medline](#)
 32. Igić, R., and Kojović, V. (1980) Angiotensin I converting enzyme (kininase II) in ocular tissues. *Exp. Eye Res.* **30**, 299–303 [CrossRef Medline](#)
 33. Ward, P. E., Stewart, T. A., Hammon, K. J., Reynolds, R. C., and Igić, R. (1979) Angiotensin I converting enzyme (kininase II) in isolated retinal microvessels. *Life Sci.* **24**, 1419–1423 [CrossRef Medline](#)
 34. Žižić-Borjanović, S., Jerinić, M., and Igić, R. (2007) Twenty five years of antismoking movement started by medical students: some further goals. *J BUON* **12**, 181–184 [Medline](#)
 35. Igić, R. (1993) Substance P inactivation by aqueous humor. *Exp. Eye Res.* **57**, 415–417 [CrossRef Medline](#)
 36. Tahirović, H. (2015) Postgraduate studies. (1978–1985) at the Medical Faculty of the University of Tuzla, Tuzla, Bosnia and Herzegovina. *Acta Med. Acad.* **44**, 68–74 [CrossRef Medline](#)
 37. Minshall, R. D., Nedumgottil, S. J., Igić, R., Erdős, E. G., and Rabito, S. F. (2000) Potentiation of the effects of bradykinin on its receptor in the isolated guinea pig ileum. *Peptides* **21**, 1257–1264 [CrossRef Medline](#)
 38. Michel, B., Igić, R., Leray, V., Deddish, P. A., and Erdős, E. G. (1996) Removal of Arg¹⁴¹ from the α chain of human hemoglobin by carboxypeptidases N and M. *Circ. Res.* **78**, 635–642 [CrossRef Medline](#)
 39. Dragović, T., Igić, R., Erdős, E. G., and Rabito, S. F. (1993) Metabolism of bradykinin by peptidases in the lung. *Am. Rev. Respir. Dis.* **147**, 1491–1496 [CrossRef Medline](#)
 40. Igić, R. (2018) Four decades of ocular renin-angiotensin and kallikrein-kinin systems (1977–2017). *Exp. Eye Res.* **166**, 74–83 [CrossRef Medline](#)