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IN MEMORIAM

REMEMBERING THE WORK OF RADOSLAV K. ANĐUS (1926-2003)

It has been ten years since Professor Radoslav Anđus ('naš Profa' as we called him) passed away in Sveti Stefan and was laid to rest nearby his ancestors. Ever since my undergraduate years, he has been my teacher, mentor and trusted collaborator, and still is.

Fig. 1. Professor Anđus in the field, surrounded by fellow biologists.

His life has been inspiring in all manner of speaking. Professor Anđus obtained his Ph.D. in Physiology at the age of 27, and became the youngest member of the National Academy of Sciences at the age of 33. During his 50-year career, he held numerous positions at the University of Belgrade and abroad, received many awards and honors, and gained respect from scientists around the world. Everything he did was aimed at broadening scientific knowledge and bringing up a new generation of scientists. He never lacked the energy and enthusiasm for organizing research laboratories, planning experiments, analyzing data, and mentoring students.

Without a doubt, Professor Anđus' most significant results are related to the study of deep hypothermia. In the 1950s, Professor Anđus and his collaborators successfully resuscitated rats with a rectal temperature between 0-2°C, even after heartbeat and respiration had been arrested for almost an hour. After this, he started a long quest to reveal the limits and mechanisms of survival in deep hypothermia and hypoxia, using different animal species, developmental stages and physiological states as comparative models of tolerance. Numerous studies, based on classic techniques, such as freeze-trapping and biochemical assays, suggested a close correlation between survival/revival limits and brain energy metabolism. These scientific achievements of Professor Anđus were later included in the teaching material of 18 foreign university textbooks of physiology and more than 40 scientific monographs.

These experiments led Professor Anđus to investigate other effects of low temperature, such as tolerance of the cardiovascular system and cryoprotection. In several papers, he and his collaborators also addressed the relevance of the endocrine status of animals, the bioenergetic aspects of adaptation to high temperature, and the effects of hypothermia on the kidney and water-mineral excretion. Many years later, in the 1990s, with the development of new research techniques, Professor Anđus used *in vivo* phosphorus 31 nuclear magnetic resonance (31P NMR) spectroscopy with its fast acquisition time to non-invasively follow metabolic events in the rat brain after oxygen deprivation by deep, lethargic hypothermia (15°C rectal temperature). Serial spectra were used to reconstruct the time-course kinetics of intracellular pH and concentration changes of ATP, ADP, phosphocreatine and inorganic phosphate. Depletion profiles were derived for a number of parameters of the brain energy status (energy charge, phosphorylation potential, total adenylate and primary energy stores, expressed as the sum of high-energy phosphate-bond equivalents). A correlation was established between the energy status of the brain and the physiological descriptors of tolerance (survival and revival times). These experiments were of great value to investigators who wanted to achieve satisfactory time-resolution in NMR studies of extremely fast metabolic events, particularly those in the brain. In addition, these detailed studies of the physiological basis of tolerance may also be of interest to researchers and clinicians who use hypothermia as a protective measure against ischemia.

A valuable thing I learned from Professor Anđus was that "it is not important what doors you open to enter Science", and he lived up to these words. His intensive research, besides thermophysiology, also included biological rhythms, endocrinology, electroretinography, immunology, biophysical modeling, fish physiology and aquaculture.

Within the field of thermophysiology, Professor Anđus was especially dedicated to studies of the molecular mechanisms of hibernation, thermal adaptation and acclimation, and cryoprotection in addition to hypothermia and reanimation. He always emphasized that the striking differences in tolerance to internal cold (hypothermia) and lack of oxygen (hypoxia) in mammals is best evidenced by a comparison between hibernators and non-hibernators. In comparison to non-hibernators, hibernators can be regarded as an ideal natural model for studying the mechanisms by which tolerance to hypothermia and hypoxia can be improved.

Professor Anđus was one of the very few scientists who, in this high-pace era in science, remained in the field of circannual rhythms, patiently and systematically gathering data over the years. Significant research results that broke some established dogmas arose from characterization of the temperature effects on body mass, examination of hibernation/activity periods and longevity, in free-running conditions over exceptionally long periods (up to nine years). These studies confirmed that body mass and hibernation/activity cycles were phase locked, but they also underlined that the relationship between temperature and period can vary with the temperature range. However, the imposing of different external temperature cycles on ground squirrel revealed two types of "unlocked" biological rhythms, in which either body mass or hibernation/activity cycling failed to conform to the exogenous rhythm. His studies on the lifespan of laboratory-born ground squirrels, exposed to different ambient temperatures, demonstrated that hibernation *per se* did not prolong life, thereby contradicting the general assumption that longevity was linked to energy savings during hibernation.

To correlate neuronal energy metabolism with thermal tolerance, the rate of glucose conversion to CO_2 was studied in brain synaptosomes from rat, as a reference homoeothermic species, and ground squirrel, as a typical hibernating mammal. The apparent Michaelis constants (K_m) for glucose conversion provided U-shaped dependence on temperature for all species. Using experimental data and the kinetic model, it was found that the minimal K_m value, and consequent highest enzyme-substrate affinity, for glucose conversion in ground squirrel synaptosomes was at a lower temperature (6.5°C) than in the rat (16.6°C). The inversion temperatures ($T_{\min}$) closely coincided with the lowest body temperatures from which unassisted recovery from hypothermia was demonstrated in both species. This study indicated that a thermal modulation of enzyme affinities might have an adaptive role in endotherms, linked to their tolerance to hypothermia. This synaptosomal model of mammalian brain was also very successfully used in neuropharmacology and drug development to test the cytotoxicity and effect of bioactive compounds on mammalian neurons.

Professor Anđus' further experimentation with temperature adaptation and acclimatization was directed toward endocrinological changes in response to cold: characterization of the role of thyroid and increased oxygen consumption by brown fat tissue of a hibernator. In his more recent studies, Professor Anđus was interested in the molecular mechanisms underlying the temperature modulation of steroid-receptor affinity. The results of *in vitro* and *in vivo* thermal activation of steroid-receptor complexes revealed that in a non-hibernator, steroid action proceeds at a temperature incompatible with its hypothermic survival. On the other hand, the analysis of steroid-receptor affinity modulations showed "positive thermal modulation" of hormone-receptor affinity in both species (i.e., an increase of the dissociation constant with increasing temperatures), with significantly different values for the maximum affinity. Similarities between findings for enzyme-substrate and hormone-receptor affinities clearly showed the common temperature modulation of biochemical reactions underlying physiological processes in mammals. In addition, short-term hyperthermic stress may also induce changes of steroid-receptor affinity with a possible functional significance in improving hyperthermic tolerance in the rat.

Together with his collaborators from the University of Novi Sad, Professor Anđus worked on establishing a laboratory for fundamental investigations in reproductive endocrinology. Among numerous studies generated by this laboratory, most recognized were those related to the characterization of endocrinological changes in the growing male rats. These experiments defined the developmental patterns of gonadotropin, prolactin and androgen secretion and the role of prolactin. Analysis of the steroidogenic maximum of the testis at peripubertal age in control and hypoprolactinemic rats pointed to delaying effects of prolactin on androgenesis in developing rats. This conclusion was further confirmed in experiments with hyperprolactinemic rats, achieved by ectopic pituitary grafts. The dependence of peripubertal relationship between prolactin, gonadotropin, and androgen secretion on endogenous opiates was also studied.

In later years, Professor Anđus' laboratory developed two models for electroretinographic (ERG) recordings: the isolated eyecup preparation of the small-spotted dogfish shark and the *in situ* eyecup preparation of the immobilized European eel. He and his collaborators studied the effects of propentofylline, a phosphodiesterase inhibitor that is commonly used as a protective agent in anoxia and brain ischemia, in stroke patients and patients with dementia or organic brain disorders. This compound exerts profound, complex, and reversible modification of ERG records, indicating the roles of cGMP accumulation in the control of amplitude and duration of late receptor potential. Professor Anđus also completed ERG evaluation of spectral sensitivity in yellow and silver eels and analyzed differences in retinal structure and responsiveness between eels from distant geographical localities.

His interest in electrophysiological explorations of fish eyes expanded in the development of aquaculture laboratories at the Institute for Marine Biology in Kotor and in the Center for Multidisciplinary Studies at the University of Belgrade. Professor Anđus designed and was personally involved in numerous studies in fish ecology, conservation, ecophysiology and fish culturing at both locations. At the Institute for Marine Biology in Kotor, his team optimized the management strategies for rearing and producing fish juveniles for species of commercial value, especially European glass eel.

For Professor Anđus it was very important that every study, whenever possible, be accompanied by theoretical models that describe the particular system and help in predicting its behavior in conditions not experimentally addressed. For endocrinological studies, he provided an elegant model for peripubertal changes in male rats, which defined the complex interactions between pituitary and gonadal hormones. Later, a unique model describing the positive and negative thermal modulation of hormone-receptor affinity was also developed. This model was used to establish the potential for adaptation to different body temperatures in comparative studies on mammals with different thermal tolerance. Similarly, Professor Anđus also modeled the thermal properties of an enzyme-substrate system, which provides an explanation for the immediate temperature compensation linked to the tolerance of homeotherms to hypothermia. For the migration of the European glass eel in river Bojana, he developed with his team a mathematical model to predict the basic characteristics of migration waves that could be used for exploiting and protecting natural fish resources. Finally, Professor Anđus with his student Željko Džakula improved the biophysical model of protein denaturation, which described the combined effects of multiple denaturants, including the effects of pH in the presence of denaturants other than protons.

However, perhaps one of his biggest and longest lasting contributions to science was his tireless work in educating and mentoring new generations of scientists. Between 2003 and 2006, the Center for Multidisciplinary Studies with the Faculty of Biology, both at the Belgrade University, published his multi-volume textbook *General Physiology and Biophysics*. This outstanding and unique work started many years ago as handouts for students in his undergraduate course of the same name. Professor Anđus was determined to expose students to the physical and biophysical principles underlying physiological processes, but also to teach them the scientific processes that led to the main discoveries in physiology and biophysics. He was not satisfied with the descriptive physiology textbooks at that time, so he started to compile his version of a comprehensive textbook from a huge number of sources. As his teaching assistant in the 1980s, I helped him to put together equations, graphs and diagrams in the form of 'boards' that we would hand to students and use as lecture notes. Little did we know at the time that this would become his legacy.

His scientific curiosity and inquisitive mind opened many doors in his pursuit of answers, and inspired generations of his students and collaborators. Thanks to his visionary approach in science and experimental work, many of us, his former students, were able to integrate easily into Academia and the scientific communities in Serbia and around the world. We excel through the inspiration of his mentorship and hope to continue successfully the work of Professor Radoslav Anđus. In commemoration of the ten-year anniversary of his passing, the Faculty of Biology at the University of Belgrade will publish a complete collection of his works.

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