Ivan Djaja (Jean Giaja)[1] and the Belgrade School of Physiology

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Summary
The founder of physiology studies in the Balkans and the pioneer of research on hypothermia, Ivan Djaja (Jean Giaja) was born 1884 in L'Havre. Giaja gained his PhD at the Sorbonne in 1909. In 1910 he established the first Chair of Physiology in the Balkans and organized the first Serbian Institute for Physiology at the School of Philosophy of the University of Belgrade. He led this Institute for more than 40 subsequent years. His most notable papers were in the field of thermoregulation and bioenergetics. Djaja became member of the Serbian and Croatian academies of science and doctor honoris causa of Sorbonne. In 1952 for the seminal work on the behavior of deep cooled warm blooded animals he became associate member of the National Medical Academy in Paris. In 1955 the French Academy of Sciences elected him as associate member in place of deceased Sir Alexander Fleming. Djaja died in 1957 during a congress held in his honor. He left more than 200 scientific and other papers and the golden DaVincian credo "Nulla dies sine experimento". His legacy was continued by several generations of researchers, the most prominent among them being Stefan Gelineo, Radoslav Andjus and Vojislav Petrović.

Key words
History of physiology • Sorbonne • University of Belgrade • Bioenergetics • Thermoregulation • Neuroendocrine regulation

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On October 1, 1957, participants of the 15th International Congress of Medicine and Military Pharmacy in Belgrade received sad news: Professor Ivan Djaja (Jean Giaja), the chair of the symposium on hypothermia organized in his honor, the man who devoted his whole productive life as a scientist to physiology and hypothermia and who was also a pioneer of these disciplines, had just died on his way to the venue. In that same year, he had published five papers; over his entire career he had written approximately 200 scientific papers and many other publications.

Ivan Djaja was born on July 21, 1884, in L'Havre, Normandy. His father, Božidar, was a naval captain from Dubrovnik, and his mother, Delphine Depoa, was French. As a six-year-old boy, Ivan moved with his family to Serbia where he finished primary and high school. Just after graduation in 1902, his family transferred him to France, where he spent a year at the infamous Lycée Corneille in the *classe de philosophie*, learning from teachers including the famous philosopher Alain (Émile Chartier). In 1903, he enrolled at the Sorbonne with Professor Albert Dastre, who was one of the youngest students of Claude Bernard and a follower of that great French physiologist and experimentalist. During his studies, Djaja also spent some time working with Paul van Tieghem at the Muséum national d'Histoire naturelle. He graduated in natural sciences in 1905.

His first steps in research were taken in 1906 with Professor Yves Delage at the Marine Station in

[1] Surname pronounced "Dja-ia". We will use the Serbian transcription of the name *Ivan Djaja*, although in papers in foreign languages he was signed as *Jean Giaja.*

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Roscoff. Djaja earned his PhD on July 23, 1909 after defending his thesis, “Study on ferments of glycosides and carbohydrates in mollusks and crustaceans”, at the Sorbonne. A year later, he was invited to become a young assistant professor of physiology at the School of Philosophy of the University of Belgrade, where he established the first Chair in Physiology in the Balkans and organized the first Serbian Institute for Physiology. He led this Institute for more than 40 years. In 1912, he published his first monograph, entitled “Ferments and Physiology”, for which he received an award from the Serbian Royal Academy of Sciences. Djaja spent the World War (WW) I confined in Austria. Upon his return to Serbia in 1919, he continued working in the restored Institute for Physiology, where he taught Physiology and Physiological Chemistry as an associate professor. He was promoted to a full professorship in 1921, and in 1923, he published his textbook “Fundamentals of Physiology”, the first of its kind in the region. In 1921, Djaja entered the Academy of Natural Sciences (Serbian Royal Academy) and became a full member in 1932. Soon afterwards, he became an associate member of the Yugoslav Academy of Sciences and Arts in Zagreb. During that period, he actively took part in the project of building the Oceanographic Institute in Split (Spalato), which was supported by both Academies. He was awarded the Pourat (1940) and Montyon (1946) prizes by the Paris Academy of Science for his studies on hypothermia and thermoregulation. WWII brought a new interruption of his work, by way of voluntary pension and imprisonment. After the war, in 1945 Djaja returned to research and was elected as the head of Institute for Physiology.

For the seminal work of his Belgrade group on the behavior of deep cooled warm-blooded animals in 1952, he was elected an associate member of the Section for biological, physiological and chemical sciences of the National Medical Academy in Paris. Two years later, he received the title of *doctor honoris causa* at the Sorbonne. In 1955, the French Academy of Sciences elected him an associate member in place of the deceased Sir Alexander Fleming, the discoverer of penicillin.

Djaja devoted a large part of his professional life to the popularization of science, as well as to the development of arts and culture in general. He was one of the founders of public universities. He wrote philosophical books as well as books for general public; even for children. He often wrote for the newspaper “Politika”, which considered him their first foreign correspondent. His last article, entitled “For the scientific youth”, was published in “Politika” on the eve of his death in 1957. An excellent lecturer and teacher, professor Djaja knew how to gather, stimulate and inspire an elite cadre of young people, physiologists, biologists, medics, and veterinarians. In 1934, he was elected the rector of the University of Belgrade. He enjoyed a worldwide reputation from his scientific papers in thermoregulation and bioenergetics, which led to the announcement of the *“Belgrade School of Physiology*” (A.U. Smith 1960).

Djaja published his first paper on amylolytic inactivation of the dialyzed pancreatic juice in 1906, at the age of 22, with H. Bierry and V. Henri. This paper was followed by a series of studies published in the journal of the Paris Biological Society (Comptes rendus hebdomadaires des séances et mémoires de la Société de biologie), and in the same year, his first paper was accepted by the journal of the Paris Academy of Sciences (Comptes rendus de l’Academie des sciences). Djaja continued to publish until the end of his life, keeping true to his DaVincian proverb: “Nulla dies sine experimento”.

Djaja’s scientific carrier could be distinguished by three periods devoted to three main topics: enzymes, metabolism and hypothermia.

Djaja’s first papers were in the field of physiological chemistry with an emphasis on enzymology and comparative physiology. He focused on mammals and birds, as well as lower animals, particularly marine organisms. His interest in marine animals stemmed from a study visit to the marine laboratory of professor Yves Delage in Roskoff. He wrote in 1914 of his interest in the chemical phenomena of life and energy of living beings. With this work, Djaja built a foundation of experimental physiology and physiological chemistry (now biochemistry). In his view, the physiology of man and animal were not separate. Thus, he also introduced the principle of physiological studies of phenomena that are common to all living beings, as well as the studies of particular mechanisms that are unique to certain organisms. These studies actually led to the foundation of General Physiology that is taught even today in Djaja’s Institute at the University of Belgrade School of Biology. Some of the major problems that interested him in this period were pancreatic coenzymes, fermentation and digestion processes and the chemistry of carbohydrate metabolism. He also suggested a new rationale for a nomenclature of enzymes.

The second period of his work actually started with his return to Belgrade after WWI and lasted over 20 years. In this period, his research interest became more focused on bioenergetics and the relationship between metabolism, temperature and asphyxia, and definitely led him to intense studies on hypothermia and its practical use. The work from this period was compiled in Djaja's seminal two-volume monograph "Homeothermia and thermoregulation" (Giaja 1938a,b), in which he published his classical curve of thermoregulation ("Djaja's diagram"; Fig. 1). The study of bioenergetics in living beings was mainly based on the measurement of their gas exchange with an apparatus (Fig. 2) that Djaja designed himself while in confinement in Vienna during WWI.

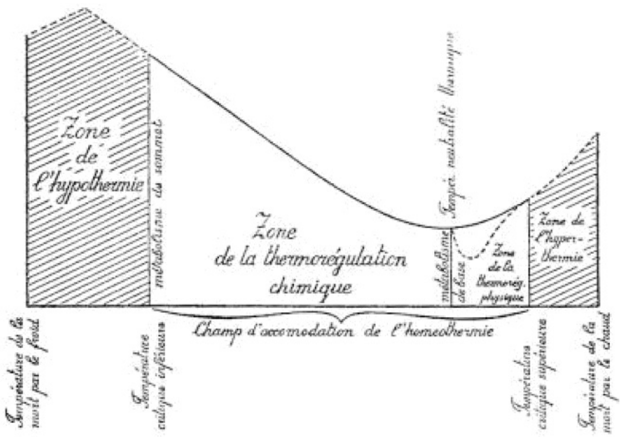

Fig. 1. Djaja's diagram of thermoregulation (1938).

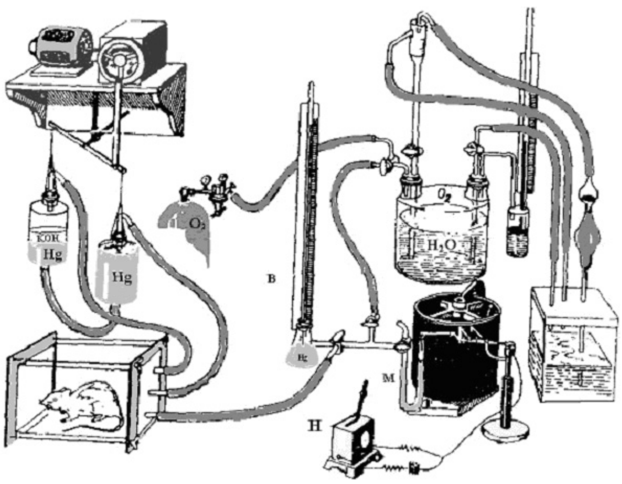

Fig. 2. Djaja's apparatus for the measurement of oxygen consumption. The animal is contained in a glass respiratory chamber, and its oxygen consumption is measured based on the rate of air pressure decrease (B, barometer; M, manometer; H, chronometer connected to a kymograph). The exhaled carbon dioxide is eliminated by binding to potassium hydroxide. The consumed oxygen is replenished with a precisely measured volume pumped out from the oxygen reservoir by an equal volume of water (Giaja 1953).

Djaja published a paper on the minimal energy consumption for life support with B. Maleš in 1921, showing that, as in poikilotherms, the energy consumption in homeotherms also depends on external temperature[2]. This paper is considered his first venture into studying hypothermia. In line with this study, in another paper in 1922 with B. Maleš, Djaja defines the oxygen consumption at normal and lethally high (40 °C) external temperatures. He then introduced a parameter termed *"peak metabolism"* and defined its relationship to the classical basal metabolism and the dependence of both on various environmental factors. Thus, he defined peak metabolism as a counterpoint to basal metabolism – the limiting point of thermoregulation during fasting or in the cold. Djaja also introduced the *"metabolic quotient"*, or the ratio of peak to basal metabolism that defines caloric consumption. In 1925, he reported on the relationship between the loss of heat and basal metabolism as well as the role of some endocrine factors (insulin and adrenergics) in heat production.

During this period, Djaja also studied the effect of alcohol on the thermogenic potential of the organism and the influence of nutrition on metabolism. He established that fat in particular is mobilized for the production of additional heat. If glycogen serves as the immediate fuel, it must be mostly regenerated proportionally to its consumption on account of the fat. In 1928, he again revised the definition of basal metabolism, presenting it not as the minimum consumption necessary for life support but instead the sum of that minimum and an assumed limiting value adapted to the functional requirements of homeothermia. Simultaneously, Djaja showed interest in hibernators. He hypothesized that hibernation could be a consequence of a lower peak metabolism, but showed that this was not the case since a hibernator such as the common hedgehog has a peak metabolism of the same amplitude as the other non-hibernating homeotherms.

In 1930, Djaja published a paper entitled "Muscle fatigue and thermogenic potential", in which he found that the maximal thermogenic potential decreases with intense muscle fatigue. This was his first foray into the research of adaptation, which he considered to be essential; in fact, he wrote that physiology could be defined as the science of functional adaptation of living beings. In the same paper, he showed that laboratory rats living at a variable room temperature could survive in

[2] Throughout the text, the historical terms from Djaja's papers, "poikilotherms" and "homeotherms" will be used instead of the contemporary versions ("ectotherms" and "endotherms", respectively).

temperatures close to 0 °C without weight loss or hypothermia, while animals kept at the temperature of their thermal neutrality (32 °C) die or experience hypothermia at the same low external temperature. Thus, Djaja concluded that the prolonged maintenance of animals at thermal neutrality significantly decreased their thermogenic potential by lowering the peak metabolism by 30 %. In other work, he demonstrated that the growth of animals kept at thermal neutrality is slowed in comparison to that of animals exposed to lower temperatures. In the series of papers that followed, Djaja conducted a comparative study on different homeothermic organisms and demonstrated that the limit of resistance to cold depends on the previous thermal adaptation of the subject. Djaja termed this decrease in resistance to cold during combating low temperatures *"thermogenic fatigue"*.

Djaja also studied the effect of anesthesia, among several other factors that affect thermogenesis. He then discovered that in deep anesthesia, heat production is very low and heat loss increases, but the adaptation potential remains. He also studied some substances, including dinitrophenol and epinephrine, which can significantly raise the basal metabolism on one hand and cancel thermogenic adaptation on the other.

In 1935, Djaja began a detailed study of the metabolic influence of the partial pressure of oxygen. He concluded that a decrease in the partial pressure of oxygen with an unaltered total pressure of inhaled air prevents chemical thermoregulation when this depression reaches half of the normal pressure. Under these conditions, when the external temperature is lower than thermal neutrality, the rat becomes hypothermic. A decrease in atmospheric pressure has the same effect on the resistance to cold and on thermogenesis. Djaja then defined the partial pressure of oxygen below which the intensity of oxidation, i.e., gas consumption, depends on the oxygen pressure as the *"liminal pressure"* (Giaja and Markovic 1950). This led him to establish a new metabolic parameter, the *"liminal baroquotient"*. The standard baroquotient defined the ratio between oxygen consumption (in ml/kg/h) and oxygen pressure (in mm Hg) as the measure of efficacy of partial pressure of oxygen; therefore, a correction had to be made for the liminal pressure because Djaja showed that it was only below this level that oxygen consumption depends on atmospheric pressure (Giaja 1957).

The third, final and most important period of Djaja's research was devoted to the problem of hypothermia. In 1955, in the paper on the *spectrum of thermogenesis* in a cooled organism (Giaja and Markovic-Giaja 1955), he summed up these investigations with the following seminal observations: "Thermogenesis can vary in intensity in a very wide range. In a rat this variation is on the scale of 1 to 25 (cal kg/h) defined on one side by the state of deep hypothermia and on the other when fighting in euthermia against cold it develops its peak metabolism. Thus, a spectrum of thermogenesis can be constructed. This spectrum shows that the thermogenesis of the same intensity can be observed at different physiological states that are designated as isothermogenic and may relate to euthermia, hyperthermia and hypothermia." As previously mentioned, Djaja's first foray into hypothermia research occurred in 1921. This inspiration continued until 1930, when he again explored the subject with Stefan Gelineo. In this work, they observed that when the body temperature of a rat exposed to cold decreases, its oxygen consumption remains high until its body maintains a temperature above 20 °C. When this threshold is crossed, however, oxygen consumption abruptly decreases to a very low level. Hypothermia is not the only cause of the decrease in thermogenesis; it is also caused by inner cooling as well as by external cold. They finally concluded that the production of heat in hypothermia results from the superposition of three factors: 1) internal cold as the instigating factor, 2) external cold as a second instigating factor, and 3) decreased temperature of the thermogenic apparatus as the inhibiting factor.

In 1940, Djaja began to focus on hypothermia as a particular physiological state as his main research topic. In studying the means of inducing hypothermia, he showed that preventing (bio)chemical thermoregulation with barometric depression and low ambient temperature can cause a rat with a 15 °C body temperature to exhibit a lethargic state, similar to the sleep of hibernators: slower respiration, extremely low oxygen consumption and a very slow heart rate. When the animal is rewarmed, it wakes and recovers fully. By slowly lowering the body temperature of a rat by asphyxia in a tightly sealed vessel (*hypercapnic hypoxia*) at a low ambient temperature, Djaja induced a physiological state resembling lethargy. The same state could be induced by direct cooling (*a frigore*).

The outbreak of WWII caused a substantial break in Djaja's career, but his research resumed in 1946 and 1947. The Institute of Physiology was severely damaged by German bombings; therefore, Djaja spent these first post-war years working at the Institute of Pathology, School of Medicine. This was a fruitful period of research in the field of energetics, adaptation to cold and resistance to barometric depression, on the protective role of hypothermia, on the bioenergetics of tumors and on the metabolism in deep hypothermia. He was joined in studying these important topics by coworkers and students in the country as well as abroad who developed the research further (see below).

Djaja energetically pursued his research on procedures to induce hypothermia. In all of these studies, hypothermia by confinement (hypercapnic hypoxia or simply "Djaja's method") has a particular place. In *The Destiny of Germans in St. Ivan and other Writings* (Igic 2002), Professor Kurt Weiss, a physiologist from Oklahoma City wrote an anecdote on the discovery of this method:

"Djaja was studying the phenomenon of hibernation in the rat. The animals were placed in wide-necked bottles, which had perforated stoppers to allow airflow while stored in a refrigerator. One day, a new technician helped with the experiments, and instead of using perforated stoppers, he inadvertently used non-perforated ones. Before long, someone noticed what the new technician had done. The "dead" rats were thrown into the container provided for carcasses, and the Professor was notified about the mistake. At the end of working hours when the laboratory cleaner arrived, he found living rats in some of these containers. He then reported the findings to the Professor. Afterwards, Djaja made similar experiments and concluded that hypoxia reduces thermal resistance, which – with other procedures – helps the non-hibernating mammals to survive in extremely low temperatures."

However, a multitude of different methods were used to induce hypothermia, such as the application of the tremorgenic alkaloid harmine, chlorpromazine, and insulin at certain dosages. In a 1955 review, Djaja defines two kinds of hypothermia: 1) primary, where the thermogenesis is decreased, such as hypoxic, hypoxic-hypercapnic, hypercapnic, insulin- and harmine-induced hypothermia, as well as the natural lethargic state of hibernators, and 2) secondary, based on the rise of thermolysis, such as hypothermia *a frigore* or that induced by chlorpromazine. Djaja also describes three types of decelerated life stages in homeotherms: 1) experimental hypothermia of non-hibernators, 2) natural hypothermia of hibernators and 3) experimental hypothermia of hibernators. A large body of his work in the later phase of his career was devoted to understanding the differences between these three states. He pointed to the case of a rat cooled to 15 °C and a hibernating ground squirrel in winter sleep, both of which seemed to be in the same physiological state. However, a comparative study shows an apparent difference; the hibernator shows much lower oxygen consumption. If the partial pressure of oxygen was decreased below 40 mm Hg, the ground squirrel awakened and abruptly augmented its oxygen consumption while the rat died without any sign of body heating. The hibernator could stay in the lethargic state for weeks or even months while the rat cooled to 15 °C died within 12 to 24 h. Notably, the lethargic hibernator reacted to a surgical intervention by heating to euthermia, while the non-hibernator stayed in a state of anesthesia (Giaja 1953, Giaja and Popovic 1953).

Djaja (Đaja 1953) was resolute that surgical (or artificial) hibernation should be clearly differentiated from natural hibernation because in chirurgy, uncompleted hypothermia is combined with the specific action of *"ganglioplegic substances"* that block the nervous system (*"lytic cocktail"*). In addition, experimental hypothermia is a physiological state of its own that, according to Djaja, needs to be particularly studied. Namely, he emphasized that an organism that loses its homeothermic qualifications by cooling does not become a real poikilotherm.

A large volume of Djaja's work was devoted to the physiology of the cooled organism. His studies on the physiology of the heart in hypothermia are particularly interesting, especially from the standpoint of contemporary medicine and surgery (Giaja and Andjus 1949, 1950, Giaja and Radulovic 1956a,b,c). In fact, the application of hypothermia in medicine was a special focus of Djaja's research. He pointed out that the hypothermia of a deep cooled (15 ^{o}C) organism may be considered a state of ideal physiological anesthesia. A number of his papers also explain the protective role of hypothermia. He showed that deep body cooling could protect from asphyxia (Giaja 1947). Moreover, in addition to protection from hypoxia, protective effects against hemorrhage and carbon monoxide poisoning were also shown. Later, he also discovered that hypothermia may protect from insulin shock, experimental uremia and even strychnine poisoning (Giaja *et al.* 1955). Djaja often explained this phenomenon figuratively, as a "decelerating effect on the life mechanism", with hypothermia thus playing the role of a "protector that prolongs life".

Ivan Djaja's last studies were devoted to a new promising subject in the physiology of hypothermia that drew particular interest in his time and is still a matter of extensive research today: the stimulating effect that occurs in the post-hypothermic state. Namely, in the rat that returned normal temperature after deep hypothermia, an increase in excitability and efficiency of muscle work was noted (Giaja *et al.* 1957). Hypothermia also had positive chronotropic, inotropic and bathmotropic effects on heart performance as well as on the resistance to myocardial fatigue (Giaja and Radulovic 1956a). Particularly important for today's organ transplantation field, it was also noted that the survival and viability of isolated tissues and organs (such as the heart) was much more pronounced if hypothermia preceded surgical intervention. Djaja hypothesized that the blood or even the cells of the cooled organism contained some stimulative substances that act for a certain period even after regaining euthermia. Djaja's abrupt death prevented him from completing these important studies. Nevertheless, this scientific endeavor was continued by the followers of his school (see below).

Professor Ivan Djaja used to say: "In life and work, ideas are more important than experimental techniques and instrumentation". Devoted to that principle, he found inspiration in the works of Louis Pasteur and Claude Bernard, but also of Gil Verne and Edmond About. However, his most important teacher and source of inspiration was Nature.

Djaja himself was an inspirational role model for numerous young investigators. He was particularly notable for his encouraging statements and demonstrated by personal example that important discoveries do not have to be the privilege of large and rich countries. In spite of several wars (Balkan wars, WWI and WWII), Ivan Djaja established an extraordinary carrier working in Yugoslavia for a total of 47 years. His work was highly cited and still is even 30 years after his death (Fig. 3). Most importantly, his work established a school of followers that took his great legacy and brought it even further recognition from the international scientific society.

A few senior investigators joined Prof. Djaja in his work on hypothermia, including Drs. Branimir Males and Stefan Gelineo. Several students also joined the group and established successful university carriers in Belgrade and elsewhere, including Drs. Vojin Popovic (Former Chair of the Department of Physiology, Emory University, Atlanta, Georgia), Leposava Markovic-Djaja (University of Belgrade, Belgrade), Radoslav Andjus (University of Belgrade, Belgrade), Vojislav Pavlović (University of Sarajevo, Sarajevo, Bosnia) and Vojislav Petrović (University of Belgrade, Belgrade). Among them, Stefan Gelineo (Djelineo), as a lifetime collaborator, and Radoslav Andjus and Vojislav Petrović, as Djaja's successors, were the most responsible for the successful continuation of the growth and development of Belgrade's School of Experimental Physiology and for establishing departments of physiology in the former Yugoslavia at large. We will end this article with a retrospective of these three followers of Djaja's legacy.

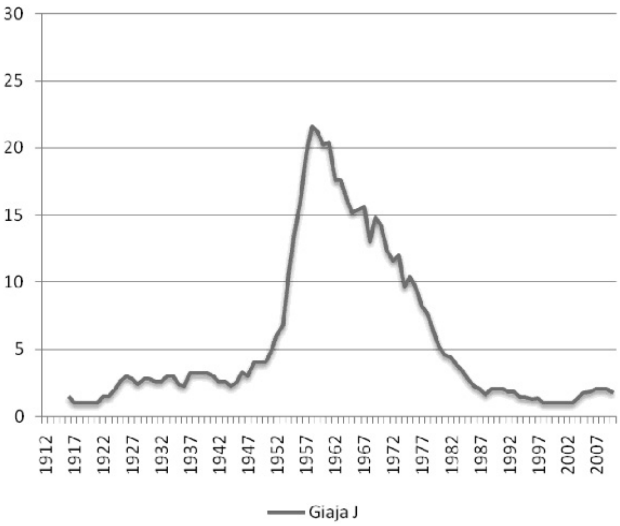

Fig. 3. Citation of Ivan Djaja's papers (E. Garfield & R. Igic, unpublished).

Stefan Gelineo was one of the first disciples and collaborators of Ivan Djaja, one of the founders of the Belgrade School of Physiology and one of the most prominent architects of its post-war development. He was born in June 1898 in Stari Grad on the island of Hvar (Croatia), completed high school in Split and studied Biology in Wien, Leipzig and at Belgrade University. In 1929, he joined Djaja's scientific team at the Chair of Physiology, where he soon defended his doctoral dissertation. In 1933, his dissertation was published as a separate edition of the Serbian Academy of Sciences, entitled "Adaptation of thermogenesis to thermal environment" (Gelineo 1933). This book had great and far-reaching significance as a special and original contribution to classical bioenergetics. It also had decisive effect on Gelineo's entire further scientific career, and the study of thermal adaptation soon became one of the major research trends in the Belgrade School of Physiology.

At Djaja's Chair, where Gelineo earned the highest professorship title in 1945 and at which he remained until his premature retirement in 1959, he taught two subjects that were completely new for the Serbian and neighboring academic society: Comparative Physiology and Work Physiology. He was elected as a corresponding member of the Department of Natural Sciences of the Serbian Academy of Sciences early in his career, in 1946.

In the scientific world, Gelineo's name is most closely linked to the *physiology of thermal adaptation*, the field in which he started his research career and to which he contributed most significantly. More clearly and more thoroughly than anyone before him, Gelineo proved that the intensity of heat production of a homeothermic organism depends not only on acute ambient temperature, to which an organism adjusts by reflex thermoregulatory mechanisms, but also on the thermal environment in which the organism had previously lived and to which it had adapted (Gelineo and Giaja 1933). At identical environmental temperature, thermogenesis is significantly more intense in homeotherms that have previously been exposed to cold than in an organism that has lived in warm environment. This holds true both for thermogenesis that is realized under the conditions of basal metabolism and for heat production that is observed at any environmental temperature in the zone of chemical thermoregulation. In simple expression, within the entire temperature range at which homeothermy is possible, the relationship of thermogenesis to environmental temperature (defined by Djaja's thermoregulation curve) depends on the thermal environment to which the organism has previously adapted.

Though Gelineo's results in the field of thermal adaptation of homeotherms have cemented his fame, no overview of his work would be complete without addressing the evolutionary physiological aspects of his research. By analyzing the phenomenon of thermal adaptation through comparative physiological and ontogenic approaches, as well as by introducing a comparative physiology course (the first course that he ever taught in Serbia), he laid the foundation for the study of evolutionary physiology in his country. He contributed mostly to the introduction of the contemporary method of comparing organisms at different levels of individual development, which became one of the basic approaches to the experimental analysis of physiological processes in the Belgrade School. He also made a considerable contribution to the foundation of ecophysiology in his country (Gelineo 1964, 1968).

The significance of Gelineo's scientific work is bolstered by the fact that in 1963, the editors of the most significant and comprehensive textbook of contemporary physiology, the American Handbook of Physiology, turned to the Yugoslav physiologist to write a chapter on adaptation of thermogenesis to thermal environment. He published 141 scientific and 14 popular science works before he passed away in Belgrade in 1971.

Radoslav K. Andjus was born (1926) and raised in Belgrade, Serbia. He majored in biology at the University of Belgrade, entered the graduate program at the same University in 1950, and defended his PhD thesis in 1953 in the field of thermophysiology. Beginning in 1951, he moved through the ranks at the University of Belgrade from teaching assistant to full professor. Professor Andjus also held numerous positions in the various scientific and educational institutions at that University during his 50-year career. Among others, he was the Head of the Department of Physiology in the School of Natural Sciences, Associate Dean of the School of Natural Sciences, Director of the Institute for Biological Research and Center for Multidisciplinary Studies, President of the Committee for Cryobiology at the International Institute of Refrigeration (1967), President of the Committee for Natural and Exact Sciences of the Yugoslav National Commission for UNESCO, and President of the Committee for Biology of the Federal Research Council of Yugoslavia.

Internationally, he held positions as a researcher at the College de France, Paris, as a staff member at the National Institute of Medical Research, London, and as a visiting professor at the Department for Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, and the Department of Physiology, University of Colorado, Fort Collins. Professor Andjus was elected to the Serbian Academy of Sciences and Arts (1959), the International Academy of Astronautics (1960), and the Montenegrin Academy of Sciences and Arts (1973). He was appointed to the editorial boards of several international and domestic scientific journals, including Cryobiology (USA), Resuscitation (UK), and Astronautica Acta (IAA).

Professor Andjus was constantly engaged in active scientific research until the end of his life. He was a productive scientist, publishing over 200 scientific papers in domestic and international journals, which have been cited over 850 times. With respect to his scientific interests, Professor Andjus was a renaissance man. His main field of investigation was thermophysiology. He studied hypothermia, suspended animation and resuscitation, hibernation and biological rhythms, brain metabolism, neuroendocrinology and endocrinology, retina and electroretinography, as well as biophysical modeling and theoretical biology. In addition, he was involved in studies on hypoxia, ischemia and radiation protection, neuropharmacology and drug developments, and fish physiology, ecology, and aquaculture. Here, we will discuss just a few of his scientific accomplishments. For details on his other work, see Stojilkovic *et al.* (2005).

Unquestionably, the most significant results of Professor Andjus's career are those achieved in his early studies on deep hypothermia, suspended animation and resuscitation. Previous studies in his group showed that 15 °C marks a turning point in the physiology of the rat. As discussed above, at 15-20 °C, animals exist in a state of cold narcosis in which hypophysectomy and other major operations can be performed (Djaja and Andjus 1949). After cooling below 15 °C, animals were unable to re-warm or to spontaneously increase their oxygen consumption. Certainly, the resuscitation of animals that have been subjected to body temperatures below 15 °C or cessation of breathing and heartbeat might have practical applications to resuscitating accidentally cooled humans, as well as in cardiac surgery to facilitate the use of hypothermia for anesthesia.

As a young graduate student, Andjus was the first to resuscitate rats with a colonic temperature between 0 and 2 °C even after heartbeat and respiration had been arrested for 40-50 minutes (Andjus 1951). Previously, rewarming was usually performed by transferring the cold animal to a warm environment. Andjus' technique instead reestablished circulation by applying heat locally to the cardiac area (by applying a hot metal spatula to the chest wall) and giving artificial respiration before re-warming the whole body.

During his postdoctoral studies in the United Kingdom, Andjus and Smith improved this methodology using a focused beam of light to reestablish the heartbeat, combined with artificial respiration. The authors also observed that spontaneous breathing was resumed in the majority of animals when the colonic temperature reached approximately 15 °C and after the neck had been heated. Only then were the reanimated rats transferred to a warm bath (Andjus and Smith 1955). Complete recovery and long-term survival in 80-100 % of the animals was also obtained using microwave diathermy for resuscitation. This technique was introduced to avoid the burning of peripheral tissues that occurred with the two other techniques (Andjus and Lovelock 1955). Once this method of reanimation was established, Andjus investigated a number of related problems, including the maximum duration of suspended animation, the effects of repeated cooling to zero, and the possibility of reanimating rats cooled to subzero temperatures (Andjus 1955).

He and his collaborators also showed that animals whose body temperature had been lowered below 15 °C exhibit a significant impairment in problem-solving performance. On the other hand, no significant differences were found between the performances of control animals and those with a body temperature brought to approximately 15 °C, confirming the view of a turning point in the physiology of the rat at this temperature. Other functions of rats were fully established within 2-3 days. Observations of breeding performance gave evidence of a temporary impairment of fertility, but the majority of animals produced progeny within three months of exposure to extreme hypothermia. The offspring were healthy and were reared normally by their mothers (Andjus *et al.* 1955).

These initial discoveries by Andjus received favorable assessments by leading scientists in the field of thermophysiology. Here we will quote only a few: "*We have found that the best method of reanimation was Andjus method of reanimation… We have religiously followed his prescriptions…*" (E. F. Adolph, Symposium on Hypothermia, XV Internat. Congress Milit. Med. and Pharmacol., 1959, p. 483); "*Dr. Andjus himself was responsible for the establishment of an entirely new principle in physiology and medicine… A great school of physiology has grown up in Belgrade…*" (A. U. Smith: Progress in Refrigeration Science and Technology; Pergamon Press, Oxford, 1960, p. 498); "*The techniques of Dr. Andjus altered completely pre-existing ideas about thermal death points in mammals and permitted a major breakthrough in the study of extreme hypothermia in homeotherms.*" (A. S. Parkers: Biological aspects of freezing and drying, L. Rey, Editor; Hermann, Paris, 1962, p. 4). These scientific achievements were later included as teaching material in 18 foreign university physiology textbooks and more than 40 scientific monographs.

The brain metabolism experiments that Professor

Andjus carried out in the early 1960s established an excellent basis for subsequent investigations in the field of neurobiology. He developed a relatively simple isolated, perfused rat brain, with preserved spontaneous and stimulated activity. This was achieved in rats, which are adequately anesthetized by deep hypothermia without the use of any chemical agent. The preparation was perfused with an artificial blood by means of a small roller-type pump. Spontaneous electroencephalographic activity was preserved in isolated brain preparations and persisted for 5 hours. Using this preparation, he also investigated the effects of pentylenetetrazol and loud sounds on electroencephalographic activity and glucose consumption. This work significantly influenced investigations in the field, as documented by over 220 citations by his colleagues (Andjus *et al.* 1967).

Together with Professor Desanka Marić and her collaborators, Professor Andjus worked on establishing a laboratory for fundamental investigations in reproductive neuroendocrinology at the University of Novi Sad. Among the numerous studies generated by this laboratory, those related to the characterization of endocrinological variables in growing male rats received the most attention. Initially, they studied the developmental patterns of gonadotropin, prolactin and androgen secretion in the developing male and found a marked age-dependent sensitivity of androgen variables to a pharmacologically-induced prolactin deficit (Kovacevic *et al.* 1982). Further studies revealed that elevated prolactin, if maintained long enough prior to puberty, might significantly attenuate or delay the intensified secretion of androgens characteristic of puberty, presumably by suppressing the intensive prepubertal gonadotropin secretion. The authors also observed a prolactin-induced increase in the responsiveness of the prostate tissue to androgens (Maric *et al.* 1982).

Andjus and collaborators also analyzed the steroidogenic maximum of the testis at the peripubertal age in controls and hypoprolactinemic rats. They found that hypoprolactinemia induced a precocious increase in number of Leydig cells and suggested that this could have resulted from the facilitation of proliferation by high prepubertal gonadotropin levels (Kovacevic *et al.* 1987). Andjus and coworkers also studied the dependence of the peripubertal relationship between prolactin, gonadotropin, and androgen secretion on testicular opiates. They showed that intraperitoneal administration of naloxone, an opiate antagonist, in peripubertal rats induces a significant decrease in basal *in vitro* androgen production by removing the testes 15 or 30 min following treatment. However, this inhibitory effect on basal steroidogenesis was not observed in control multiple-dose experiments in which incubated testes from naloxone-naive rats were directly challenged with naloxone; on the contrary, this produced enhancing effects. The possibility thus remains open that indirect inhibitory effects of injected naloxone may occur in intact animals (Maric *et al.* 1987).

Professor Andjus was an exceptionally gifted individual, with enormous energy and tireless devotion not only to science but also to teaching. Besides teaching a course in animal physiology for biologists, Professor Andjus founded a course on cell physiology and biophysics for molecular biologists, as well as a physiology course for psychologists. He also contributed to the organization of a graduate multidisciplinary program at the University of Belgrade and mentored numerous PhD candidates. His former graduate students now teach physiology and biophysics in several cities of the former Yugoslavia, the United States, the United Kingdom, Canada, and Morocco. Others are working as independent scientists in several prestigious international research institutions. He died in 2003 while working on a module of his multi-volume textbook, “General Physiology and Biophysics” (Andjus 2002). This outstanding and unique work represents a synthesis of his decades-long teaching, research, and intellectual activities. It is not only a textbook written in the best tradition of American authors, but also a history book addressing many aspects of major discoveries in physiology and biophysics, and a monographic summary of the contributions of Yugoslav scientists to these discoveries.

Vojislav M. Petrović was born in Mala Kamenica, a small Serbian town near Negotin, in 1925. After graduating from the Department of Biology at the School of Natural Sciences at the University of Belgrade in 1953, he worked as a high school teacher in Negotin for a short period before he was employed at the School of Natural Sciences in Belgrade. After successfully defending his PhD thesis, entitled “Endocrine Factor of Thermoregulation and Thermal Adaptation”, in 1959, he became deeply involved in the scientific field of Animal Physiology, becoming one of the most distinguished members of the Belgrade School of Physiology.

Thanks to the significant results in his doctoral thesis, professor Petrović earned the opportunity to spend one year in Paris at the College de France, Laboratoire de Endocrinologie as a postdoctoral fellow of the Andre Mayer Foundation and the French Academy of Sciences in Strasbourg. At the invitation of the National Research Council in 1967, he spent one year in Canada as a visiting researcher, working on a joint project dealing with endocrinology and thermal regulation (Marinkovic 2007).

He was elected assistant professor at the University of Belgrade in 1960, an associate professor in 1968 and a full professor in 1974. He founded the Chair for Comparative Physiology and Ecophysiology at the Department for Biological Sciences (currently School of Biology) of the School of Natural Sciences at the University of Belgrade. He headed the Department until his retirement in 1993. He was also the founder and head of the Department of Endocrinology and Metabolism (currently Department of Physiology) at the Institute for Biological Research "Siniša Stanković" at the University of Belgrade (Davidovic 1998). Students eagerly attended and carefully followed professor Petrović's lectures. He published over 20 university textbooks (including "Comparative Physiology I" in 1991; "Comparative Physiology II", in collaboration with R. Radojičić, in 1993; and "Endocrinology: General and Comparative", in collaboration with G. Cvijić, in 1997), high-school textbooks and professional books.

Some of Professor Petrović's major research interests were neuroendocrine regulation and neurosecretion in disturbed homeostasis, the effect of hormones on molecular processes, thermoregulation, thermal adaptation and stress, biological rhythms and hibernation, and the mechanisms of redox regulation and cellular antioxidative defenses. He published over 200 scientific papers in these topics, which have been cited more than 600 times.

His research on response to high external temperatures (in the thermolysis zone) showed that homeotherms first respond with an array of characteristic changes in the neuroendocrine system, which are reminiscent of alarm state behavior: the sympathetic tonus is increased, animals are disturbed, the catecholamine content in the adrenal glands is decreased and accompanied by their increased excretion, and adrenocortical activity is increased. The enzymatic activities of tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltransferase (PNMT), which regulate catecholamine synthesis, are also increased. These results were the first to point to the nonspecific response of homeotherms exposed to high ambient temperatures (Petrovic *et al.* 1976). These studies led to a new theory about the nonspecific response in the alarm phase and attracted particular attention among his peers in the field. In fact, these results pointed to opposite bioenergetic demands in homeotherms exposed to the zone of thermolysis with respect to thermogenesis in cold conditions, although at first they respond in the same way. At the Symposium on stress held in Monte Carlo in 1979, organized by the Canadian Institute headed by Hans Selye, professor Petrović was invited among a few dozen scientist including six Nobel price winners. Discussing Petrović's results Hans Selye commented "the best proof for nonspecific response to stressors that I have seen so far".

Relative to the scientific subtopic of the sympatho-adreno-medullar system and its adaptation to changes in the thermal conditions of the environment, Professor Petrović's results showed that homeoterm's direct response to cold depends on the activity of sympathetic nervous system, as well as of the medulla and cortex of the adrenal glands. Sympathetic tonus under these conditions is increased, resulting in the involuntary muscular movements of shivering, which is expressed as heat production due to respiration and phosphorylation processes. The biological rationale of these changes is to maintain homeostasis. Most of the shivering thermogenesis occurs during the first several days of exposure to cold conditions, after which it gradually decreases, accompanied by a compensatory increase in non-shivering thermogenesis. Both hormones of the adrenal medulla, epinephrine and norepinephrine, take part in the thermogenic "response" of homeotherms to cold. These hormones stimulate glycolysis and lipolysis, thus providing energy-rich compounds for further biochemical transformation in the most thermogenically active tissues. Under these conditions, the quantity of both stated catecholamines in the adrenal glands is decreased and their secretion as well as that of their metabolites, such as vanilmandelic acid, is increased.

Ground squirrels exposed to cold in the summer exhibit changes in the activities of adrenal TH and PNMT. Petrović proved that these changes are the result of neuronal and hormonal control. Specifically, cortical glucocorticoids stimulate the activity of PNMT, the enzyme responsible for epinephrine synthesis; adrenocorticotrophine is involved either directly or indirectly in the regulation of TH and PNMT activity. As treatment with metopirone and dexamethasone affected PNMT activity but did not alter TH activity, it can be assumed that hormonal factors dominate in the control of PNMT activity (Fig. 4). It seems that neural factors dominate in the control of TH activity (Petrovic and Janic Sibalic 1976).

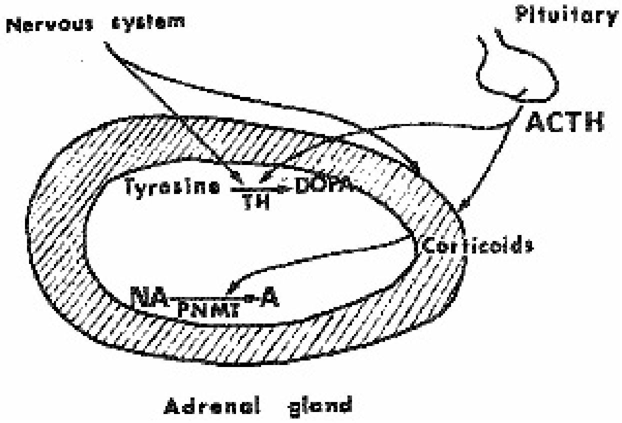

Fig. 4. Adrenocortical control of phenylethanolamine-N-methyltransferase activity in the ground squirrel (*Citellus citellus*) during the summer (from the original paper: Petrovic and Janic Sibalic 1976).

In early 1970s, professor Petrović recognized the importance of the study of free radicals, particularly superoxide anion radicals and antioxidative defenses, which at that time was in its infancy. It was a logical choice after his long period of research in mitochondria, given that the highest production of superoxide anion radicals occurs in the respiratory mitochondrial chain. Professor Petrović's research group was the first to publish on the activity and distribution of CuZn superoxide dismutase in the tissues of hibernators. This work empirically suggested that the increase in the antioxidative defense superoxide dismutase activity in the tissues of a hibernator (*Citellus citellus*) during hibernation, when metabolism and oxygen consumption is decreased, results from preparation for the waking phase, when metabolism intensity and oxygen consumption increase precipitously over a short period of time (particularly in thermogenically active tissues). This became an unavoidable reference in all works dealing with hibernation, given that it produced the first data of that kind (Petrovic *et al.* 1983). Later on, the proposed mechanism was confirmed by other authors using different parameters. It was also shown that the same mechanism of preconditioning is employed for the needs of both waking from hibernation and for other states that lead to increased metabolism and oxygenation (Petrovic *et al.* 1983a,b).

In parallel with his highly successful scientific and teaching work, Professor Petrović was eagerly committed to various other academic activities. He was a vice dean (1969) and a dean (1971) of the School of Natural Sciences at the University of Belgrade, head of the Department for Biological Sciences of the School of Natural Sciences (1973) and, together with professors R.K. Andjus and D. Kanazir, he greatly contributed to the establishment of a new study group at his Faculty: Molecular Biology and Physiology, the first such group in former Yugoslavia.

In 1974, he was elected to be the corresponding member of the Serbian Academy of Sciences and Arts; from 1975-1979, he became vice-rector, and from 1981-1985, he served as rector of the University of Belgrade. Between these two elections, he held the position of the Director of the Institute for Biological Research in Belgrade. He was a member of the Bureau of the European Standing Conference of Rectors *(ad personam*), a member of the Bureau of the Danube Rectors' Conference, a member of the Higher Education Commission at the European Council in Strasbourg, President of the Education Council of Serbia, and a member of the Federal Committee for Science and Technology, SFR Yugoslavia. In 1986, he was elected full member of the Serbian Academy of Sciences and Arts. He was a member of the Presidency of the Serbian Academy of Sciences and Arts and President of its Board for Biology in the Department of Chemical and Biological Sciences and a member of the Inter-Departmental Boards for Biomedicine and Ecophysiology. Professor Petrović died in 2007.

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