REVIEW

Czech Footprints in the Bioenergetics Research

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Summary

Life manifests as growth, movement or heat production that occurs thanks to the energy accepted from the outside environment. The basis of energy transduction attracted the Czech researchers since the beginning of the 20th century. It further accelerated after World War II, when the new Institute of Physiology was established in 1954. When it was found that energy is stored in the form of adenosine triphosphate (ATP) that can be used by numerous reactions as energy source and is produced in the process called oxidative phosphorylation localized in mitochondria, the investigation focused on this cellular organelle. Although the Czech scientists had to overcome various obstacles including Communist party leadership, driven by curiosity, boldness, and enthusiasm, they characterized broad spectrum of mitochondrial properties in different tissues in (patho)physiological conditions in collaboration with many world-known laboratories. The current review summarizes the contribution of the Czech scientists to the bioenergetic and mitochondrial research in the global context.

Keywords

Mitochondria • bioenergetics • chemiosmotic coupling

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Mitochondria

Bioenergetics is a study of the transformation of energy in living organisms that is tightly connected with the cellular organelles - mitochondria. Research on these organelles began in the middle of the 19th century when their structure was recognized [1]. In 1890, Richard Altmann named these organelles “bioblasts” and concluded that they are elementary organisms inside the cells [2]. Later, Carl Benda started to call them mitochondria (from the Greek “mitos” – thread and “chondros” - granule) because of their tendency to form long chains [3]. However, many important findings were reported before the relationship of these particles to mitochondria was known [1].

In 1913, Otto Warburg linked cellular respiration to particles isolated from guinea pig liver, which he called “grana” [4]. During the late 1930s, significant advancements were made in the elucidation of the reaction pathways and energetics of aerobic metabolism. In 1937, Hans Krebs, Otto Warburg’s student formulated the citric acid cycle (CAC) [5] and the first indications of aerobic phosphorylation have been published [6-8]. In the following years, it was found that these particles contain all the enzyme equipment needed for aerobic oxidation of the metabolites from CAC and that it relates to the synthesis of ATP molecule [9,10] and Albert L. Lehninger and colleagues linked oxidative phosphorylation to mitochondria [11]. With the development of thin-sectioning techniques, the first high-resolution images of mitochondria were published (Fig. 1) showing that the mitochondrion possesses cristae (infoldings) created by the inner membrane that are surrounded by the outer membrane [12].

Nevertheless, it took a long time to begin to understand how the energy released by oxido-reduction enzymes is coupled to ADP phosphorylation. The answer to this question was published in 1961 by Peter Mitchell, who proposed a chemiosmotic hypothesis of oxidative phosphorylation [13]. He hypothesized that energy released in the oxido-reduction chain is stored in the form

of a proton gradient across the coupling membrane and that the membrane contains a proton-translocating reversible ATP synthase, which can utilize the proton gradient to synthesize ATP from ADP and P_i. In 1978 he won the Nobel Prize for the chemiosmotic theory (Fig. 2).

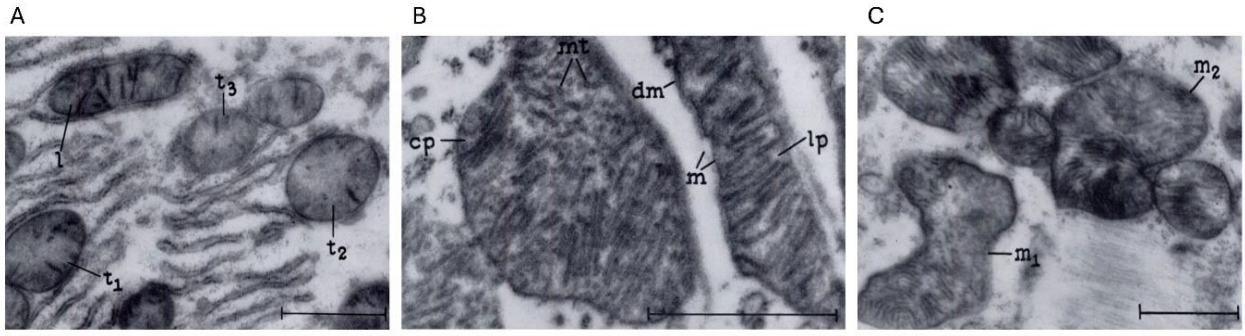


Fig. 1. Electron micrographs of mitochondria from the liver (**A**, magnification: 31,300x), kidney (**B**, magnification: 50,000x), and muscle (**C**, magnification: 30,700x) published by Palade et al., 1953 [12].

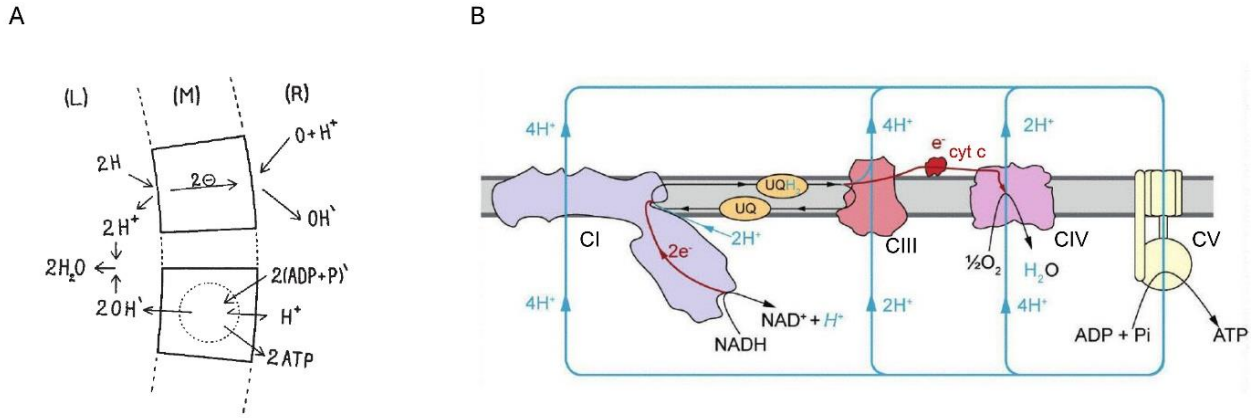


Fig. 2. (**A**) Electron transport system (above) and reversible "ATPase" system (below) chemiosmotically coupled in charge impermeable membrane (M) enclosing aqueous phase (L) in aqueous phase (R) [13]. (**B**) Simplified scheme of the mitochondrial oxidative phosphorylation apparatus consisting of 3 H^+-translocating complexes (CI, III, IV) that transfer electrons from NADH-linked substrates, ubiquinone pool, cytochrome *c* and ATP synthase (CV, complex V) that utilizes energy stored as H^+ gradient across the inner mitochondrial membrane. CI – $4H^+/2e^-$, CIII – $4H^+/2e^-$, CIV – $2H^+/2e^-$, UQ – ubiquinone, UQH_2 – ubiquinol (adapted from [14]).

The Czech bioenergetics beginnings

The beginnings of bioenergetic research in our country date to 1917, when Prof. Edward Babák (1873 – 1926) wrote the book "On the transformation of energies in the living bodies" [15]. In 1933, his student and collaborator Prof. Vilém Laufberger (1890 – 1986), while breaking up hepatocytes [16] isolated small grains, later known as mitochondria. During WWII, an enthusiastic student Arnošt Kleinzeller (1914 – 1997) fled to England from the Nazi regime and studied for many years in the laboratory of Hans Krebs at the University of Sheffield. When he returned to Prague after the war, he established the Cell Metabolism Laboratory and became a lecturer in Biochemistry at the Faculty of Sciences of Charles University. Besides his knowledge, he brought to Prague laboratory equipment including a Warburg respirometer that allowed to measure cellular respiration. He chose five students of Biochemistry – Ladislav Kováč, Arnošt Kotyk, Jiří Čerkasov, Radovan Žák, and Zdeněk Drahota.

"Nature" coincidences

After graduation, Radovan Žák and Zdeněk Drahota started to work at the newly established Institute of Physiology of the Czechoslovak Academy of Sciences (CSAS) under the supervision of Ernest Gutmann. They started to isolate mitochondria from muscle on a Janetzki centrifuge in a cold room and with the limited methods they had at hand, they studied amino acid degradation and with youthful virtue sent their work to *Nature journal*. Due to Gutmann's perfect Oxford English (to the surprise of the reviewers), the paper was accepted for print [17].

However, the two young men were aware that it was probably pure coincidence. Browsing the *Nature journal*, their attention was caught by an article about the swelling of mitochondria by Samuel V. Perry [18]. They wrote a bold letter to Perry, as his colleagues from Nature, and asked him if he would be so kind to come to Prague for a few days and show them how mitochondria are isolated in Oxford.

No one assumed that Prof. Perry would dare to go behind the Iron Curtain. He came not only to teach the two Prague students to isolate mitochondria, but above all to find a place called "Mährisch Trübau in Böhmen" (Moravská Třebová in the Czech Republic), where he was deported from Africa as a prisoner during the WWII after being captured during the Western Desert Campaign. With the help of an English professor and a homemade refrigerated centrifuge, the mitochondria in Prague were isolated for the first time in the same way as in practices in Oxford. At the same time, the barracks in Moravská Třebová, a former POW camp, were successfully identified, and Prof. Perry exchanged greetings with the mayor and signed the memorial book with great fame. Perry's stay behind the Iron Curtain was successful and the Institute of Physiology obtained a precious friend who returned to Prague with pleasure for the Symposium held in Liblice and, on the other hand, a bed & breakfast was always prepared for the guests from the institute in England.

"Italian "coincidence

Based on these experiences, the mitochondrial research in Prague successfully continued. Since two are better than one and Warsaw is close to Prague, the joint works of the Institute of Physiology CSAS and the Institute of Biology of the Polish Academy of Sciences about the fatty acids oxidation in liver mitochondria soon appeared in *Biochimica et Biophysica Acta* [19]. Luckily, the first author of the publication, Lech Wojtzak, and his wife Anna performed the research at Johns Hopkins Medical School in Baltimore in the laboratory of Albert L. Lehninger, and thus Zdeněk Drahota also got the chance to do the research in this famous lab. He continued with the research on liver mitochondria and discovered that sodium and potassium do not influence calcium-activated respiration and calcium retention capacity [20].

In Baltimore, a third happy accident occurred. During his "sabbatical" in Italy, Prof. Lehninger wrote the famous Biochemistry textbook [21] and his laboratory in Baltimore was always full of Italian students and researchers. The joint Czech-Italian-American publication in the *Journal of Biological Chemistry* [22], which studied in detail the calcium accumulation by mitochondria, represented the first result of a long-lasting collaboration. Thus, Zdeněk Drahota appeared at the first of the series of international Symposia on mitochondria in Italy. These symposia were organized by Ernesto Quagliariello, the rector of the University of Bari, with the support of the committee for the development of tourism in southern Italy. At that time, nobody knew how important this coincidence would be for the development of bioenergetic research at the Institute of Physiology. Prof. Quagliariello soon became the president of the Italian Academy of Sciences (CNR) and he made sure that every bilateral agreement on cooperation between CSAS and CNR, would contain the stay for the bioenergetic researchers in Italy. Then Prof. Quagliariello, in the letter addressed to the president of the CSAS, extended the stay for a few months, claiming that extra costs, of course, would be covered by the Italian side. That is how Ernesto Quagliariello became the first patron of the Czech researchers who have always been very grateful to him for his support.

"Swedish" coincidence

The fourth lucky coincidence gave the bioenergetics research at CSAS new energy and another patron. It started when Jiří Křeček's Department of Developmental Physiology started to sprout. One of his students, Petr Hahn, founded a Laboratory of the Development of Metabolic Functions. The group was interested in lipid metabolism and mitochondria, and in collaboration with the Institute for Mother and Child Care studied the regulation of lipid metabolism in newborns. Their results were utilized during the development of the new infant formula enriched with lipids [23]. At that time, a new physiological-bioenergetic function was demonstrated - the thermogenic function of brown adipose tissue [24]. From there, it was only a step to the idea to see what substrate brown fat mitochondria utilize to generate heat. Therefore, Zdeněk Drahota and Eva Honová isolated mitochondria from brown fat, and after a series of experiments they demonstrated high oxidation of carnitine esters of fatty acids. Together with Marie Schutzová they prepared the graphs and Petr Hahn, while lying on the divan, dictated the first version of the manuscript to his wife Nada, and in a few days the text was on its way to Switzerland to the *Experientia journal* [25]. As soon as the article was published, a letter arrived from Olov Lindberg,

the director of the Wenner-Gren Institute of the University in Stockholm and a world-known scientist in brown adipose tissue research [26]. Due to his high moral qualities and the character of a calm Northerner, he was not upset that this "short communication" was published a few months earlier than his "full paper" and generously invited members of Hahn's group to cooperate. As he later stated, another reason for his friendly help was that he felt personal responsibility for the damage caused by the Swedish army by the end of the Thirty Years' War at Prague Castle.

Similarly to the first Italian patron and Prof. Lindberg, a member of the Royal Swedish Academy, made sure that the bilateral cooperation agreement between the Royal Swedish Academy and CSAS always included the topic of "Thermogenetic function of brown adipose tissue", and he also extended the stays of the Czech partners in Stockholm from weeks to months. In exchange, the members of the Swedish laboratory used to come for discussion on the mechanisms of thermogenesis organized by Zdeněk Drahota's students and collaborators: Josef Houštěk, Petr Svoboda, and Jan Kopecký. The scientific discussions were accompanied by hiking and the consumption of incredibly good and cheap beer in the mountain huts. It was the beginning of long-lasting cooperation.

1968

When the year 1968 was approaching, the 5th meeting of the Federation of European Biochemical Societies (FEBS) was being prepared in Prague. One of the topical subjects was "Mitochondrial Structure and Function" which was proposed by Dr. Drahota from the Institute of Physiology CSAS during the first discussions, which symposia would be included in the program. Just after the committee's meeting, Lars Ernster, a mitochondrial physiologist, who mentored many foreign students, received a phone request to organize this symposium. He immediately accepted. The very next day, the requests for symposia from other institutes appeared. However, to cancel the symposium organized by this distinguished biochemist, Olov Lindberg's student, director of the Institute of Biochemistry of the Royal Swedish Academy, and a member of the Nobel Committee for Chemistry was no longer feasible. Thus, the mitochondrial symposium survived.

The meeting preparations culminated during the "Prague Spring". An incredible number of people poured into Prague for the symposium on mitochondria, not only because of the mitochondria, but because they also wanted to know what was happening in Prague.

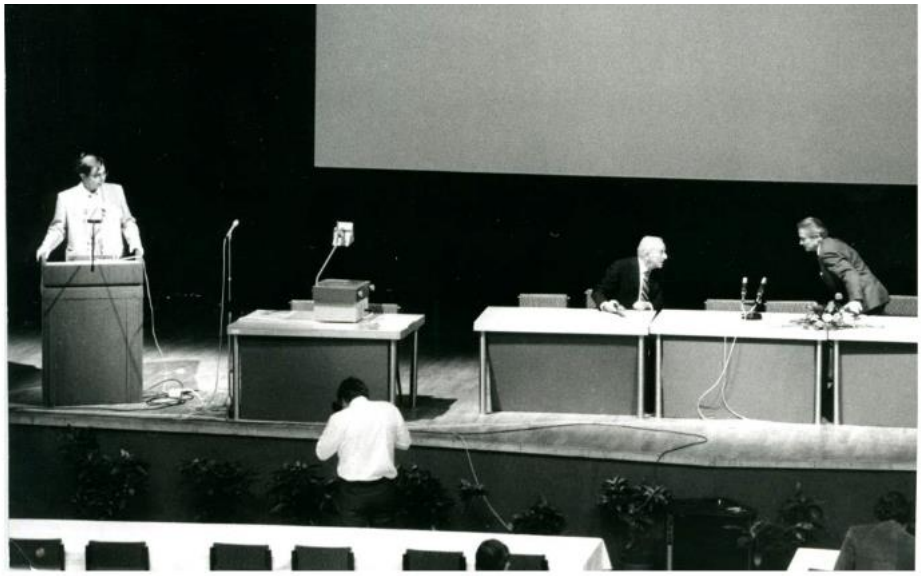
When the mitochondrial researchers arrived in Prague in July, they were surprised at how little excited the organizers about the current situation were. When saying goodbye at the end of the congress, the Italian patrons assured the organizers from the Institute of Physiology that they could always count on Italian friends. The Swedish patron heartily shook both hands and even without words, everything was clear. Nobody realized that some weeks later the "Prague Spring" would end abruptly in an unbelievable disaster during the shocking and sad days of August 1968 and how important would be the support of the patrons for the bioenergetic research at the Institute of Physiology CSAS to survive the next 20 years.

Normalization

A few weeks after the entrance of the troops of Warsaw Pact countries into Czechoslovakia, the researchers interested in mitochondria Petr Hahn and Josef Houštěk were already in England, and Zdeněk Drahota in Italy. When Zdeněk Drahota and Josef Houštěk met in the half-empty institute in 1969, mitochondrial research started to wake up only slowly. The normalization policy of the Communist Party led to the emigration of many researchers, others could not attend international meetings or proceed with their research at all. Nevertheless, new young colleagues appeared and the patrons in Italy and Sweden kept the doors open.

At the beginning of the 1970s, the collaboration with Stockholm University focused on the function of the brown adipose tissue started to take shape. First, the uncoupling mechanism via uncoupling protein 1 (UCP1) and its regulation was studied [27]. Later, also other complexes specific to the brown adipose tissue and involved in mitochondrial metabolism were investigated. Together with Barbara Cannon and Olov Lindberg, Josef Houštěk found that mitochondrial glycerol-3-phosphate dehydrogenase activity is high in this tissue compared to other tissues [28]. When possible, international congresses, symposia, and courses were organized. In particular, the 4th European Bioenergetics Conference organized in Prague in 1986 further stimulated mitochondrial research and attracted young talented students (Fig. 3). It certainly contributed to the development of the independent research programs of the laboratory members and building of several new scientific

laboratories within the institute, including Department of Bioenergetics with Josef Houštěk as the head. Two other departments also focused their research on mitochondria – Petr Ježek's group investigated the role of uncoupling proteins and oxidative stress, and Jan Kopecký's department further explored thermogenesis in the fat and muscle tissue and obesity in general.



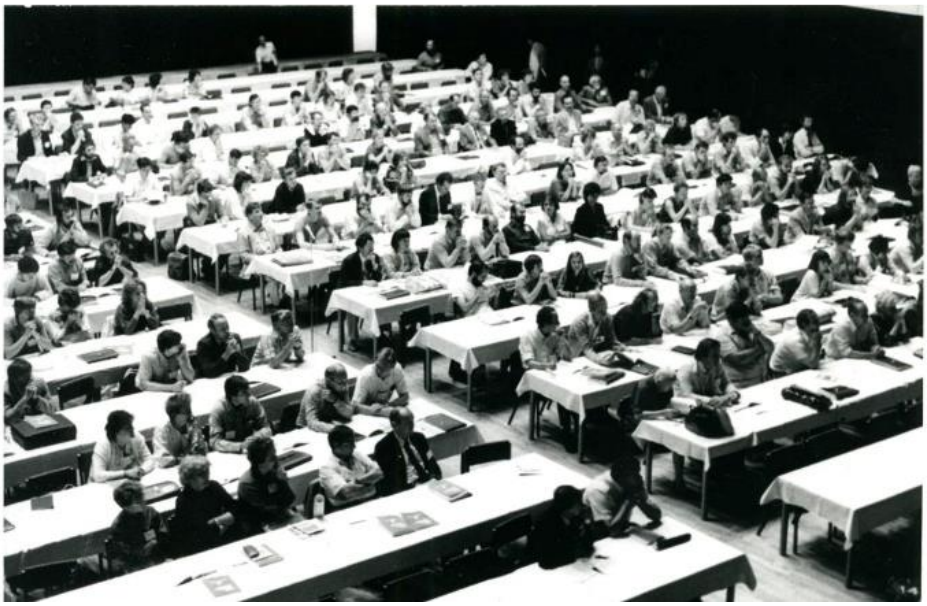


Fig. 3. 4th European Bioenergetics Conference (EBEC) in 1986 in Prague, Czechoslovakia. Left (from left to right): Josef Houštěk, Lars Ernster, and Zdeněk Drahota.

Collaboration with the Italian scientists was focused on the mitochondrial ATP synthase and with the groups from Bari they investigated ATP synthase inhibitor - DCCD (dicyclohexylcarbodiimide) [29]. They found that, besides inhibiting proton-pumping enzymes, e.g. ATP synthase, it also binds to another 33 kDa mitochondrial transport protein [30]. Later, in collaboration with the group of Štefan Kužela from Bratislava, they proposed that DCCD also binds to UCP1 protein in brown adipose tissue [31]. With the group of Giorgio Lenaz from Bologna, they identified two distinct, temperature-dependent states of the F_1 domain of ATP synthase (hydrophilic part) [32].

August 1968, the Soviet occupation of our country and the following communist persecutions slowed down the scientific career of many researchers, among them also Štefan Kužela from the Institute of Experimental Oncology CSAS. Despite the persecution, he remained active and further promoted unique mitochondrial genetics studies in Bratislava. Illegal seminars in his office also

initiated the unofficial winter Czechoslovak conferences on Bioenergetics, which continue to these days (Fig. 4).

Fig. 4. 36th International Conference on Bioenergetics in 2023.

1989

The change of the regime after the Velvet Revolution in November 1989, allowed to travel and collaborate freely even with people who emigrated after 1968. The research in Prague on brown adipose tissue continued in cooperation with the groups from Stockholm. It focused on the low capacity of mitochondrial ATP synthase in brown adipose tissue compared to other tissues. It was published that the abundant pool of mRNA of the β subunit is not fully translatable and thus it may account for the decreased content of mitochondrial ATP synthase [33,34]. Further, high levels of transcripts of other subunits were detected, except for the subunit *c*. It suggested that the expression of subunit *c* and its synthesis critically controls the biosynthesis of the whole ATP synthase [35]. The joint studies on UCP1 and its function in brown adipose tissue were focused on hormonal regulation of UCP1 expression [36] , adrenergic receptors [37,38], or the role of interleukins [39,40]. A recent study also showed that UCP1 is involved in the control of reactive oxygen species generation [41].

During the 1990s increasing attention was paid to mitochondrial disorders causing human disease. The first publications arose from the collaborations with the laboratory of Coby van den Bogert at The Academic Medical Center in Amsterdam and long-lasting cooperation with Jiří Zeman from the Department of Pediatrics (First Faculty of Medicine, Charles University). In patients with atypical NARP/LS syndrome and impaired ATP synthase [42,43] they observed mtDNA defects caused by *ATP6* mutations in subunit *a* [44,45]. In 1999, the case of the first patient with ATP synthase defect of nuclear origin was published [46]. The ATP synthase complex was selectively reduced, and the newly synthesized enzyme had the same subunit composition as control, implying the altered biosynthesis of the enzyme. Nine years later, in cooperation with the groups of Wolfgang Sperl from Salzburg and Stanislav Kmoch from the Institute of Inherited Metabolic Disorders in Prague, the *TMEM70* gene was identified as the disease-causing gene, which also uncovered TMEM70 protein as novel specific biogenetics factor of mammalian ATP synthase [47-49]. Within this cooperation, also the first patient with somatic mutation in ATP synthase subunit ε was described [50]. In collaboration with Catherine Godinot's group from Lyon, the functional alterations of patients with disordered cytochrome *c* oxidase assembly factor SURF1 were described [51]. This collaboration further continued and a series of papers studying the role of oxidative phosphorylation in cancer was published [52,53]. All these efforts stimulated further the interest in elucidation of the

molecular basis of various inherited mitochondrial disorders and led to numerous joint international research projects in successive years.

With the expansion of the laboratory, the research interests and collaboration also expanded. Thus, the ability of glycerol-3-phosphate dehydrogenase to produce reactive oxygen species [54-56] and its interaction with the coenzyme Q pool [57] were investigated together with the group of Giorgio Lenaz. Within the Czech Republic, cooperation with the laboratory of Zuzana Červinková from the Faculty of Medicine in Hradec Králové developed during the 1990s. Their research focused on the effect of triiodothyronine on regenerating liver [58-60], the role of oxidative stress [61-63] and mitochondrial membrane permeability [64-67]. Also, the ambiguous effect of biguanides (the most common treatment of type II diabetes) on oxidative phosphorylation was studied [68,69].

Since 1917, when Edward Babák described living organisms as energy convertors [15], bioenergetics research in the Czech Republic has advanced greatly with significant input from studies originating from and/or performed at the Institute of Physiology, Czech Academy of Sciences. It would not have been possible without the enthusiastic scientists and the scientists would not be enthusiastic without the initial ignition by their teachers.

"*Scientists are not so much born as made by those who teach them research*" Sir Hans Krebs

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Conflict of Interest

There is no conflict of interest.

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