Why the mammalian embryo?

An interview with Professor Nikola Skreb

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Professor Nikola Skreb is the founder and *spiritus movens* of the embryological school initiated in Zagreb, which has devoted continuous interest to studies of the early mammalian embryo. He was born in Zagreb, Croatia, on August 21, 1920, where he was educated and attended the University of Zagreb Medical School. His university education took place amidst the turmoil and hardships experienced in southeastern Europe during the Second World War. He finished his medical education in 1948 and started his research and teaching activities at the Medical School in Zagreb, first in the Department of Bacteriology and later on in the Department of Biology. His continuous research interest in mammalian embryology was supported by numerous research grants either from Yugoslav and Croatian science foundations or from the US-Yugoslav Joint Board on Scientific and Technological Cooperation. The US Department of Health, Education and Welfare supported, through its National Institute of Child Health and Human Development, three successive research projects, with professor Skreb as the principal investigator: Cytological and Biochemical Studies of Early Mammalian Embryos (1971-1975); Experimental Analysis of Early Rodent Embryos (1982-1985); and Functional Analysis of Postimplantation Mammalian Embryo (1986-1989).

Professor Skreb had numerous contacts with scientists and visited laboratories in France, Belgium, the United States, USSR, Poland, Denmark, Sweden and Great Britain. He has participated in countless scientific meetings, at many of them as an invited speaker: Paris (1974), Nutley (1975), London (1975), Minneapolis (1978), Toulouse (1979), Boulder (1987), Hamburg (1990) and Dubrovnik (1991). For his work he was elected to the Yugoslav Academy of Sciences and Arts in Zagreb (1979), the Serbian Academy of Sciences and Arts, and Honorary member of the Croatian Medical Academy, Zagreb. He was awarded the «Ruder Boskovic Award» for science in 1970, and the Award for his Scientific Life Work in 1979.

Professor Skreb took part in all the scientific meetings organized by the European group of scientists dedicated to embryology which later on became known as the European Developmental Biology Organization (EDBO, 1978). Professor Skreb was elected the first president of EDBO in Berlin and served this position for four years.

Your contributions to the developmental biology of mammals are well known and appreciated in the relatively small community of mammalian embryologists. Although this interview and conversation are supposed to focus primarily on your scientific activities, let us first have a short look back at the origins of your interests and motivations. Was there something in your childhood and formative years that could be associated with, or that initiated, your later scientific career?

Yes, my intellectual development was strongly influenced by the cultural milieu in our family. My father was professor of geophysics at the University of Zagreb, and my older brother, who was living with us, was interested in studies and theory of literature. He became

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a well recognized professor of German language at the same University. Conversations about scientific research were frequent in our home, and each of us spoke at least one foreign language fluently.

I remember that I never really liked high school in Zagreb, so I could hardly wait to begin my studies at the University.

What was the motive for your decision to study medicine?

In spite of the fact that I did best in subjects which were in the domain of languages or literature, I decided on medicine as my future study and occupation, very early. Reasons and motives were quite complex and highly abstract. I had a very general idea and desire to help people with my work. Then I was also strongly convinced that a thorough understanding of human physiology is the prerequisite for solving the rest of the «capital» problems. Namely, under the influence of my brother Zdenko, I had been reading many philosophers and the leading writers of the world at that time. So, I was fairly busy and occupied with my attempt to solve the general philosophical enigmas.

All that brought me to the deep conviction that only through thorough understanding of human nature could we attempt to solve the other problems.

My studies of medicine and of the secrets of the human body turned out to be very of Yugoslavia by fascist troops in 1941. I had to interrupt the course of my studies for reasons of *vis major* on two occasions: in 1942 and in 1944.

It is well known that you joined the resistance movement in our country very early. Can you tell us something about your activities and the attitudes of the Croatian intellectual amidst happenings before and during the turmoil and sufferings of the Second World War?

Immediately on the outbreak of war I joined the antifascist organizations, first by collecting goods and drugs in the city for the fighters in the forests and mountains. Later on I joined the partisans myself. Once there, I was obliged to take on the duties of a medical doctor, though I had not yet finished my studies. Fortunately, I had passed my exams in all the theoretical and clinical courses (except for surgery), because I found myself performing all types of urgent medical interventions and treatments from tooth extraction up to delivering of babies in civilian families around us.

It was an interesting coincidence that I was serving as the medical doctor for the British mission accredited with our resistance forces as well, because their only medical force consisted of a student in his first year of studies!

And what about events after the war?

After the war I was not allowed to leave the army, because medical doctors were badly needed. I was encouraged to finish my studies as a military student, but I did not want to accept this possibility. So I remained in the army till 1947, when I was finally released upon my repeated demands.

You graduated from the Medical School in Zagreb, when you had already had a lot of first-hand experience in practical medicine. Why is it then, in spite of that, you have chosen a basic medical discipline for your future professional occupation?

Upon completion of my studies I chose specialization in microbiology, as the field which offers the possibility for experimentation, and yet remains associated with clinical work. My first and direct contact with medical experiences during the war made me change my preferences away from the domain of practical medicine.

What caused you to switch from clinical microbiology to embryology?

The authorities of our Medical Faculty considered me as well suited for microbiology, and successful in experimental work, so I was offered a position in the Department of Biology of the Medical School, University of Zagreb in 1948. The reason for that invitation was the conviction of Professor Andrija Stampar, the founder and one of the directors of the World Health Organization, that all the theoretical courses offered at the Medical School should be led and taught by medical doctors! So I was offered the WHO fellowship which stimulated my decision for transfer into the Department of Biology of the Medical School, Zagreb.

With the transfer I found myself in a total vacuum; there was nobody who could lead me in such a hybrid field between medicine and biology, at that time. I was not interested in the cytogenetics of Lepidoptera, their genetics or reproductive basis of speciation, which was at that time the active field of research of Professor Lorkovic, who was the head of the Department of Biology.

So I came across a new acquisition in the Library of the Department of Biology: «Einfuhrung in die Physiologische Embryologie» by Lehman, which had influenced my thinking and interest, so I decided to enter the field of embryology, which was closer to my medical training. However, my first attempts in the work with Amphibia were not very successful.

At that time, in 1948 there was no great tradition in research in developmental biology in Croatia or even Yugoslavia. There were no competent scientists in this field. How and where did you gain your first experience and ideas?

I was happy to obtain a fellowship in 1950 and I left first for France to join Professor Devillers in his research on fish. Than I moved to Brussels and joined Professor Dalcq in the work on Amphibia. Upon the advice of Professor Dalcq, who was just starting his research work on laboratory rodents, after my return to Zagreb I started research work on bats, in an attempt to verify Dalcq's hypothesis concerning the origins of bilateral symmetry in wild animals. Unfortunately, I was not able to find the signs of such symmetry in eggs of the bat.

Nevertheless, I owe my gratitude to Professor Dalcq for the idea to start experimental embryological work on mammals. Very soon I became aware on my own that with bats I would not be able to go a long way, so I started work on laboratory rats.

I would like to ask you now, Nikola, probably one of the most important questions: what led you to start your developmental investigations on the early postimplantation rodent embryo? What was your first approach?

I started exercises in experimentation on the rat embryo first. I tried to transplant the early rat embryo from one pregnant female into another, with the aim of exposing it to an external influence, like, for example, UV irradiation, during the transfer. Another question was to analyze its potential for implantation. Later I came upon the idea of analyzing various teratogenic agents, in an attempt to estimate from the developmental consequences of their actions what the physiological mechanisms were underlying the development of the early mammalian embryo. So I kept testing the effects of X-ray radiation, hyperthermia and mercaptoethanol. On the basis of my first results reported at the embryological meeting in Pallanza in 1960, I proposed my hypothesis on the critical period of development in the rat embryo, which coincides with the period of mesoderm formation. Before this stage I observed the so called «all or none» effect: embryos were either totally deleted, or showed no deleterious effects whatsoever. After this stage of mesoderm formation, I observed malformations in various parts of the embryo, caused by the applied noxious agents.

The methodological approach most frequently used in your laboratory has been the procedure of grafting early rodent embryos onto different extrauterine sites. What are the most important results of these experiments?

Yes, among the new approaches one was the study of developmental consequences in the embryo, when it was transferred to various extrauterine sites, depending on its developmental stage: before mesoderm formation or after the mesoderm was present. We have worked with the chorio-allantoic membranes, we transplanted the early embryos into the anterior eye chamber, as well as under the kidney capsule of the rat. The most interesting results were obtained in experiments carried out by Professor Bozica Levak-Svajger. The rat embryo with mesoderm is capable of producing well differentiated tissues upon such transplantation into the anterior eye chamber, while the earlier embryo without the mesoderm cannot produce them. These results proved the hypothesis of the critical developmental stage of the early rat embryo: the period of mesoderm formation.

Transplantation experiments carried out with the mouse embryo under the kidney capsule also yielded interesting results. A certain percentage of early mouse embryos did not give rise to differentiated cells, and they remained at the embryonic stage of develop-

ment, which was partially dependent upon the genetic background of the mouse strain used. These tumorous masses of undifferentiated embryonic cells kept growing until they killed the host mice recipients of the embryo transplants. The embryogenic teratocarcinomas were studied and characterized very intensively by Professors Ivan Damjanov and Davor Solter. Both of them, as the medical doctors in our biological team composed of embryologists, histologists, and pathologists, continued their research very successfully upon moving to the United States and to their new laboratories there. They have both published an impressive list of papers in this field, as well as internationally recognized overviews.

At roughly the same time, or even much earlier, one of my collaborators, Professor Drasko Serman, started electrophoretic studies of protein changes, as indicators of differential gene activation taking place during organogenesis in the rat.

In the preface of the first issue of the monograph «Development in Mammals», it was pointed out that the embryo surgery worked out in your laboratory was one of the achievements that revolutionized studies of early mammalian development. What is the major result from application and work with this technique?

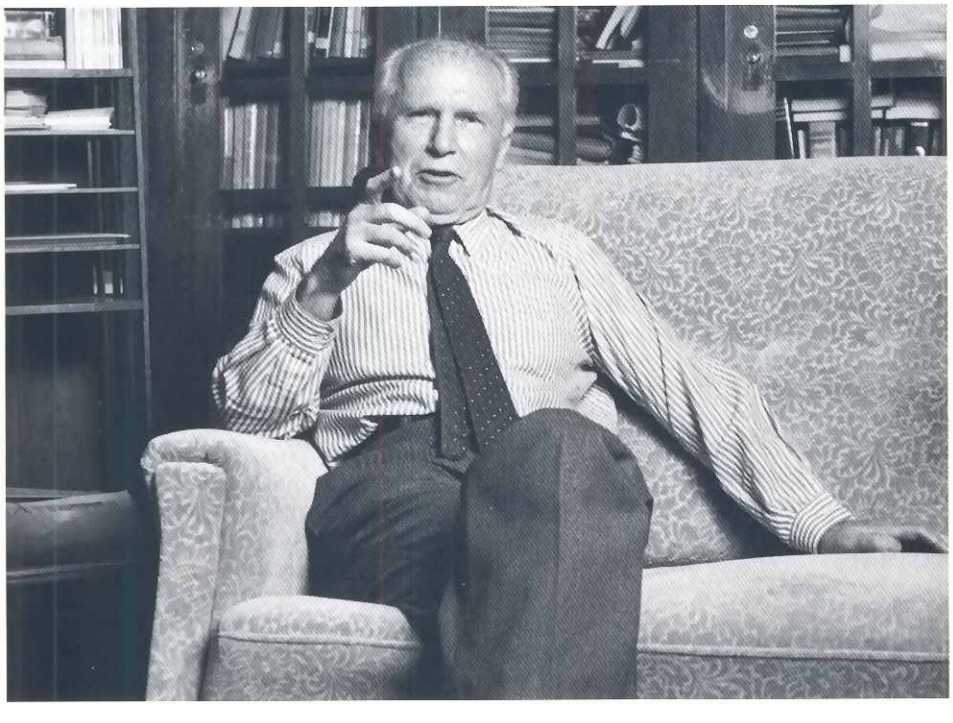
Thanks to persistent attempts by Professor Anton Svajger, we were able to penetrate further into the analyses of this stage of embryonic development. Professor Svajger has developed the technique for isolation of particular germ layers, which were afterwards transplanted by Professor Bozica Levak-Svajger under the kidney capsule. In this way we were able to study and start to understand their «differentiating potentialities». The most general conclusion from these experiments indicates that practically all the tissues arise from the primary ectoderm. So we are obliged to consider the classical theory of germ layers as having only a limited value, and must accept it only with great restrictions.

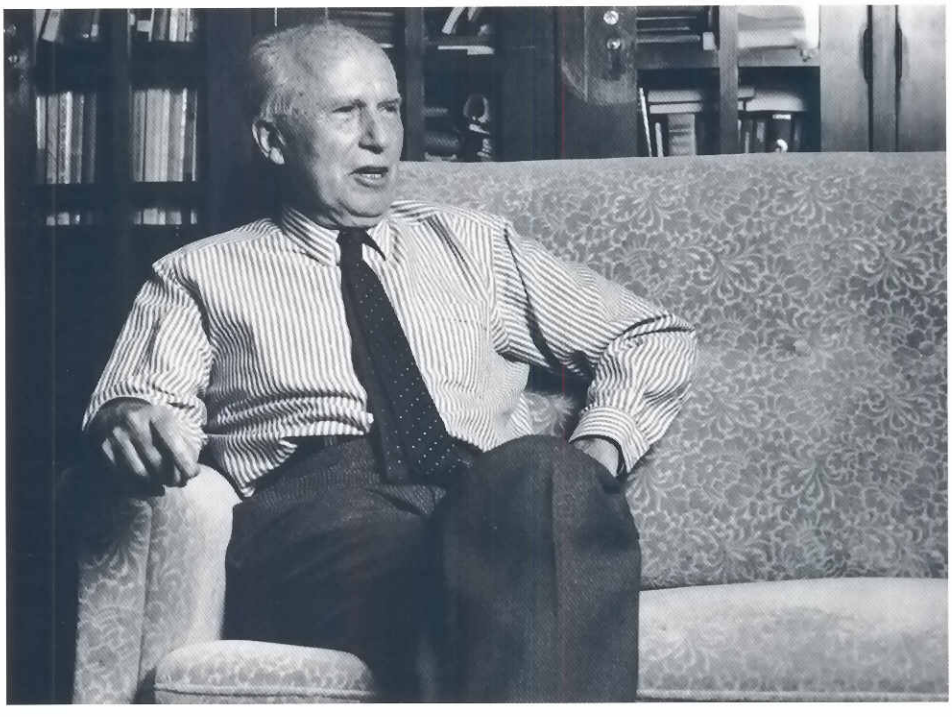
Nikola, what are you expecting from the numerous experiments of *in vitro* cultivation of early rodent embryos? You started this work long ago and you are still busy with these experiments, six years after your retirement from active duty.

The method of *in vitro* cultivation of mouse or rat embryos under conditions referred to as the organ culture method promises to provide interesting results, because one can analyze the conditions necessary for differentiation of tissues in chemically defined media without serum and protein complementation. After two weeks of cultivation in such «serum-free» conditions there is no morphogenesis, but we can observe terminal differentiation of major tissue types. Here I am happy to acknowledge collaboration with Dr. Floriana Bulic, Vesna Crnek, M.Sc. and Maja Vlahovic, M.Sc. By selective addition of a single factor or combinations of well defined factors to the growth medium we hope to obtain results on the requirements of various types of tissues for their differentiation factors. We hope that we shall be able to analyze in this way most or all the factors necessary for the early differentiation of tissues.

Nikola, you were the first president of the European Developmental Biology Organization (EDBO). Who initiated the foundation of EDBO and what was your role in this effort?

The first hint about the EDBO came from the late Professor Alberto Monroy. Later, during the Sorrento Conference in 1974, Professors D. Newth and M. Abercrombie carried further the





informal discussions with many scientists including myself. In 1976, many national societies of developmental biologists were formed, and they nominated their representatives to constitute the Provisional Board. Dr. Job Faber was nominated as Provisional President, and Dr. John McKenzie as Provisional Secretary and Treasurer. I was elected by the postal ballot as the representative of the individual members on the Board. At the second Board Meeting on the occasion of the XIIIth International Embryological Conference in Berlin, 1978, the new Constitution was adopted. Finally, the definitive Board was elected. As Dr. Faber declined to seek re-election, I was elected President of the EDBO Board.

Every important breakthrough in science precipitates concentrated interest on the newly emerging concepts and problems. Earlier, still unresolved problems are frequently regarded as «old fashioned» and sometimes are almost neglected. The high points of contemporary developmental biology are obviously within the realm of molecular genetic explanations of developmental phenomena. Once separate and even exclusive intellectual and educational entities classically known as «Entwicklungsbiologie» and «Vererbungslehre» have finally and fortunately found a common ground and seek common mechanisms and answers. Homeobox genes and developmental biology of nematodes are such attractive and highly competitive areas of research. In such a situation, how do you see the present position of «classical mammalian embryology» and what sort of future do you anticipate for this research?

It is obvious that even «classical mammalian embryology» can follow the new directions and trends, which is evident already from numerous works from several well developed laboratories. Once found, the genes responsible for developmental processes in *Drosophila*, for example, can be sought in mammals too. Therefore, there is no barrier keeping developmental genetics from being equally and successfully applied to mammalian and even to human development. Nevertheless, a thorough understanding of classical and experimental mammalian embryology remains an important prerequisite for this progress.

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