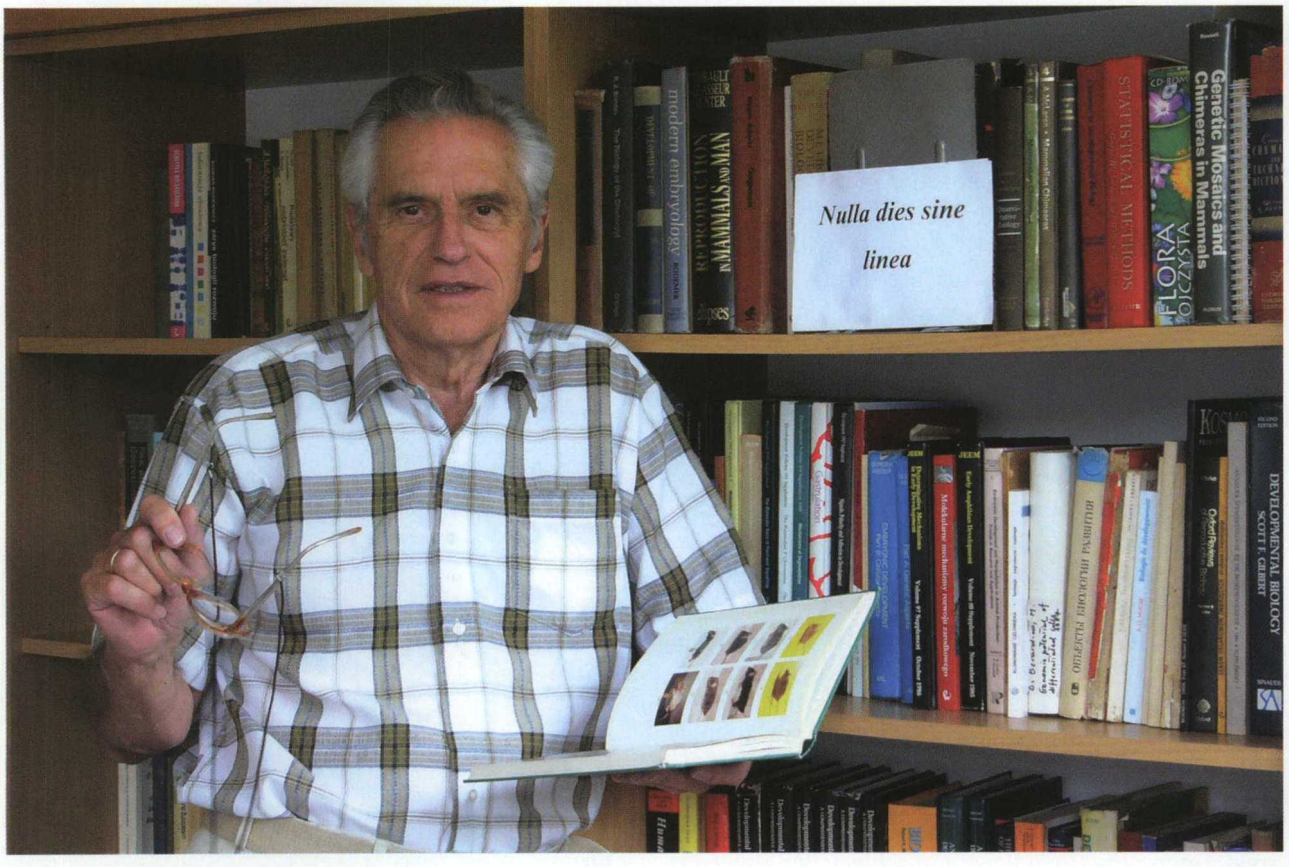
Interview with Professor Andrzej K. Tarkowski

Intrigued by Experimentation



Katarzyna Szczepańska

Prof. Andrzej K. Tarkowski in his office at the Department of Biology, University of Warsaw

Academia: Last year the Nobel Prize in medicine and physiology was awarded for breakthrough research on embryonic stem cells. I have heard it said that this discovery would not have been possible without your previous work.

Andrzej Tarkowski: *That's overstating it. My contribution – if we can speak of one at all – was very indirect: initiating experimental research on the early embryonic development of mice in the late 1950s and early 60s, and developing certain techniques which later enabled other researchers to obtain embryonic stem cells. I did not work on obtaining such cells. Probably my most useful experimental finding was to obtain chimeric animals by aggregating two embryos at an early stage of development (other methods of obtaining them were also later developed). It was in building chimeras from a single embryo plus groups of embryonic stem cells that the latter were shown to be pluripotential – able to differentiate into different body tissues, as well as to form gametes and thereby pass their genome on to the next generation. One of the methods for obtaining transgenetic animals involves introducing embryonic stem cells that have been genetically altered* in vitro *into an early embryo. The chimera so created passes on an altered genome to its progeny.*

Maybe if it weren't for my research, as well as the research of several other individuals, obtaining embryonic stem cells would have been delayed.

Much of your research later had great significance in various fields of experimental biology and medicine. It is thanks to your discoveries we can now employ prenatal diagnostics to detect certain congenital defects in human embryos before they are implanted in the womb.

Partly, and again only indirectly. My doctorate thesis, the main findings of which were published in Nature *in 1959, showed that a normal mouse*

could develop from only one of the two first cells a fertilized egg cell divides into, although under normal circumstances it develops from both. I also demonstrated that removing one of the first 4 cells did not disturb normal development in most of the embryos so treated. For genetic diagnosis of preimplantation human embryos, usually 1-2 cells are collected from 8-cell or later embryos, and so the damage is even smaller. Early mammalian embryos tolerate changes in the number and initial distribution of cells very well, i.e. they show regulatory capabilities. Interestingly, embryos of altered initial mass soon regain normal size after being implanted in the womb. Another project of mine, which helped to develop cytogenetic studies on oocytes and early embryos (also of human origin) consisted in inventing a new technique of making chromosome preparations from them. That paper still gets cited quite frequently, although it was published in 1967, 40 years ago.

How did all of this get started? How did you first get interested in developmental biology?

I first took an interest in biology two years before graduating from secondary school, and already then I discovered how fascinating embryology was. I was chiefly inspired by two books: an academic textbook on embryology and a small popular-science booklet by a professor in Kraków on experimental embryology, which was then also called "mechanics of development." Among the research described there was that of Hans Spemann, who received a Nobel Prize in 1935 for attempting to explain early development in amphibians, grafting embryo fragments from one location to another and observing how they behaved in their new tissue environment. Those studies fascinated me.
Unfortunately, there were no embryologists at the University of Warsaw just after WWII. Prof. Zdzisław Raabe, director of the Zoology Department where I worked as a deputy assistant while at university, put me into contact with Prof. August Dehnel, who was setting up a field research station in Białowieża (now the large Mammal Research Institute, Polish Academy of Sciences). There I studied the reproduction of a small mammal, the common shrew, under natural conditions. After publishing two papers on that, I came to the conclusion that I was more intrigued by laboratory experimentation. The most convenient species for embryological study was the mouse, which was also quite well studied in genetic terms. So I began to experiment. I wanted to verify a hypothesis that required early embryos to be transplanted from one female to another. That led to the topic for my doctorate research, which was inspired by a chance observation: a certain 2-cell embryo with one of its cells damaged still turned out to develop normally for several days, even though it was half its natural size. After developing a method for selectively destroying cells using a glass needle, I implanted such modified embryos into host females. I described the development of such embryos after they implanted in the uterus and I identified at what point the regulation of their size took place. Several completely normal mice developed from embryos treated in this way.

How did it happen that you published your results in *Nature*, one of the world's most prestigious research journals, as such a young man?

The results were ground-breaking; no one had performed such an experiment on mice embryos before. Experimental mammalian embryology was then in its infancy. That was recognized by the editors of Nature. *To a certain extent I was helped by Prof. F. W. Rogers Brambell, with whom I later worked in Bangor in North Wales as a Rockefeller Foundation scholar. While settling the details of my stipend, we sent him that paper to evaluate. He only corrected the English somewhat and sent it off to the editors of* Nature *himself. His authority may have helped get it published, but it really was a good paper. That was back in 1959, nearly half a century ago.*

> Probably my most useful experimental finding was to obtain chimera animals by aggregating two embryos at an early stage of development

But soon thereafter you published another paper in *Nature*. That is something not many renowned Western scientists have achieved...

Indeed, my next paper was published in Nature *two years later, this time not inspired by a chance observation. I had planned the experiment in detail, although initially I wasn't sure how things would turn out. Prof. Brambell approved my research projects. One aimed at identifying the developmental capabilities of two early mouse embryos when aggregated together. Such an experiment had been carried out for the first time using rat embryos in the US in 1942, but the results then obtained raised serious doubts. The experiment I planned was successful and completed very quickly, something that rarely happens because hundreds of problems usually crop up. I showed that aggregated embryos become fully integrated and form a single embryo, from which a single normally-structured chimeric mouse can develop. That paper went down into the canon of embryological literature.*

After those great achievements, which opened the doors to many of the world's top laboratories, you did not choose the

easy path. You returned to Poland and set up your own team here. You wrote a paper in Warsaw on the development of parthenogenetic embryos.

Experimental parthenogenesis involves provoking an egg cell to begin to develop under the influence of a chemical or physical stimulus, without any sperm cell. We showed that parthenogenetic embryos of mice (and other mammals, as we now know) can begin embryonic growth that initially proceeds normally, but then they die in mid-pregnancy. This paper appeared in Nature *in 1970, together with a paper by my friend Prof. Christopher Graham from Oxford, who also dealt with the development of artificially stimulated mouse egg cells, albeit in a somewhat different experiment. These papers provided serious arguments in favor of the hypothesis that certain "imprinted" genes in mammals are in some way inactivated in the sex cells as they form, but differently for each gender. As a result, normal and full embryonic development is only possible when an embryo is equipped with the genome from both a mother and a father. The entire genome of parthenogenetic mammalian embryos, even if they are equipped with the normal diploid number of chromosomes, is of maternal origin, which prevents their normal development beyond a certain stage.*

Perhaps using parthenogenetic embryos to obtain human stem cells would enable ethical issues to be avoided? Such embryos could never develop into normal fetuses on their own.

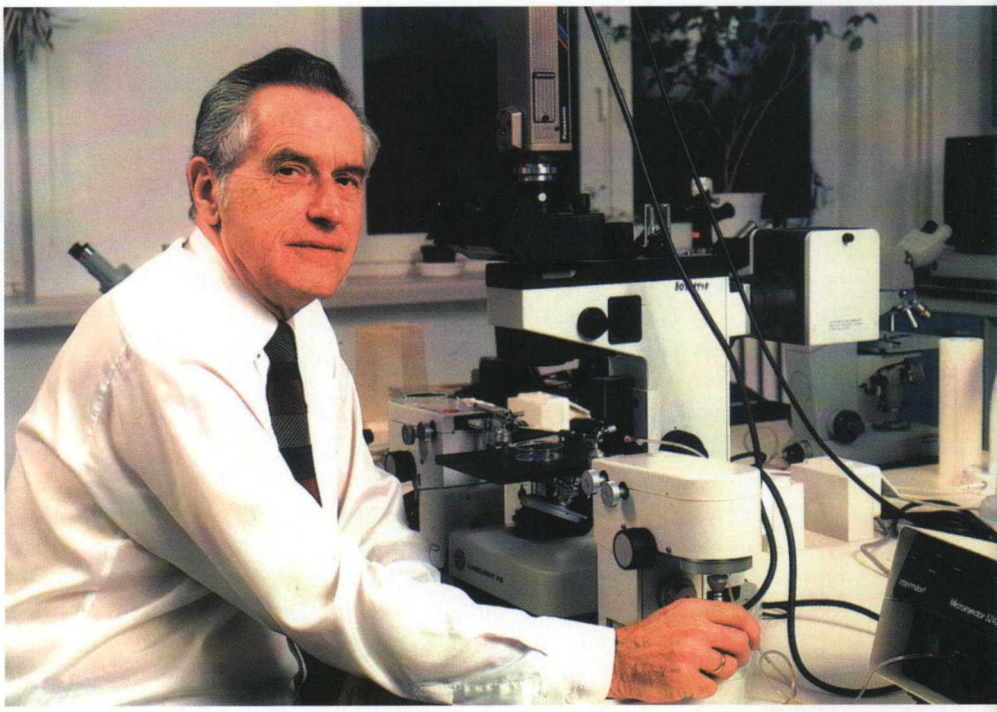
That is one possible route. Embryonic stem cells have already been obtained from parthenogenetic embryos of mice and humans, but those tests have only been moderately encouraging. Frequently the cells so obtained are not as viable as the cells obtained from embryos derived from fertilized egg cells. They might also have certain genetic defects. We should bear in mind that stem cells obtained from parthenogenetic embryos could essentially only be used to treat the very same woman who donated the egg cells these embryos were derived from. Using them to treat other individuals would require genetic testing similar to when organ donors are sought for a given recipient in need. I don't think this would be an easy and entirely safe route, plus it would still probably not resolve the ethical doubts of those opposed to such procedures.

Is there no other way to obtain pluripotent cells, instead of taking embryonic stem cells from embryos?

The ideal solution would be to force the "ordinary" cells building the tissues of an adult host to "undifferentiate," regaining the properties of embryonic cells. Last year, several publications described efforts headed in this direction. By introducing a few genes that are normally active in the early embryonic stage (but cease to be active as the cells differentiate), such differentiated cells were successfully "rejuvenated," obtaining cells showing the properties of embryonic stem cells. Those "rejuvenating" genes were introduced into cells using specially prepared viruses. That technique will have to be modified if it is to become completely safe.

Let's talk about the ethical aspects of research using embryos. This is something normal for us biologists, but not for many other people. Whenever there is talk of embryos, *in vitro* fertilization, or stem cells, there is a lot of controversy in the media. How justified is such controversy, in your view?

Human embryos are definitely not something we should experiment on to gain general knowledge about mammalian development. But I would consider it permissible, in very specifically defined situations, to use early embryos in order to obtain embryonic stem cells. Here I would consider only cases in which a fatally ill person could not be saved by any other means. A consciously aware human being that is suffering and



Paweł Wójcik, private files of Andrzej Tarkowski

It is not easy to lead the experiments having only an enthusiasm. Prof. Andrzej K. Tarkowski in his lab (2002)

terrified at their approaching death is in my view of greater value than a single-cell zygote or an embryo of several dozen cells. I would not like to deliberate precisely when a person comes into existence, because that would first require a definition of the concept of "person" and we would get bogged down in philosophical disputes. But I do consider it unjust for a conscious human being to be treated on par with a zygote.
Besides, medical treatment for humans using stem cells is still more of a theoretical consideration, and human embryonic stem cells are nowadays obtained from egg cells and embryos which are no longer slated to be used for in vitro *fertilization...*

...and that's a point that also sparks a lot of controversy...

But it shouldn't. I believe that in the case of infertility, seeking the assistance of biology and medicine is justified. For many people this is an issue of utmost importance, and in my view they do have a right to have their own child. Although faulty reproductive function does not threaten a person's life, like a serious heart defect does for instance, it is a huge tragedy for many people, perhaps even undermining the sense of living. Why should such people be condemned to infertility if science can indeed help them? Naturally, such assistance cannot be used frivolously, but I would consider refusing it entirely on ideological grounds to be inhumane.

12 years have already passed since the famous sheep Dolly was cloned. Many years ago, you and your collaborator developed the technique of electrofusion that was used to fuse embryonic cells.

We were the first to employ the previously developed technique of electrofusion to mouse egg cells and embryos. Our intention was to clone a mouse, but we were not successful. We described what happens to a nucleus collected from embryonic or somatic cells when it is planted in place of an egg cell's own nucleus. We tested what conditions were necessary for that nucleus to be reactivated. This work was utilized by other researchers working on cloning, and perhaps to some degree also sped up their success.

Much of your fundamental research later proved to be significant.

When publications get described in the press, journalists always ask us about the practical importance of such research, about what sense it makes. I have to say that as a young man such questions bothered me greatly. I felt that there was sufficient justification in seeking to identify and explain phenomena which no one had ever reported or explained. I still feel that way because much fundamental research has to come long before any practical applications arise, and it is hard to tell right away what will ultimately prove useful in practice. But these days I don't react negatively to such questions. Now, when because of my age I've started to think about my own work retrospectively, I frequently wonder whether what I've done in life has been useful to people in some way. And I can say that I'm not completely satisfied with my reflections.

But your research has laid the foundations for great achievements in contemporary biology.

Perhaps to a certain degree, but I do not feel the kind of satisfaction felt by researchers who discovered an effective drug or cured a seriously ill patient. I don't have the sense that I helped any specific person directly. Sometimes I envy my wife, who as a psychologist has put many people back on their feet after a nervous breakdown and helped them rebuild their lives. That means something more valuable than the birth of some mouse derived from only part of an embryo. But then again, it seems my wife sometimes envies me...

Interviewed by:
Patrycja Dołowy
Warsaw, 24 April 2008

Prof. Andrzej Krzysztof Tarkowski. World-renowned Polish embryologist, a professor at the University of Warsaw, a member of the Polish Academy of Sciences (PAN), the Polish Academy of Arts and Sciences (PAU), the French Academy of Sciences, the US National Academy of Sciences, and Academia Europaea. Winner of numerous research awards around the world, including the prestigious Japan Prize, known as the "Japanese Nobel," in 2002.

He graduated from the Faculty of Biology and Earth Sciences at the University of Warsaw in 1955 and his career has remained linked to the university ever since. He held a Rockefeller Foundation scholarship at the Zoology Department, University of North Wales, and served as a visiting professor at: the University of Oxford, Rockefeller University of New York, University of Adelaide, Institut Jacques Monod (CNRS), and Université de Paris XII.

He originated a school of experimental mammalian embryology known throughout the world. The results of his research have been published in prestigious journals (several times in *Nature*) and continue to be frequently cited.

Aside from his professional work, he is also a photographer. His exhibitions "Botanical Impressions," "Trees and Wood," and "The Earth We Walk On" have been on display in many Polish cities.