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Cell movements in neurogenesis

An interview with Professor Carl-Olof Jacobson

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The intriguing formation of a three-dimensional embryo by concerted cell movements, ultimately controlled by the linear genetic information, has been in focus of the research interest of Carl-Olof Jacobson and his students for several decades. In the present article developmental biologist Carl-Olof Jacobson gives his personal view and perspectives on this topic.

Carl-Olof Jacobson was born in 1929 in Dalsland, a beautiful province in western Sweden. Following the tradition of so many other young students interested in biology ever since Linnaeus' days, he went to Uppsala for university studies. There he became interested in experimental embryology and he describes below how this came about. After receiving his Ph.D. with Sven Hörstadius as his supervisor (Fig. 1), he has been affiliated with the Department of Zoology in Uppsala except for two periods as a visiting scientist at the Wistar Institute of Biology and Medicine in Philadelphia and at the Zoology Department, University of Texas, Austin. In 1970 Jacobson was appointed Professor of Zoomorphology in Uppsala, where he also served as Dean of the Faculty of Natural Sciences and Engineering for a long period. Since 1989 he holds the full time position as Secretary General of The Royal Swedish Academy of Sciences in Stockholm.

What made you choose developmental biology as the area of your scientific activities?

In the early fifties I studied zoology at Uppsala University, where Sven Hörstadius was teaching a course on experimental embryology, a truly exciting experience for a young biologist. However, I had entered another field, ecology, which by that time was exploding, and had just concluded my first field work, when Hörstadius in 1954 suggested that I should switch to a subject that had always attracted him, but which he then understood, would be too big a new experimental system for him to master. He was approaching his sixties and was heavily engaged in international and national science policy questions at his university and in International Council of Scientific Unions (ICSU), to mention one major organization that took advantage of his experience. The field of investigation of his dream that he wanted me to enter was the formation of the cerebral nerve-fiber pattern. How do nerve fibers from the brain find their way to their partner cells? His idea was that I should use axolotl larvae as experimental objects. A good stock of such animals was still kept at the Department since Hörstadius' experimental work on the neural crest some years earlier (see Hall and Hörstadius, 1988). The general idea was that I should make relocalizations of nuclei and ganglia of the prospective central nervous system (CNS) on the neural plate stage, using methods that Hörstadius had picked up in Ross G Harrison's lab at Yale at the end of the 1930s. I accepted the offer with a great deal of enthusiasm, a choice that I have never regretted, not even when my dear axolotls tied me to the lab night and day without taking notice of holidays or family celebrations. Such egocentric family-father behavior was still accepted 40 years ago (Fig. 2).

Hörstadius is not only a famous scientist, a brilliant lecturer and a good ornithologist (he is one of our best-known early nature-photographers. His close-ups of shy birds from the 1910s were the result of enormous patience and inventiveness). I happened to know him also in his capacity as chairman of one of the student choirs in Uppsala, a position he had been offered more for his light and vivacious behavior than for his voice. However, the network of contacts in the scientific community was of high-

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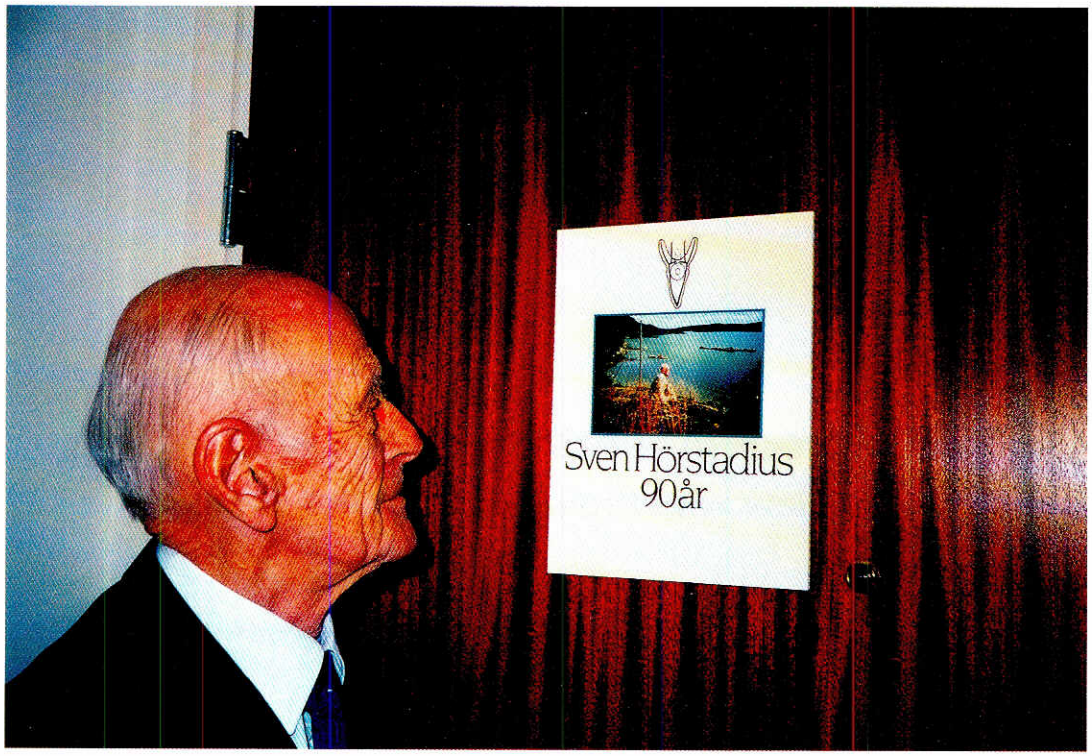

Fig. 1. Sven Hörstadius, supervisor of C-O. Jacobson's PhD Thesis, on his 90th birthday in 1988.

er value for a graduate student. Most of the grand old men of experimental embryology of the 1930s and 40s appeared now and then in the Department. Imagine the excitement of a young embryologist when, because of their friendship with Hörstadius, Viktor Hamburger and Johannes Holtfreter wanted to come with me to Uppsala after a conference in Helsinki, so they could discuss my experiments and results during a couple of days! To be sure, these and subsequent discussions with Hamburger meant more for my development in neuroscience than even those with Hörstadius (Fig. 4).

When you studied embryology, the extracellular matrix was hardly recognized. Its function in controlling neural crest cell migration and differentiation, for example, is now well established. What achievements in this area do you regard as most important?

Already Detwiler in the 1930s and Hörstadius and Sellman (1946) had suggested that the migration of neural crest (NC) cells might be influenced by factors in their environment, but we had to wait until the 1970s before more detailed facts on this environment were described. That the extracellular matrix (ECM) plays a role was a common theory from the beginning of that decade. Somewhat later Löfberg (amphibians) and Ebendal (chick embryos) from our lab described arrangements of the ECM that seemed to provide good substrates for contact guidance movements of the NC cells, since they were forming tracks in the right direction. Electron microscopy (EM) (Fig. 3) showed that ECM and moving NC cells were in close contact. During this period it was also made clear that different components of the ECM in various ways are influencing NC morphogenesis and differentiation.

Adhesiveness probably plays a key role in many morphogenetic movements in the embryo and it has been thoroughly studied in several model systems like neurulation (Jacobson, 1968), liver-cell aggregation and NC cell migration. For a couple of decades it has been understood that ECM and molecules adhered to the matrix are interplaying with the surfaces of the moving cells. It is beyond doubt that adhesion mechanisms are also governing the growth cone behavior but of course they must be coupled with repulsion molecules in order to get the loosening of surface anchors necessary for movements. The intriguing interplay among chemoattraction, adhesion and repulsion is and will be a tempting research object (review by Baier and Bonhoeffer, 1994).

A central topic in developmental biology is the mechanisms turning the genetic information of the fertilized egg into the three-dimensional organization of the embryo. This was the theme of a meeting in 1977 at Uppsala University as part of the celebration of its 500th anniversary. Do you think that the years since this meeting have brought us closer to an understanding of the establishment of the organization of embryonic organs?

When celebrating the anniversary, Uppsala University had chosen to look ahead instead of singing praise to years passed. To do this a great number of international meetings were arranged. The general idea was to demonstrate how the natural

sciences and humanities had become differentiated and what sort of development and new thoughts could be foreseen within different disciplines. What scientific area could be more appropriate in this respect than developmental neurobiology, dealing with differentiation and development of the thinking part of the body....? Be that as it may, a symposium on cell interactions in morphogenesis was an inspiring thing to arrange at that time. Biochemists and morphologists had started to use each other's weapons and they made joint efforts to understand how cells were communicating before and during formshaping movements. The meeting turned out to give a good picture of the current situation of the time (see Jacobson and Ebendal, 1977). It was a source of inspiration for the symposium participants that Sven Hörstadius, then 80, was present. After all, he is one of those who has laid the cornerstones concerning cell interactions in embryogenesis.

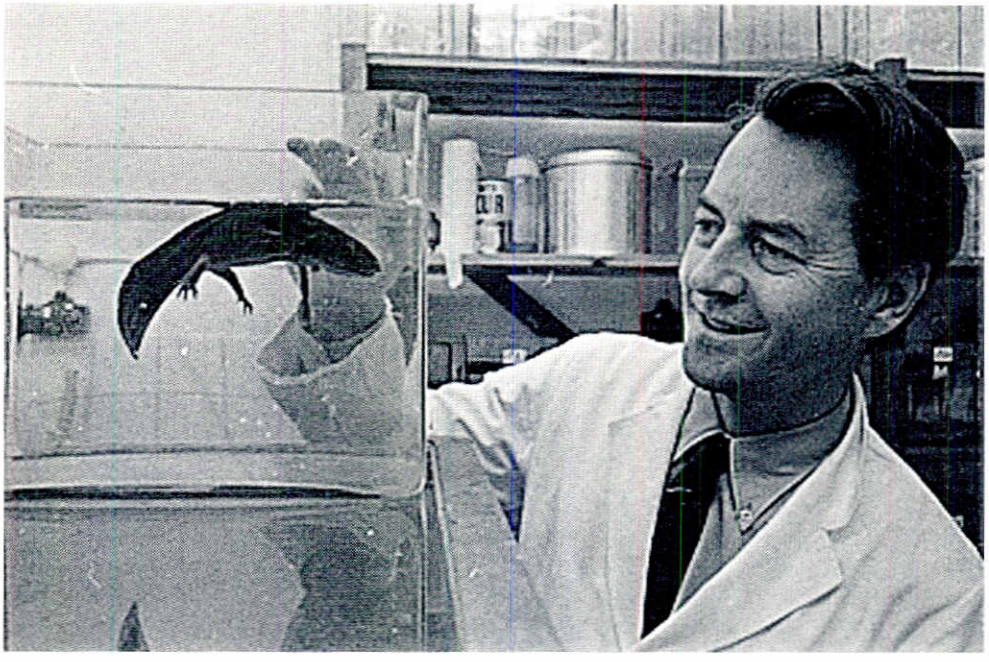

Fig. 2. Jacobson together with one of his axolotl friends in 1970.

Although in 1977 we were rapidly improving our understanding of the mechanisms working in morphogenesis, we were mostly making good guesses based on large amounts of experimental data. Our knowledge of the molecular interactions was still limited. Now, 18 years later, we can make use of the ever progressing gene technique and we are thus able to determine reactive parts of the acting molecules and to describe the morphogenetic processes with more accuracy. That exciting process has only started.

One of your research activities was to show that neurite attraction by target tissues could be studied by co-culture experiments. What is your reaction to the recent achievements in molecular cloning of chemoattractive and chemorepulsive molecules like netrins, collapsin and semaphorin considering the original ideas of Ramon y Cajal, Speidel and others?

My first reaction is sheer joy that the new methods in biology make it possible for me to witness how my indirect evidences and guesses from earlier years now, more than 30 years later, are quite rapidly getting factual contours. One of my first experiments was to study some selected outgrowing nerve fibers *in vivo,* after having mapped the prospective brain areas on the early determined neural plate of axolotl larvae (Jacobson, 1959, 1964). Thus I found that fibers from motor nuclei were finding their way to certain entrance/exit areas for cranial nerves (CNEAs), even if they had to grow for longer distances than in the normal brain and even if they had to penetrate tissue that is not normally in contact with these fiber bundles. The CNEAs seemed to attract the motor fibers and my suggestion was that they secreted growth-cone guiding molecules which were attached to cells and ECM structures in a gradient fashion. Thus my results were in line with those of Lumsden and Davies (1983) twenty years later.

In the 70s the *in vivo* experiments were followed up in our lab with experiments *in vitro*, in which cranial ganglia and target tissues were explanted in collagen (Ebendal and Jacobson, 1977). At the time, nerve growth factor (NGF) was the only well-characterized activating molecule which we were able to identify in the extracts from the fiber-attracting tissues. Now at last several more molecules are identified, which might have capacity to direct growth cones when they are sniffing out the appropriate nerve fiber pathway. We are looking forward to the results of knock-out experiments with netrins etc., experiments which will definitely prove that we have got hold of molecules essential for nerve fiber orientation (see Baier and Bonhoeffer, 1994).

Major parts of the Uppsala tradition in experimental embryology have been to study sea urchin and axolotl embryos, none of which are native to the region. What do you think is the reason for their popularity and how were the axolotls brought to Uppsala?

Sea urchins were the basis of earlier Swedish developmental biology studies (carried out by Runnström, Hörstadius, Gustafson). The sea urchins and their larvae are abundant along all coasts, also at the Kristineberg Marine Biology Station on the Swedish west coast, a Mecca for experimental embryologists, where so many classical experiments were performed in the 1920s, 30s, 40s and 50s. An important reason for the popularity of these larvae is that you can get them by the thousands in one single batch of *in vitro* fertilized eggs. They are relatively easy to manipulate with simple instruments like glass needles which Hörstadius, an astonishingly accurate experimentalist, was able

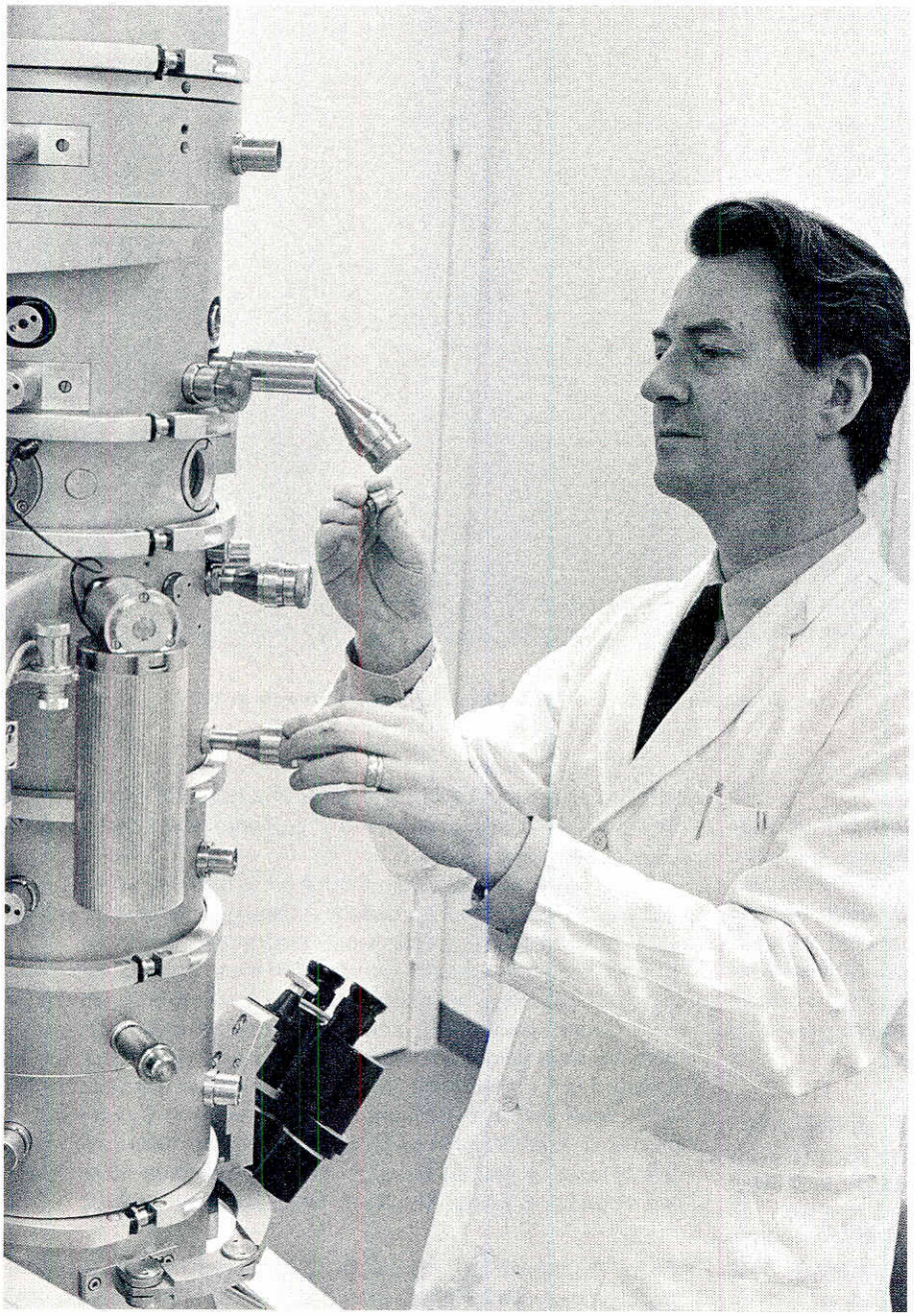

Fig. 3. Jacobson at the Department's TEM device in 1970.

to handle without micromanipulators when he did his basic studies on the interaction between different cells in the early embryo (his great skill with the tiny needles was facilitated by a stiffness in his right wrist, he once confided to me). Another important feature of the sea urchin eggs is that they have only small amounts of yolk which give them distinct morphogenetic movements, in which all cells take part. They are also transparent enough to allow the experimentalist to follow cell movements inside the ectoderm, which Tryggve Gustafson did in his admired time-lapse movies on the gastrulation mechanisms.

When I started my experiments as a young graduate student, a large colony of axolotls already existed at the Uppsala Zoology Department since the days when Hörstadius and Sellman made their extensive study on the origin and derivatives of the neural crest. The animals were originally obtained from Trampusch's laboratory in Amsterdam, and in addition some new axolotls were delivered from the same source during my early work (transported in the cockpit by a cooperative air pilot). The neotene amphibians are ideal experimental animals since they can be kept in small aquaria and since it is comparatively easy to get them to lay their many hundred eggs even at the wrong time of the year. By simple arrangements the axolotls can be fooled into believing that spring has come. The only disadvantage I can remember concerning those dear creatures was that

Fig. 4. Viktor Hamburger (right) and C-O. Jacobson (left) discussing the past and the future of biology at Linnaeus' country place outside Uppsala in 1984.

they had been very spoiled by an animal loving technician before my time: they would spit out all food except calf liver! The best thing about axolotls, however, is the speed with which wounds heal after experiments. In my experiments, pieces of neuroectoderm were cut out and incorporated in new sites and they healed in 15 minutes without visible scars. The operation technique was very simple. Glass needles were manufactured in a microflame. To keep the larvae in place during the operation they were held in place by a loop of children's hair mounted on a glass pipette. A most disturbing problem was the recurring mucous infections, which were quite difficult to get rid of in the barely semi-sterile laboratories of the 1950s.

A recent trend in Sweden and elsewhere is that young scientists with a background in recombinant DNA technologies enter the field of developmental biology and neurobiology. Is there a risk we might lose the tradition of classical experimental embryology due to the power of the modern gene cloning and sequencing methods?

If by classical experimental embryology you mean investigation of details but also a keen wish to understand the entity, to investigate how the new knowledge will change your way of looking at the developing organism, then there might be such risk. The knowledge of basic zoology is not overwhelming among most molecular biologists. If you for instance get new information on how adhesion between cells is working, you will get the full scientific reward only when you can formulate new hypotheses on how the new facts might interfere with the morphogenetic movements in a developing body. In other words – what physiological meaning will new facts about a gene and its expression imply? However, in my opinion, that sort of lack of holistic approach seems to be less frequent today than in the infancy of molecular biology, when the researchers often neglected to see the studied cells and molecules as parts of a functioning organism. Many of the new techniques, on the contrary, are based on a study of the role genes play in the entire body. The results of a knock-out experiment, for example, normally have to be evaluated at the organ and organism level.

Do you find the time and possibility in your present position to keep up with your old field of developmental biology?

To be responsible for the activities in an academy such as the Swedish Academy of Sciences with its broad engagement in science policy, educational issues, global problems like environment-and-development, Nobel Prizes (Fig. 5) and other rewards, scientist exchange programs and research institutes is not a job to be done on the side. Nor is careful keeping up to date with trends and results in developmental biology. I try to read *Science* and *Nature*, but I have no opportunities to read the essential specialized journals or to have the necessary continuous discussions with fellow scientists in the lab or at conferences. In other words, I am probably becoming a less inspiring discussion partner!

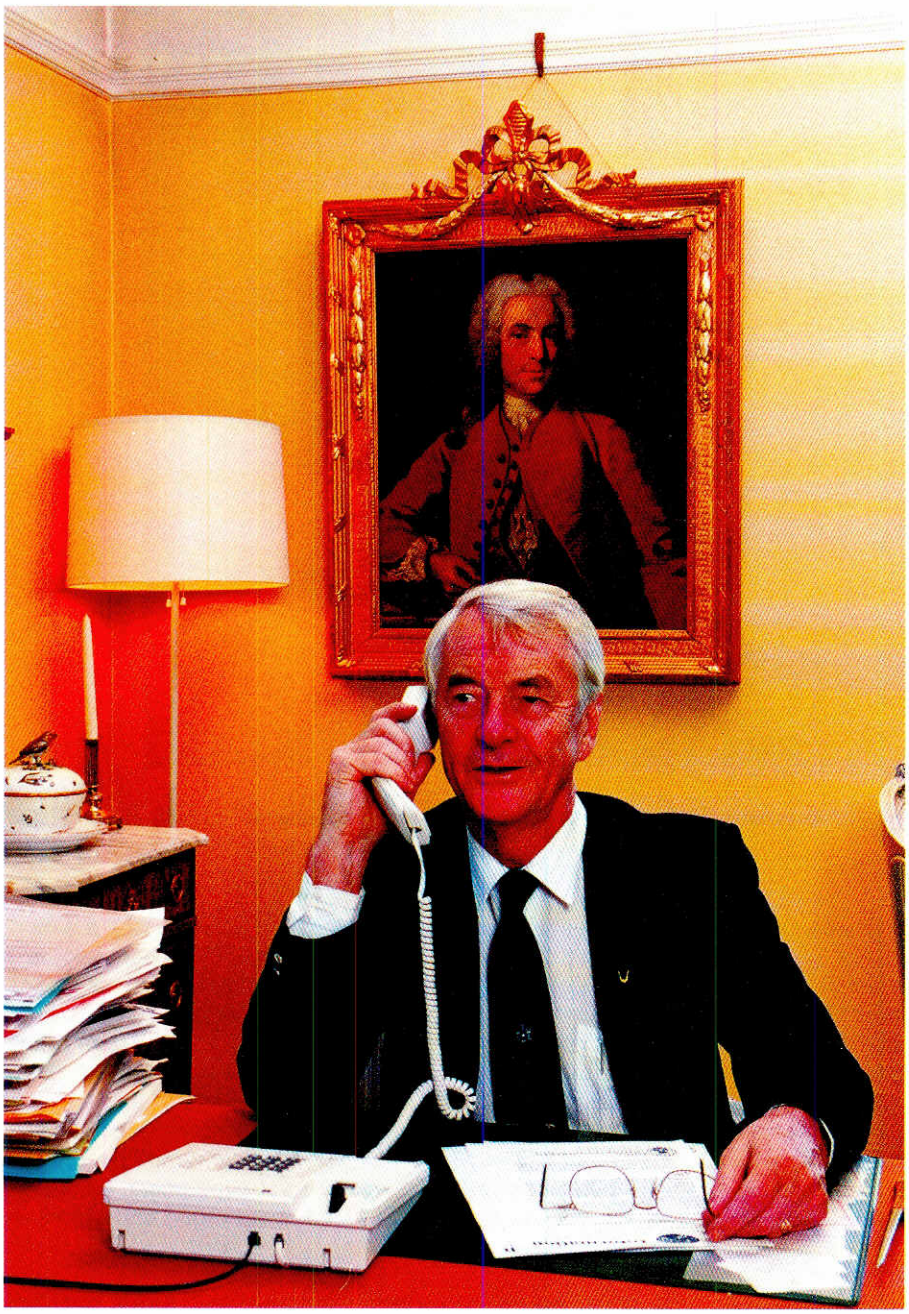

Fig. 5. As Secretary General of the Royal Swedish Academy of Sciences one of Carl-Olof Jacobson's duties is to call the new Nobel laureates immediately after the Academy's decision.

On the other hand, I would never have left the "hands-on" biology had I not been convinced that facilitating scientific work for others is a most important task. At the Academy my time is fully occupied with activities such as initiating discussions on science policy with politicians and international science organizations, arranging conferences, creating ties between Swedish scientists and their economically handicapped colleagues in Eastern Europe and maintaining good conditions for international co-operation at our own research institutes (one of them is the Kristineberg Marine Research Station, once the playground for Runnström, Hörstadius, Lindahl, Gustafson and their international guests). It is an easily explained habit of somewhat older scientists, that they want to lift their eyes above their lab work in order to explore how it fits within a broader context, and how different science areas can contribute to keeping our planet habitable. In that respect, the Academy is a suitable platform. More than 300 of Sweden's most respected scientists and scholars from all areas of human knowledge are at hand for interdisciplinary discussions and seminars and most often we can interact with our sister academies in the US, in Great Britain, in Holland etc. One of the new developments, which I have had the privilege to see take shape during my time as leader of the Academy, is the Beijer International Institute of Ecological Economics, an institution with great potential for attacking problems of impor-

tance for a sustainable development of human societies and the natural environment.

(In the last paragraph the word "development" was used in a much broader sense than what is usual in this journal, but all science ultimately is an expression of human curiosity, and without that attitude to life and the physical world we will have no chance to understand development of neither the organisms nor the planet).

Finally, from your perspectives, do you think that progress in the field of developmental biology will be beneficial to society in general and improve human health?

Developmental biology is truly an interdisciplinary science that reveals all kinds of facts concerning the steps from genetic master plan to functioning organism. It gives us a rapidly growing knowledge of development and maturation in human beings as well as in bacteria. No doubt molecular biology has given us tools which will help us reach a better understanding of the enormously complicated web of interactions that constitutes life. Such knowledge is the only road to deep understanding of deviations from a healthy life and thus also to corrective efforts. For some years now, the new knowledge of development of the nervous system has made it tempting to foresee cures for CNS deficiencies like dementia and Parkinson's disease. As our detailed knowledge of the genes is more and more complete, and our understanding of molecular interaction in the developing organism is widened, we will come closer to the longed-for human control of unwanted gene expression. In other words: yes, I do believe that developmental biology is a key discipline for future health care, not least for ageing professors!

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