A polar development
The Runnström tradition in Swedish developmental biology

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Retzius never advanced his studies of development beyond one of the interacting partners in the fertilization event (i.e. the sperm, see the preceding paper by B.A. Afzelius). However, indirectly, Retzius did fertilize the growth of developmental biology in Sweden.

During the summers of 1906 and onward, a young student from the College of Stockholm (Stockholm University did not formally exist at that time) took summer courses in marine zoology at Kristineberg Marine Biological station in Fiskebäckskil, on the West Coast of Sweden. The student worked here for Retzius, and Retzius introduced him to the analysis of marine organisms. For the young student these summers became determining, not only for his life-long devotion to the biology of development but also in general for developmental biology in Sweden; indeed they had effects on how Swedish experimental biology was to develop in the coming years, effects which are still evident today, nearly a century later. The young student was John Runnström (Fig. 1).

Runnström had really planned to study philosophy but he had early made the compelling analysis that in order to study philosophy one should have a good background in natural sciences. Thus, he postponed his philosophical studies (although he lived to be 82 years old, he never felt he had acquired sufficient scientific insight to start these difficult studies) and studied science instead.

In Stockholm, as in many universities at that time, a main occupation in biological studies was the anatomical analysis of the structure of the multitude of organisms which were at hand. Indeed, the institute at which Runnström worked was called the Zootomical Institute: the institute for dissecting animals and studying them macro- and microscopically. Thus, basically, it was a study of dead material – of course in the hope of understanding function, systematics and evolution, but still without the fascination of following real life events.

The development of the sea urchin: a visual fascination

Few students, even of developmental biology, have today had the chance to personally experience the dramatic and rapid event which takes place immediately after the fertilization of the sea urchin egg. Most experimental procedures of modern biology lack the direct feeling of witnessing something happening in front of your naked eyes: gel electrophoresis of proteins and nucleic acids, transfections, EMSAs etc. all require steps of tedious separations, photographic film developing, analysis of data – before one can find out what the experimental outcome has been; there is a long time-lag between the experiment itself and the result. To look at a sea urchin after fertilization is quite different: in the morning, you just see some round eggs; in some hours you can actually *see* how they are dividing and before the end of the day, a sea urchin larva may already be swimming around. Although we do not know when Runnström first admired this on-going development himself in the microscope, there is evidence that many of his later collaborators entered a life-time of fascination for biology by observing development by directly looking at the processes in the microscope; several of Runnströms collaborators started in the same way that most of us have first been impressed with the powers of development: by collecting frog eggs from nearby ponds and following their transformation from jelly balls into living organisms.
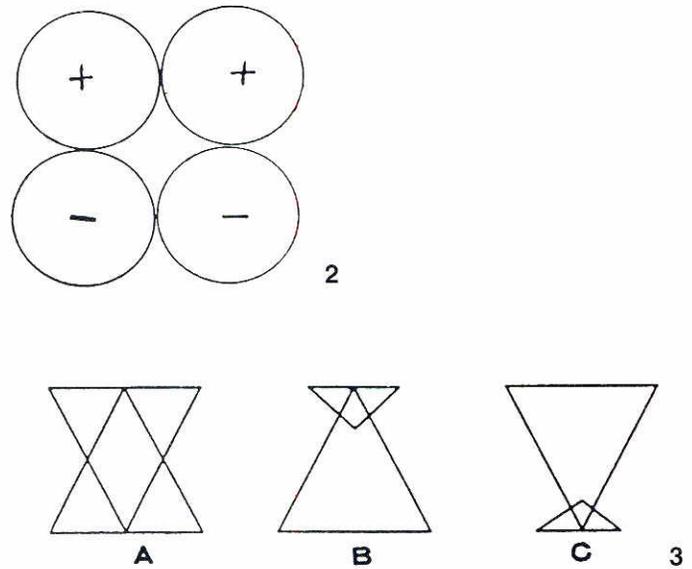
Fig. 1 (left). John Runnström (1888-1971), at the time when he had become director of the Wenner-Gren Institute for Experimental Biology.

Fig. 2. Illustration from one of Runnström's first investigations on sea urchins (Runnström, 1914). *The pluses and minuses symbolize what had been observed by Driesch at the turn of the century: that the sea urchin embryo already at this stage was functionally organized.*

Fig. 3. The gradient theory: *The fate of the cells in the embryo is influenced by substances diffusing from the animal or vegetal pole of the embryo. Normally, these two factors balance each other (A) but experimental situations may be created in which one of these may dominate (vegetalization, B, or animalization, C). From Runnström, 1936.*

A polar development

In the first figure of one of Runnströms first articles on sea urchin development (Runnström, 1914), a preview of what was to become the main theme of his investigations was already evident (Fig. 2): that the sea urchin embryo already at a very early age is functionally organized in what is known as animal and vegetal poles.

Within the next decades, he successively formulated the principle that determinants from each pole influence the future fate of the cells of the sea urchin embryo; in some of his last experimental work published more than 50 years after the one from which Figure 2 is taken, isolation of such substances was still the goal of the study (Hörstadius *et al.,* 1967). As seen in Figure 3A, the idea is that in the normal sea urchin, these animalization or vegetalization factors are balanced, and cells in the middle of the embryo receive a balanced impulse which determines them to enter a new and specific line of differentiation. As 3 different types of cells are then already determined, further lines of development may be induced by gradients from 2 of these 3 cells types, etc.

An indirect implication of these theories is that already in the egg there must be pre-established gradients, as e.g. indicated by Runnström in Figure 4A. There are two comments which should be made to this figure. One concerns the clear formulation of the existence of intracellular agents and receptors for these; although this may seem a simple scheme to us today, the mere idea that it was possible even in developmental biology to formulate events following 'simple Michaelis-Menten kinetics' was not banal at that time (Runnström had not been uninfluenced by a sabbatical term he spent with Michaelis at the Rockefeller Institute in 1934). The other comment is, of course, that this was only a suggestion. However, with modern molecular biology, it has been possible to establish that gradients of the type proposed by Runnström do indeed exist (Fig. 4B).

From very early on in the development of these concepts, the analysis of the ideas was advanced tremendously by the exceptional experimental skills of Sven Hörstadius (Fig. 5). One of the basic observations was the establishment of the effects of the polarity. Hörstadius developed a technique for separating the cells of the embryo. E.g. at the 16-cell stage, there is a principal

difference in the fate of the separated cells if the division is made from pole to pole or equatorially. If the cells are separated in such a way that the proposed gradients are conserved (polar separation), two principally normal sea urchins develop. However, if the separation is performed equatorially, the two halves develop differently and poorly (Fig. 6).

From observations like these, demonstrations of the existence of determining factors became possible, with still more intricate experiments being performed by Hörstadius (Fig. 7). Hörstadius became professor in Uppsala but continued to collaborate with Runnström throughout Runnströms lifetime. With time, Hörstadius also studied the development of the neural crest and the migration of cells originating from the neural crest, a tradition that is still alive at the University of Uppsala (see paper by Hallböök *et al.* in this issue).

Not only morphology

It is implicit in the hypothesis summarized above that it should be possible to affect normal development by adding foreign substances to the embryo. Such substances could be analyzed based on their ability to promote animalization or vegetalization of the embryo. One such substance was lithium, the vegetalizing effect of which had already been observed earlier. In the papers of Runnström from this period and onwards, the ability of a large array of substances to promote vegetalization or animalization were described. In this way, new types of experiments – principally of a chemical character rather than of the physical character of Hörstadius' experiments – could be made within developmental biology, but analysis of the experimental results was performed with morphological techniques.

However, Runnström understood that with the sea urchin egg it would be possible to expand the study of development from a morphological area into the biochemical one. As it is possible to fertilize large amounts of sea urchin eggs simultaneously, synchronous development could be induced in millions of eggs, and the collective changes in chemical constituents may become measurable. Thus, Runnström's plan became to chemically analyze the events at the time of fertilization.

In retrospect, as exemplified below, the broadening of the field would mainly seem to be guided by the principle "if it moves, measure it". However, it should be realized that through this, Runnström helped in the transfer of developmental biology, from a purely descriptive analysis at first to an experimental morphological analysis – and then further on to an experimental biochemical (or molecular cell biological, we would say today) pursuit.

We will exemplify below some of these new types of investigations into developmental biology that Runnström initiated. What Runnström did was to allow each new student to start the analysis of a new chemical constituent of the sea urchin. As seen with time, these studies developed in their own right, and the subprojects and their leaders tended to leave the area of developmental biology as such.

Energy metabolism

When Warburg came to Stockholm for a congress in 1926, he donated to the Runnström group a demonstration copy of what was later to be called the Warburg apparatus. He had actually
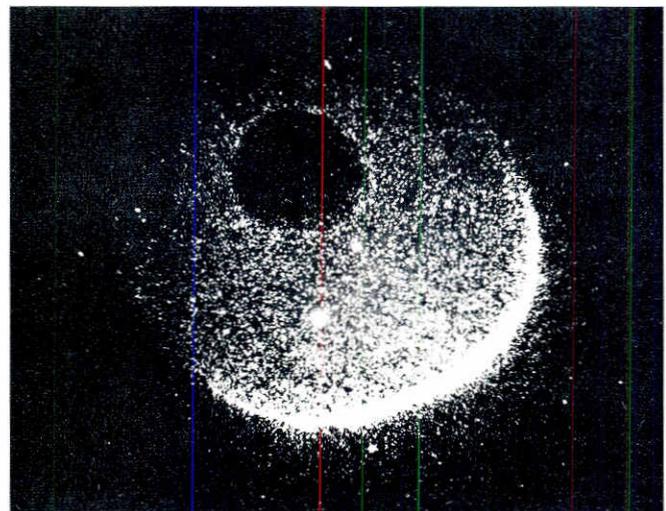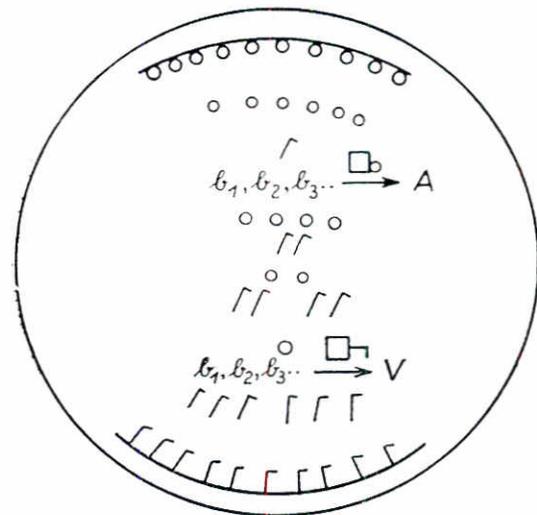

Fig. 4. (top) Suggestion of the existence of pre-established gradients in the sea urchin egg. *From Runnström (1954).* **(Bottom) Later demonstration of such gradients by molecular biological techniques:** *the distribution of the mRNA for Vg1. Courtesy of D.A. Melton (Melton, 1987).*

already performed measurements of respiration in sea urchin eggs and observed that fertilization led to a large increase in respiration. Runnström found both the observation and the instrumentation exciting; with time the Warburg apparatus became an important experimental procedure at the institute, during some time periods probably the most important instrumentation.

The metabolic consequences of fertilization became the project of a student in the group, Olov Lindberg (Fig. 8), who, in his first work (Örström and Lindberg, 1940), was able to show the rapid degradation of glycogen occurring after the fertilization of the sea urchin egg (Fig. 9). In post-war years, Lindberg maintained his interest in metabolism. When it was realized that respiration was localized to a subcellular fraction – the mitochondrion – this fraction became his object of study. In this transfer of

Fig. 5. Sven Hörstadius (born in 1898) at the time when he was working with Runnström in Stockholm. *He became Professor in Uppsala in 1942.*

Fig. 6. (right, top). The fates of the animal and vegetal part of the embryo of the sea urchin if the two parts are separated. *Redrawn by Runnström ¡(1935) from Hörstadius.*

Fig. 7 (right, bottom). An example of the intricate experiments performed by Hörstadius, here investigating the effects of micromeres etc. *From Hörstadius (1939).*
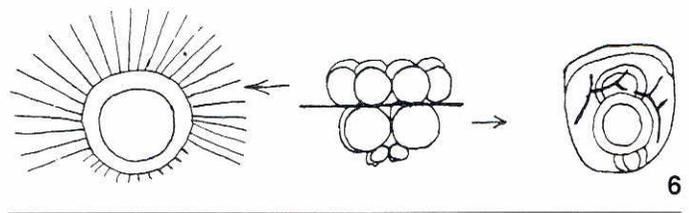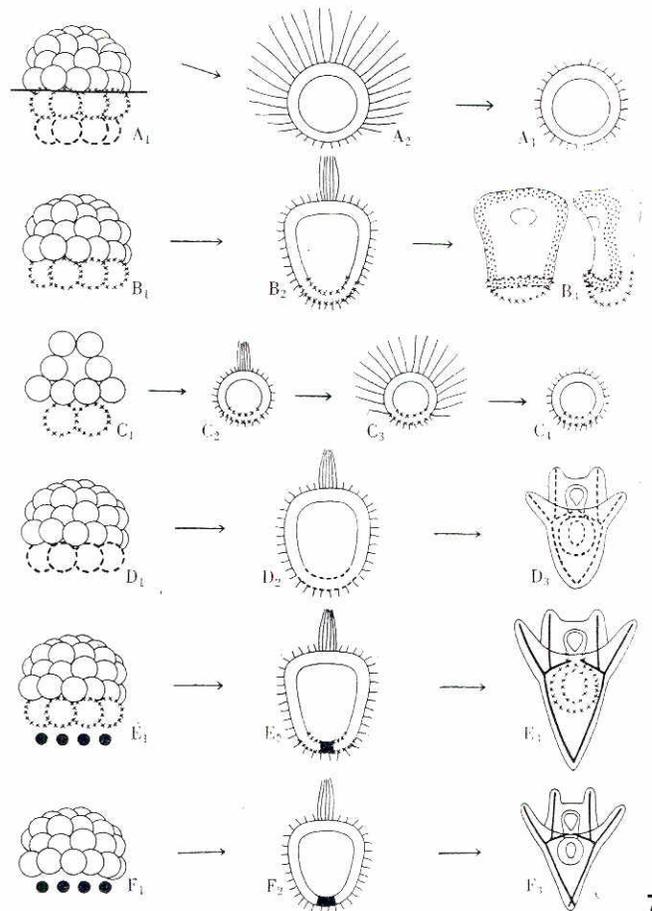
interest from the metabolism of sea urchin embryos to that of liver mitochondria, one of Lindberg's students, Lars Ernster, was pivotal. When Ernster left the institute to become Professor of Biochemistry at Stockholm University, Lindberg decided to devote his interest to what is the most respiratorily active tissue in the mammalian body: brown adipose tissue, and to the most respiratorily active mitochondria: the brown adipose tissue mitochondria. Today studies on brown adipose tissue are still going on at Stockholm University, and these studies are now approaching questions which may be considered to be close to those of developmental biology (see paper by Nedergaard *et al.* in this issue).

Protein synthesis

Protein synthesis was also found to increase dramatically at the moment of fertilization. Whereas the incorporation of radioactively labeled amino acids is practically non-existent in unfertilized eggs, a high rate of protein synthesis occurs shortly after fertilization. The study of protein synthesis became the subject of Tore Hultin (Fig. 10). Hultin expanded these studies to a general study of ribosomes in eukaryotes. With the advent of the understanding that this protein synthesis must be based on preformed mRNA – which was thus found in the sea urchin egg before fertilization but was not utilized for protein synthesis – questions concerning the regulation of protein synthesis initiation and rate control were obvious (Hultin, 1961). Studies in this tradition are still going on at Stockholm University (Holmberg and Nygård, 1994).

In an interesting extension of these studies, Hultin realised in the 1960'ies that an experimental object existed which in certain of these respects was similar to the unfertilized sea urchin egg. This was the cryptobiotic cysts of the shrimp *Artemia salina* (Hultin *et al.*, 1969). These cysts possess preformed mRNA, and this organism has with time become an object of study in itself.
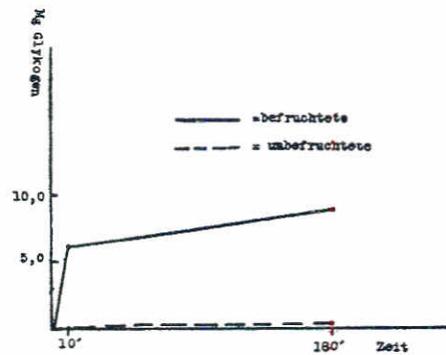
Verschwinden des Glykogens in unbefruchteten und eben befruchteten Eiern. Nähere Erklärungen siehe Tabelle I. Die Kurve zeigt, wieviel Glykogen (als Glucose) nach den betreffenden Zeiten verschwunden ist.

Fig. 8. Olov Lindberg (born 1914) at the time when he worked on the metabolism of sea urchins. *He later became Professor of Experimental Biology and Cell Research at the Wenner-Gren Institute and Director of the Institute. He retired in 1980.*

Fig. 9. Demonstration of the effect of fertilization on the rate of glycogen breakdown. The dramatic difference between the fertilized (upper curve) and the unfertilized egg is evident. *From Örstrom and Lindberg, 1940.*

The antigens

One of the most visionary projects of Runnström was his idea that it would be possible to use immunological techniques to identify substances found in the sea urchin egg. He therefore asked a student, Peter Perlmann (Fig. 11), to inject sea urchin eggs into rabbits and to characterize the antibodies which were produced. To use antibodies to characterize proteins does not seem revolutionary today, but in the late 1940s, this was a unique procedure, not least in developmental biology. This type of experiment (Fig. 12) worked: antisera against sea urchin egg surface proteins were developed and could be used to characterize surface glycoproteins.

An even greater surprise was the observation that some of these antisera had the ability to activate the egg parthenogenetically.

From an analysis of surface antigens on developing sea urchin eggs, the step was not far to investigations of surface antigens on other rapidly growing cells: cancer cells. Peter Perlmann – who is still active at Stockholm University – later started studies on surface antigens on cancer cells (Schneider *et al.*, 1980) and subsequently went further trying to develop vaccines against malaria (Chougnet *et al.*, 1991).

The ultrastructure

To look at fertilization and development in the microscope was, as indicated, the standard technique for studies of development before the time of Runnström. With the construction of the electron microscope, it was natural to try to investigate whether an analysis at this level would reveal new information on what was happening in the egg.

Runnström therefore suggested that a new student, Björn A. Afzelius, should be acquainted with this technique. Afzelius therefore started his studies at the department of Anatomy at the Karolinska Institute (i.e. at the old institute of Retzius). With this new instrumentation, Afzelius was able to follow events during the fertilization process which could not have been observed earlier, e.g. the events occurring during the fusing of the cortical membrane with the outer membranes of the egg.

Also the study of the sperm at the moment of fertilization caught Afzelius' interests. This led to a long series of studies on sperm cells (Baccetti and Afzelius, 1976), which included the first detailed description of their structure (i.e. the general cilial structure as well) (Afzelius, 1959) and the pathological effects of the loss of the dynein "arms" of this structure (Afzelius, 1976). Afzelius is still active at Stockholm University.

The morphogenic movements and the morphogens

In certain respects, the early phases of development are somewhat bland. Eggs – although cleaving – and blastulas are rather homogeneous structures. However, the moment of gastrulation (Fig. 15) is a physically very active period during development. This type of event involves morphogenic movements, and for most of his research career, these movements were the object of study for Tryggve Gustafson (Fig. 13) – the only of
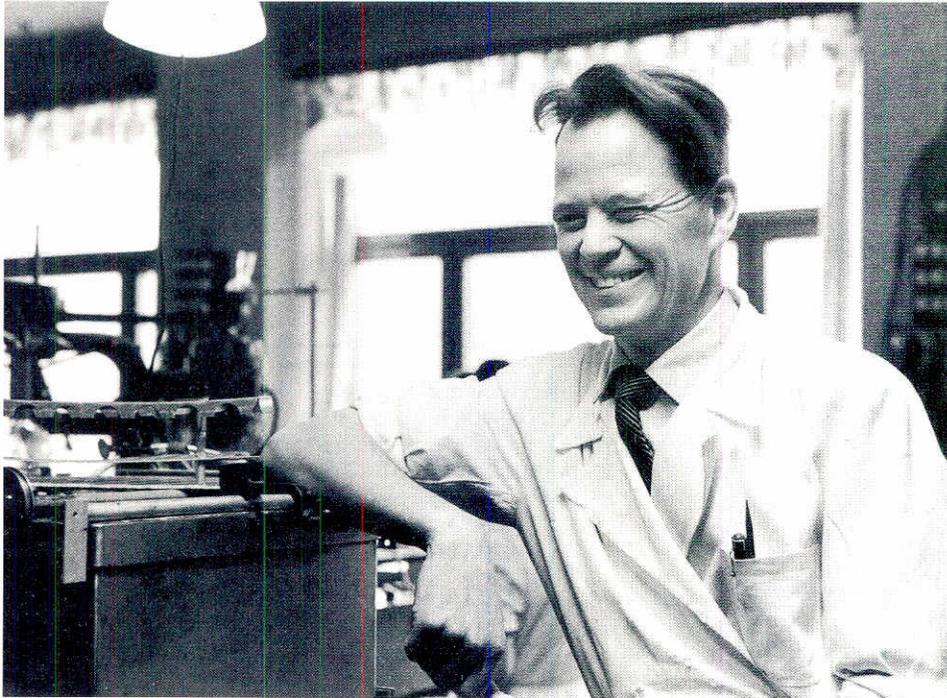
Fig. 10. Tore Hultin (born 1919) in the laboratory at the Wenner-Gren Institute in which he studied protein synthesis, first in the developing sea urchin and later in rat liver. *Hultin retired in 1985.*

Runnström's students who stayed loyal to sea urchin developmental biology during his entire scientific career. A mere look at the events at gastrulation makes one think of muscular activity, and the question was raised as to whether the neurotransmitters that regulate muscular activity in the adult animal could also be involved in the morphogenic movements of early development. Gustafson was able to demonstrate the chemical presence of neurotransmitters in the sea urchin larva, and he demonstrated that the larvae were able to respond to the neurotransmitters, at least acutely and with motor activity (Fig. 14). He therefore implied that these substances could be important both in regulating the motor activity of the cells (such as during gastrulation) and that they could also play a role as morphogens, determining the future fate of cells. Although this latter line of research has not been followed up in relation to the development of the sea urchin, the basic notion that even classical neurotransmitters (such as e.g. norepinephrine) may have effects on cellular development may be valid in certain systems (see e.g. paper by Nedergaard *et al.* in this issue).

An institute for the developing sea urchin

The successful studies of Runnström and the growing interest in the field of Experimental Cell Research (which became the name of the journal founded by Runnström) had the consequence that the old institute became too small.

From the Rockefeller Foundation, Runnström was able to obtain a promise that the foundation would support the creation of a new institute with a substantial amount of money – provided that Swedish sources in some way would provide a similar amount. In those days, money for research did not necessarily come from governmental sources, and Runnström was able to attract the interest of Axel Wenner-Gren, the founder of

Electrolux, at his most successful time believed to be one of the richest men in the world. Wenner-Gren was pleased to be considered a benefactor of arts and science and he promised to provide the second half of the money necessary. Thus, in reality based on the study of the developmental biology of the sea urchin larva, a whole institute was created: The Wenner-Gren Institute for Experimental Biology. The new building was inaugurated in the summer of 1939 (Fig. 16), and for many years ahead, Axel Wenner-Gren supported the ongoing research activities of the institute (Fig. 17).

However, this – the moment of triumph for Swedish sea urchin developmental research – was destined to coincide temporally with the start of a decline in this type of research. This was not due to dwindling internal interest but to external forces of a much stronger nature.

On the very day in 1939 when the sea urchin group (Runnström and his doctoral students and collaborators) returned to Sweden from the annual summer stay with the sea urchins in Roscoff – only some months after the inauguration of the institute – the Second World War broke out. Of the many effects of the war, one was that the ability to travel to France and to Italy with the seasons of sea urchin activity, was gone. Even at the Swedish marine research station Kristineberg, activity became much more cumbersome. Thus, the scientists of the institute had to concentrate on experimental material which could be obtained even under wartime conditions, and projects with e.g. yeast were started. Although this forced upon the institute what was to become in reality a period of very fruitful research into what today would be understood as cell biology in a broad sense, it meant that the developmental profile of the institute started to fade, and was replaced with a broad cell biological profile.

However, in one respect the institute gained from the war. An influx of scientists from Central Europe came to the institute and
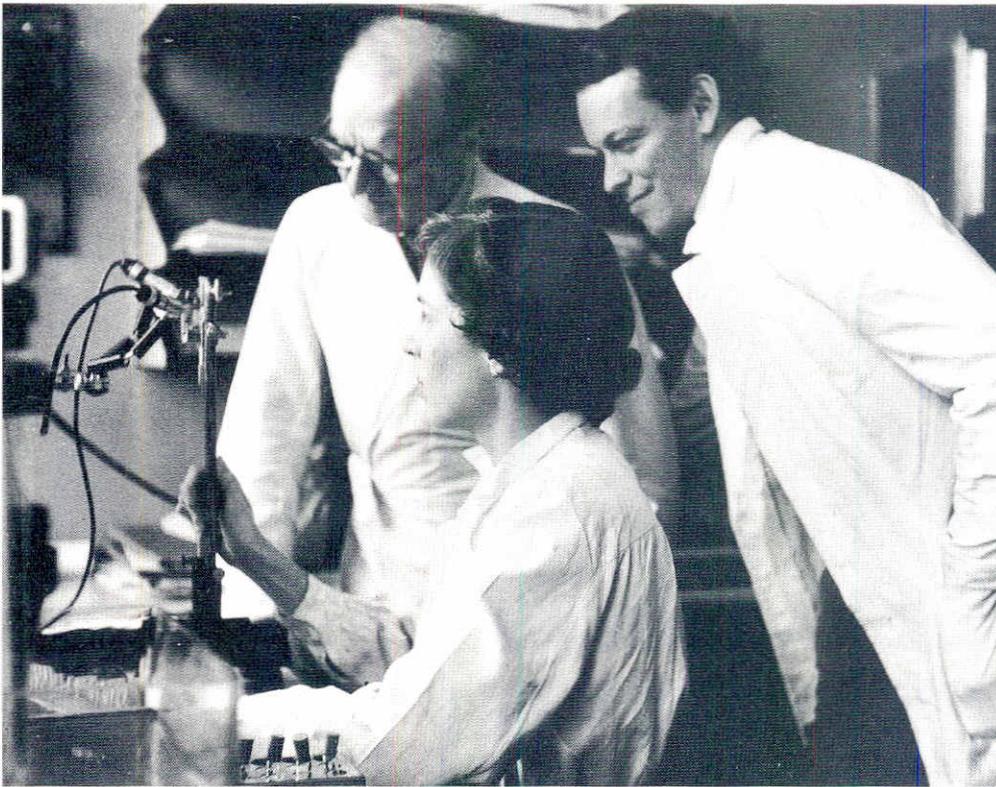
Fig. 11. (top). John Runnström (1888-1971), let, together with Peter Perlmann (born 1919), right, in the laboratory. *Perlmann later became Professor of Immunology at the Wenner-Gren Institute.*
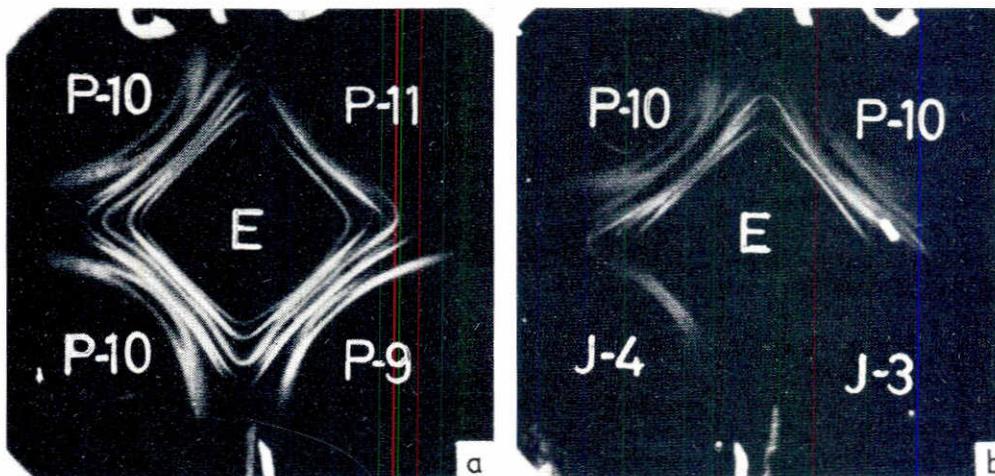
Fig. 12 (bottom). Interaction of different antisera with a sea urchin egg (E) homogenate (Perlmann, 1957). *The antisera P-9, P-10 and P-11 were antisera made againts jelly-free eggs of Paracentrotus lividus whereas the antisera J-3 and J-4 were made againts purified jelly substance from such sea urchin eggs. The P antisera also showed the remarkable ability to induce activation of unfertilized sea urchin eggs (e.g. initiating cell division) whereas the J antisera were much less able to do so.*

they were welcomed and helped by Runnström, who was American-oriented and antinazi. Although this influx was the effect of tragic events, it meant that leading scientists passed through the institute – some to stay but most on their way to Allied countries. Through this, the institute of Runnström was fertilized with ideas from Central Europe, and after the war, an international orientation and international contacts remained a characteristic of the institute.

The physical building of the Wenner-Gren Institute for Experimental Biology stood for nearly 50 years. However, as the Wenner-Gren financial empire was successively eroded away, the Wenner-Gren Institute became an integrated part of Stockholm University which coalesced from different more or less independent institutes during this time. As an administrative unit, the institute still survives as a major part of experimental biology, containing departments of Cell Biology, Physiology and Immunology. No sea urchin development is today studied within the institute (or anywhere in Sweden). However, as a reminder of the origin of the institute, the Runnström and the Wenner-Gren lectures have been created (with eminent developmental biologists as speakers). As a new professorship became vacant after Afzelius' retirement, it was decided that the time had come for developmental biology to again become a defined subject for research and education. An additional department, that of Developmental Biology, was therefore (re)created and as the first formal professor in developmental biology at the institute,
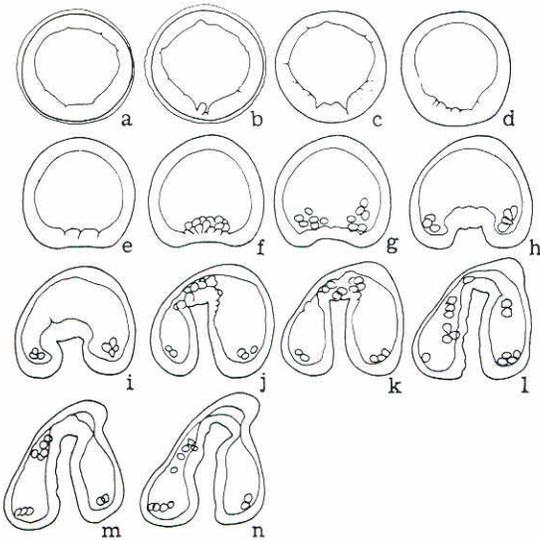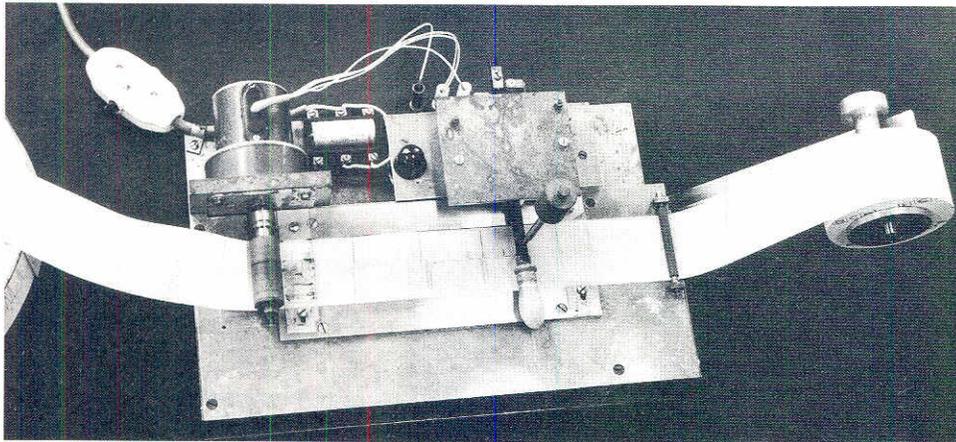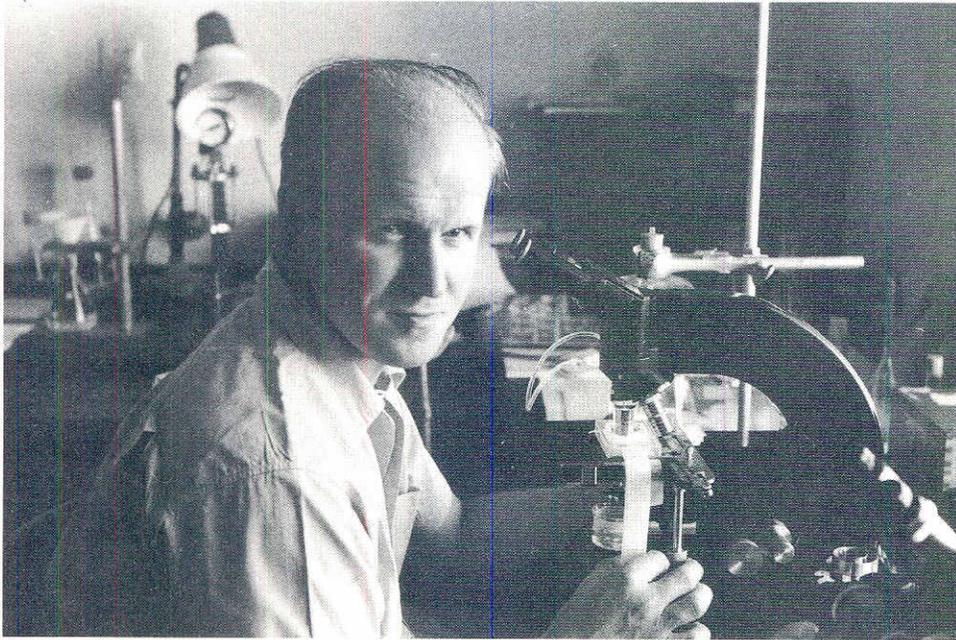
Fig. 13. Tryggve Gustafson (1918-1989) at his microscope. *Gustafson later became Professor of Zoological Physiology at the Wenner-Gren Institute.*

Fig. 14. The apparatus used by Tryggve Gustafson for registering morphogenic movements. *As stated by Gustafson (Gustafson et al., 1972), he was indebted to Runnström for (among other statements) "he insisted that the problems should be attacked from various angles and with different methods, not necessarily the most expensive and complicated...". This instrumentation is a clear manifestation of this notion. It was used in the way that the paper was drawn with a constant speed through the apparatus while the sea urchin larva was monitored in the microscope. By pressing different buttons, three different markings were made on the paper indicating e.g. morphogenic movements; these markings could later be counted and analyzed.*

Fig. 15. The gastrulation event. *Adapted from the review on the cellular basis of morphogenesis and sea urchin development by Gustafson and Wolpert (1963).*

Fig. 16. On the day of the inauguration of the Wenner-Gren Institute, Runnström demostrates experimental apparatus (Warburg) for the Swedish crown prince, later King Gustav VI Adolf.

Fig. 17. The donator, Axel Wenner-Gren (in dark suit), together with some of the staff at the Wenner-Gren Institute: from the left, Lars Ernster, Olov Lindberg, Tore Hultin, Harry Boström and Tryggve Gustafson.

Eva Engvall was appointed in 1993 (see paper by Engvall in this issue).

Perspectives

When one looks at the biological journals of today, it is amazing to realize that not so many years ago, developmental biology was considered by many to be a section of the sciences which had reached and passed its high point, restricted as it seemed by technical limitations: there was apparently not much more that could be done. In Sweden especially, the end of the Runnström era meant a decline in interest in sea urchins specifically and in developmental biology in general. It is today possible to realize that in his concepts Runnström was far ahead of his time, and that it was methodological problems which were the limitations to progress. However, internationally, Runnström had been able to forward the study of development, and in Sweden he had founded an interest in experimental biology which is still a hallmark of Swedish science.

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