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Developmental biology in Lund from a zoological perspective

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With the inauguration of the Wenner-Gren Institute in Stockholm in 1939, developmental biology, in the present sense of the term, was definitely established as an independent branch in Swedish zoological research. At the universities of Uppsala and Lund, research in developmental biology was for some years ahead not segregated from the zoology subject, but gradually its independence was accepted there as well. When the new university subject Zoophysiology was introduced at these universities, it was understood that developmental biology would be a leading element in it, and a similar trend was also seen in other Scandinavian countries. After the appointment of Per-Erik Lindahl as the first Swedish Professor of Zoophysiology in Uppsala in 1946 it was just a matter of time when Lund was to have its counterpart.

The foundation period (1949-1967)

In 1949 Ivar Agrell was appointed permanent Associate Professor (Swedish: laborator) of the Chair of Zoophysiology in Lund and as full Professor from 1959. Originally he had made a name for himself as entomologist and pioneer in insect ecology, but became increasingly fascinated by the complex problems behind growth and diapause in insects. Stimulated by his contacts with the famous physiologist Torsten Thunberg at the medical faculty in Lund, he began to adopt Thunberg's methods for biochemical and cell biological analysis in his own studies of the metabolic adjustments during insect development (Agrell, 1947).

Owing to the initially very small resources allotted to him, the organization of the new department became a difficult and time-consuming process. Already from the onset it was understaffed, and only the most urgent research facilities could be provided for (Fig. 1). The laboratory space was very limited and not well suited for its purpose. Agrell's policy was that already from the beginning the new department should have a broad research profile, and for that reason his early students were not engaged in his own insect project but were encouraged to start new, independent research projects. Of course this was a stimulating task, but as things were then, it scarcely furthered a rapid development of the research potential of the department.

From the very beginning Agrell realized that in developmental biology it was of the utmost importance to pay special attention to the progress within biochemistry. It was therefore a natural thing for him to enter into collaboration with the gradually developing biochemical laboratory at the Chemical Institute of the university, a connection that became particularly fruitful within the field of nucleic acid analysis. His good relationships with researchers in cell biology in Copenhagen, notably at the Serum Institute and the Carlsberg Institute, were also very useful and resulted in the introduction of important new techniques in the laboratory work.

In developmental biology in Stockholm and Uppsala the sea urchin embryo had become the favourite research object, and through his contacts with the Wenner-Gren Institute people, especially with Tryggve Gustafsson, Agrell came to realize its usefulness. Above all he became fascinated by the rapid, synchronous cell divisions during the embryo's cleavage period, but it also led to a widened interest both in early embryonic growth in general, and in the cancer phenomenon.

In addition to Agrell's own insect and sea urchin projects, four other research projects were firmly established at the new department in the mid 50s, two of them in developmental biology. Thus growth and cell multiplication was studied in the gastrulating chick embryo by Hadar Emanuelsson, and in flatworms the regenerating capacity was analyzed by Nils Olof Lindh.

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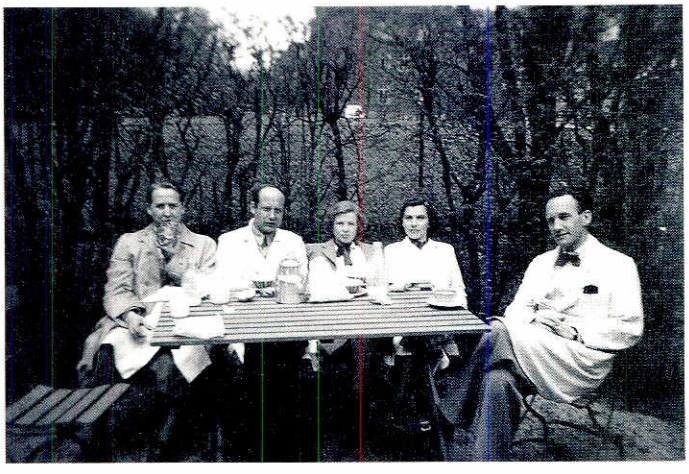

Fig. 1. Ivar Agrell with his early staff. *From the right: Ivar Agrell; Elsa Rosengren, later Professor of Medical Physiology in Lund specializing in histamine research; a temporary employee; Ragnar Fänge, later Professor of Zoophysiology in Oslo, then in Gothenburg. Field of research: fish physiology; the author, Professor of Developmental Biology at the Swedish Natural Science Research Council.*

Agrell's own studies on sea urchin embryos were performed in the 50s at Kristineberg in Sweden and at Roscoff and Banyuls in France. His interest was focused on the very early embryos. Their early mitotic gradients were carefully recorded and in this connection he also tested the influence from various metabolic inhibitors, steroids and lithium chloride (Agrell, 1954, 1956) on mitotic activity and duration of the various mitotic phases. The results were rather sensational, e.g. he found estradiol to be a potent mitotic inhibitor, and lithium to prolong the synchrony of the early cleavage divisions (Fig. 2). The mechanisms behind the observed disturbances remained difficult to explain, however. This new interest for steroid effects on mitotic activity furthermore led to analyses of mitotic activity and steroid excretion in tumor-bearing mice. Agrell's analyses of the early mitotic activity were later widened to include fly embryos as well (Agrell, 1962), and in flies he also studied membrane formation in the imaginal discs.

In the chick embryo project cell production and cell streaming connected with embryogenesis were recorded with cytological and biochemical techniques, and attempts to reveal the mechanisms underlying the early embryonic induction were started (Emanuelsson, 1960).

In the flatworm project the regeneration pattern of the isolated body sections was followed by detailed biochemical analysis of the nucleic acid synthesis in them, and special attention was paid to the histogenesis and polarity developing in them. Also transplantations, notably heteroplastic ones, were performed on the flatworm material (Lindh, 1959).

Concurrently with the increasing activity at the institute during the 50s more grants for research gradually became available so

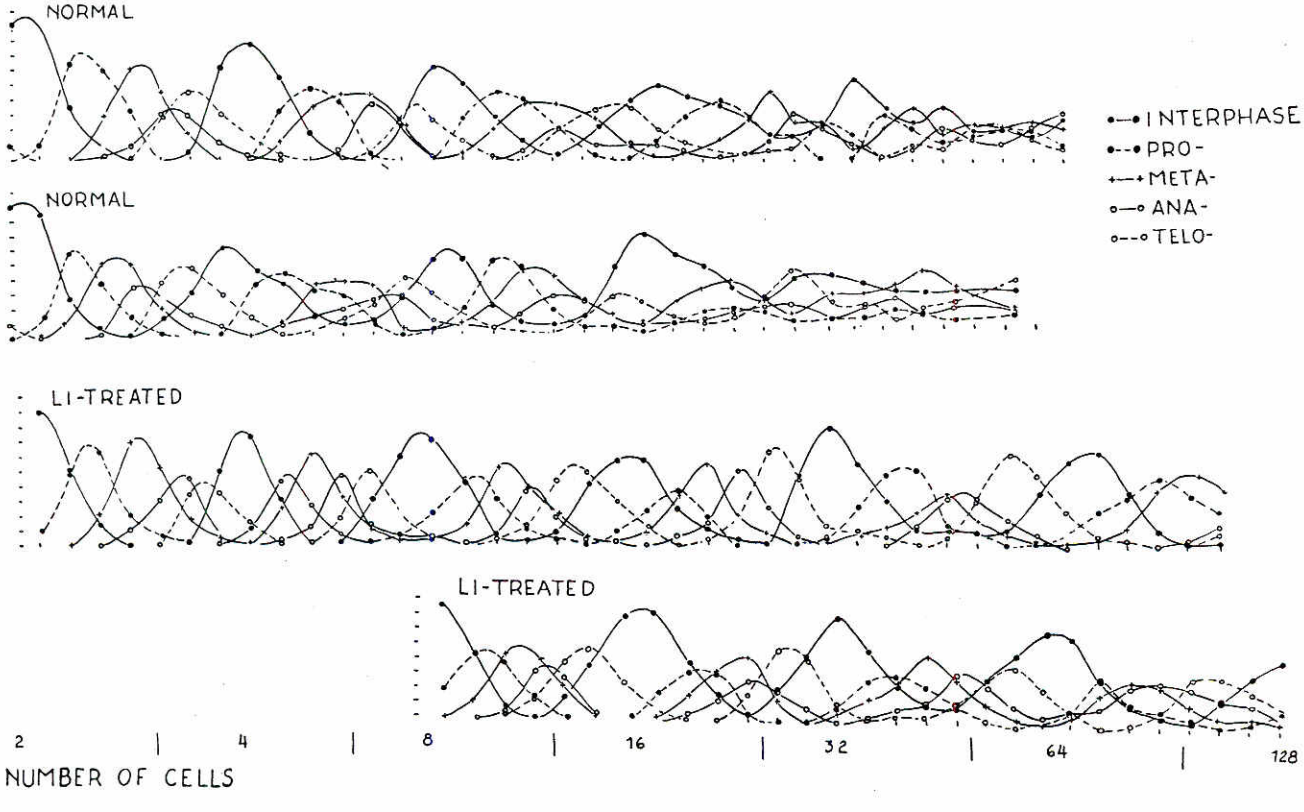

Fig. 2. The mitotic spectrum in the early period of the embryonic development of the sea urchin *Paracentrotus lividus*, as recorded by Agrell. *The diagram demonstrates the observed differences between normal and lithium-treated embryos. Notice the careful registration of each of the mitotic phases. (From Agrell 1956).*

that outdated equipment could be modernized or replaced. New cytochemical and biochemical micromethods were introduced and adapted for the various research objects. Phase-contrast microscopy, hitherto the standard method in cell analysis, was supplemented with equipment for DNA-cytometry, and electron microscopy was put into practice little by little. Special techniques acquired from the Carlsberg Laboratory and developed at the institute was the weighing of cells/tissue fragments using the Cartesian diver balance (Zeuthen, 1948), and the preparation of synchronous mass cultures of the ciliate *Tetrahymena* using repeated heat shocks. Last but not least, a small cell culture laboratory could be organized with support from the Swedish Natural Science Research Council.

The stabilization period (1967-1973)

Already in the beginning of the 60s the laboratories at the institute were filled to the limit of their capacity, but not until 1967 the institute could move into a new building (Fig. 3). At this time the principal projects in developmental biology dealt with:

1) Mitotic activity, cell growth and cell differentiation in invertebrate and amphibian embryos. Analysis of various growth parameters in the ciliate *Tetrahymena* and in cancer cells.

2) Regeneration in flatworms. Soon afterwards this successful project was unfortunately cancelled when the project leader (Nils Olof Lindh) left the institute and took up a new post at the University of Umeå.

3) Growth and induction processes in the early chick embryo.

4) Milk protein absorption in suckling piglets, then a newly established project with particular focus on the physiology of the small intestine. Based on detailed protein analyses it announces the introduction of advanced immunological research methods at the institute (Karlsson, 1966).

With access to more spacious and well adapted laboratories in the new building more attention could now be given to research on cell and tissue cultures, and the purchase of modern centrifuges made it possible to perform qualified analyses on proteins and cell fractions. Since special laboratories had been earmarked for radioisotope biochemistry and autoradiography a rational utilization of radio-isotopes in the analyses could at length be accomplished. The staff could be increased and in addition to more space for it and for the laboratories also teaching was adequately provided for. Now it was also possible to arrange special rooms for permanent housing of research animals, especially rabbits, guinea pigs, rats, mice and amphibians. Space was also reserved for permanent cultures of ciliates, bluebottles and polychaetes.

In brief outline, research in developmental biology had proceeded as follows at the end of the period:

The sea urchin studies had gradually changed their character. While initially the main attention had been paid to mitotic gradients and patterns in the developing eggs/embryos, the activity was subsequently directed towards the problems behind the rapid synthesis of nucleic acids from the nucleotide pool stored in the egg (Agrell and Persson, 1956). Great efforts were spent on the biochemical analyses involved, i.e. to find out whether a special cytoplasmic DNA was operating at the earliest stages. Even if this idea could not be confirmed, the analyses obviously had a stimulating influence on contemporaneous sea urchin research. The final papers from the institute dealing with sea urchin embryos have essentially described the appearance of various RNA-types during the early embryo development (Ohlsson, 1968).

Fig. 3. The Department of Zoophysiology was initially housed in the Zoology building (right) but moved into a new building (left) in 1967. *The Department is still in the same place but has modified its name to Animal Physiology.*

Research on mitotic synchrony in early fly embryos was soon followed by analyses on nuclear migration and on the resulting massive cellularization. In later investigations on fly embryos at the institute, the appearance and characterization of pole cells has been a leading motif (Lundquist and Emanuelsson, 1980).

As a consequence of the striking interest in embryonic mitotic activity, research in cell multiplication was successfully expanding, pursued as it was along two principal lines, one based on observations on heat-synchronized ciliates and on free-living amoebae, the other on observations on myoblasts, fibroblasts and cancer cells. Work on growth in the ciliate *Tetrahymena* originally started with observations on protein metabolism in the organism (Christensson, 1959) but gradually also other cell components, e.g. DNA (Holm, 1968), had been included in the analyses. The leading motif in the other studies concerned regulation of onset and length of the various phases in the cell cycle during varying culture conditions. Also the interaction between nucleic acids and histones was investigated (Agrell, 1969). The mitogenic action of phytohemagglutinin was successfully demonstrated in various types of cell cultures, and, interestingly, also in amoebae (Agrell, 1966).

In the chick embryo studies, special attention was paid to problems concerning morphogenesis, and notably to the role played by the intracellular yolk granules in the developmental process. As a consequence, the analysis had to be extended to the cleavage stages and also to ovogenesis, an activity which required extensive electron microscopical work. To widen the basis for the yolk studies, contemporary analyses were started on embryos of the polychaete *Ophryotrocha labronica* (Emanuelsson, 1969). More easily kept in the laboratory than sea urchin embryos they soon became a favourite object in developmental biology research at the institute.

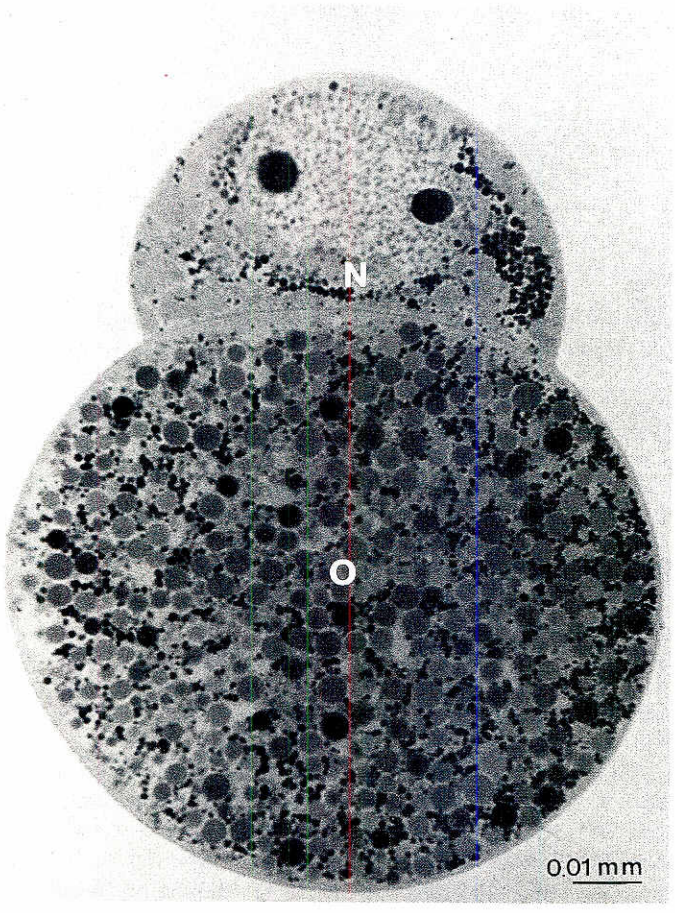

Fig. 4. Section through an isolated oocyte-nurse cell pair of *Ophryotrocha labronica*. *N, nurse cell; O, oocyte. Usually there is only one nucleolus in the nurse cell, sometimes there may be two as in this case.*

Up to this time practically all projects at the institute dealing with cell growth and cell differentiation had analyzed the problems in terms of nucleic acid synthesis and protein synthesis. Whereas the importance of the immediate metabolic precursors involved in these processes was clearly conceived, any influence on them from other low-molecular elements was largely disregarded. In that situation the introduction at the end of the 60s of polyamine research by Olle Heby had a vitalizing effect and created a new approach to growth and differentiation phenomena.

Agrell lived to witness the inauguration of the new institute building (Fig. 3), but shortly afterwards he was stricken with illness. In spite of that he bravely continued his scientific work but passed away in 1973.

He leaves behind the memory of a highly gifted person, endowed with a lively temperament, and universally recognized both as scientist and artist. In the scientific discussions he gave proof of logical acuity and brilliant ingenuity. As a developmental biologist he spread his interest on varying projects, which certainly suited his artistic temperament, but prevented him from founding a school in normal sense. Among his publications in developmental biology, those dealing with the metabolic adjustments in insect development and with synchrony and cell mobilization in early invertebrate embryos have received most attention.

Transformation period (1973-1990)

After a drawn-out procedure to fill the post after Agrell, a neurobiologist (Anders Edström) was in 1976 appointed to the Chair in Zoophysiology, and by that a lasting change in the research activities of the institute towards neurobiology started. Also organ physiology expanded, thus research on neonatal and suckling piglets has increasingly become focused on organ physiology with transmission of macromolecules in the small intestine as one leading motif. A clear indication of the re-orientation at the institute was the onset in 1990 of major improvements of the housing facilities for research animals and of the operating rooms. This event can therefore mark the end of this short historical survey.

During this transformation period developmental biology has still held a strong position at the institute, and it has been supported by the establishment of a Professorship in Developmental Physiology for Hadar Emanuelsson, initiated and sponsored by the Swedish Natural Science Research Council 1979-1992.

Research on chick embryo development reached its peak during this period and important new findings could be reported concerning yolk consumption, early growth and morphogenesis. Interestingly it was also possible to demonstrate a decisive role for polyamines in the embryogenesis and early organogenesis of the chick (Löwkvist *et al.*, 1980).

As a new research object in developmental biology at the institute the polychaete *Ophryotrocha labronica* proved extremely useful, e.g. because of its short life cycle, smallness and transparency. During oogenesis, when the oocyte is supported by a single nurse cell that is later discarded (Fig. 4), all phases in oocyte growth can be easily followed, and the fact that all eggs deposited in an egg pack are synchronous in development facilitates the planning of experiments. Polychaete studies have provided important new information about yolk formation and degradation, and also about the role of polyamines in early embryo development (Emanuelsson and Heby, 1978). In recent years they have particularly contributed to a more coherent picture of the role and formation of serotonin and serotonin receptors in the early invertebrate embryo (Emanuelsson, 1992).

All the time from its introduction at the institute, developmental biology has essentially maintained its classical research profile, i.e. the investigated events and problems have been regarded as expressions of cell/organism biology. During the 80s, however, molecular biological aspects began to be adopted, supported as they were by the new findings concerning the gene activity operating in the developing systems.

It is significant that in Lund the early studies on cell multiplication and differentiation in mammalian cells during this period showed a traditional approach and mostly described growth patterns and characteristics of various tumor cell cultures, using classical cytochemical and biochemical methods (Andersson and Agrell, 1972; Andersson, 1977). The successive, general concentration upon a new specific problem – the role of polyamines in cell growth and differentiation – made it necessary

to introduce new molecular techniques. This well-established project has received much attention, also abroad. It was only logical that it should gradually focus on the molecular regulation of the polyamine synthesis, and in present work an elucidation of the underlying genetical basis is an urgent research problem.

In conclusion it may be stated that from its introduction late in the 40s and up to now, developmental biology has held and still holds a strong position in zoological research in Lund. This is substantiated by the fact that up to now about forty percent of all dissertations from the Department of Animal Physiology in Lund have dealt with problems decidedly within developmental biology.

References

AGRELL, I. (1947). Observations on the hydrogen-activating enzymes present during the metamorphosis of insects. *Acta Physiol. Scand. 14:* 317-334.

AGRELL, I. (1954). Oestradiol and testosterone propionate as mitotic inhibitors during embryogenesis. *Nature 173:* 172.

AGRELL, I. (1956). The synchronous mitotic rhythm and the effect of lithium during the early development of the sea urchin embryo. *Acta Zool. 27:* 53-60.

AGRELL, I. (1962). Mitotic gradients in the early insect embryo. *Ark. Zool. 15:* 143-149.

AGRELL, I. (1966). Phytohaemagglutinin as a mitotic stimulator on free-living *Amoebae. Exp. Cell Res. 42:* 403-406.

AGRELL, I. (1969). Nucleohistone relations in model experiments. *Biochim. Biophys. Acta 186:* 226-228.

AGRELL, I. and PERSSON, H. (1956). Changes in the amount of nucleic acids and free nucleotides during early embryonic development of sea urchins. *Nature 178:* 1398-1399.

ANDERSSON, G. (1977). Accumulation of polyploid cells and G2-phase cells during ascites tumor growth. *J. Cell. Physiol. 90:* 329-336.

ANDERSSON, G.K.A. and AGRELL, I.P.S. (1972). Cytoplasmic and nuclear growth during the proliferation of Ehrlich ascites tumour cells in mice. *Virchows Arch. (Cell Pathol.) 11:* 1-10.

CHRISTENSSON, E. (1959). Changes in free amino acids and proteins during cell growth and synchronous division in mass cell cultures of *Tetrahymena* pyriformis. *Acta Physiol. Scand. 45:* 339-349.

EMANUELSSON, H. (1960). Effects of RNA on early chick embryos cultivated *in vitro. Acta Physiol. Scand. 48:* 352-363.

EMANUELSSON, H. (1969). Electronmicroscopical observations on yolk and yolk formation in *Ophryotrocha labronica* LaGreca and Bacci. *Z. Zellforsch. 95:* 19-36.

EMANUELSSON, H. (1992). Autoradiographic localization in polychaete embryos of tritiated mesulergine, a selective antagonist of serotonin receptors that inhibits early polychaete development. *Int. J. Dev. Biol. 36:* 293-302.

EMANUELSSON, H. and HEBY, O. (1978). Inhibition of putrescine synthesis blocks development of the polychaete *Ophryotrocha labronica* at gastrulation. *Proc. Natl. Acad. Sci. USA 87:* 928-932.

HOLM, B. (1968). Changes in the amount of DNA in synchronized cultures of *Tetrahymena* cells. *Exp. Cell Res. 53:* 18-36.

KARLSSON, B.W. (1966). Immunochemical studies on changes in blood serum proteins in piglets after colostrum ingestion and during neonatal and juvenile development. *Acta Pathol. Microbiol. Scand. 67:* 237-256.

LINDH, N.O. (1959). Heteroplastic transplantation of transversal body sections in flatworms. *Ark. Zool. 12:* 183-195.

LUNDQUIST, A. and EMANUELSSON, H. (1980). Polar granules and pole cells in the embryo of *Calliphora erythrocephala* : ultrastructure and [^{3}H] leucine labelling. *J. Embryol. Exp. Morphol. 57:* 79-93.

LÖWKVIST, B., HEBY, O. and EMANUELSSON, H. (1980). Essential role of the polyamines in early chick embryo development. *J. Embryol. Exp. Morphol. 60:* 83-92.

OHLSSON, O.A.T. (1968). The RNA-metabolism during early sea urchin embryogenesis. *Ark. Zool. 20:* 365-379.

ZEUTHEN, E. (1948). A Cartesian diver balance weighing reduced weights (R.W.) with an accuracy of ±0.01 γ. *C.R. Lab. Carlsberg, Sér. Chim. 26:* 243-266.