DEVELOPMENTAL BIOLOGY **182,** 1–4 (1997)
ARTICLE NO. DB968489

IN MEMORIAM

Pieter D. Nieuwkoop (1917–1996)

Pieter Nieuwkoop died September 18, 1996, at age 79, in Utrecht, The Netherlands, after a brief illness. He continued his laboratory research on early vertebrate development nearly to the end of his life. He is remembered by developmental biologists for his numerous research contributions and integrative hypotheses over the past 50 years, especially in the areas of neural induction, meso-endoderm induction, and germ cell formation in chordates.

Born in Enschede, The Netherlands, he began his doctoral studies shortly before World War II at the State University of Utrecht under the supervision of Professor Chr. P. Raven (who had trained with M. W. Woerdeman, who had trained with H. Spemann). His thesis, written in English after the war and published in 1946, concerned the determination of germ cells and the development of the germinal ridges in urodeles, a subject he returned to in later years. For many of us, acquaintance with his early work first comes with the *Normal Tables of Xenopus laevis* (*Daudin*) published in 1956 with J. Faber, an enduring volume now reprinted (Garland Publishing Co.). The book contains not only their original observations of morphogenesis and organogenesis, but also a compilation of the literature on the external and internal anatomy of embryos and tadpoles and on the breeding and care of frogs. During this early work Nieuwkoop and Florschütz (1950) studied *Xenopus* gastrulation in detail and distinguished an internal blastopore at which deep mesoderm cells involute 1–2 hr before surface endoderm cells do so at the visible external blastopore. They also found that most if not all mesoderm precursors are internal even before *Xenopus* gastrulation begins, and not in the surface layer, a disposition opposite that of urodele embryos and even other anurans, as later analyzed in detail by Ray Keller and his colleagues. Nieuwkoop used *Xenopus* in his research for many years, in parallel with his use of urodeles (axolotls, newts), which have larger and more slowly developing embryos more favorable for certain kinds of surgery, and his last publication cites the advantages of using both in embryology (Nieuwkoop, 1996).

Neural Induction

Pieter Nieuwkoop's first major contribution to early development came with his study of neural induction in urodeles in 1952 by a novel method of surgically inserting flaps of ectoderm into the dorsal midline of the neural plate of an early neurula embryo at different anteroposterior levels and later scoring the kinds and arrangements of neural tissues formed by the flap which protruded from the neural tube. The most anterior neural structures (forebrain) developed at the distal end of the flap, whereas posterior structures developed at the base and matched those of the neural tube level at which the implant was located. This and other experiments (by his student H. Eyal-Giladi) led him to propose that inductive neural patterning is accomplished by two factors: (1) an activating factor causing a neuralization of ectoderm and, if no other induction followed, leading exclusively to anterior neural differentiation such as forebrain and midbrain; and (2) a transforming (or posteriorizing) factor that could work only on already neuralized tissue, making it develop to more posterior neural parts such as hindbrain and spinal cord. His was one of the first comprehensive two-component hypotheses for neural patterning; it came several years before Saxen and Toivonen's double gradient model, though after T. Yamada's rather different proposals in 1950, F. E. Lehmann's suggestions in 1942, and the reports of Holtfreter and Chuang in the mid-1930s that partial purification of various heterogeneous inducers gave either a neuralizing activity or a trunk–tail-inducing activity, but not both, and that the dilution or concentration of one did not make it act like the other. Nieuwkoop's activation/transformation hypothesis has survived to this day and is cited to explain the results of contemporary experiments with pure inducers such as the noggin and chordin proteins acting on isolated ectoderm. Still, it is unclear whether posterior neural tissue is always or only formed by posteriorization of independently neuralized anterior tissue or whether it can be induced directly by a single agent (see Lamb and Harland, 1995). So great was Pieter Nieuwkoop's familiarity with the anatomy of the amphibian nervous system that he was one of the few researchers of recent decades who could write in the methods section of a paper (and have it accepted), ''. . . the authors did not use molecular markers because the first author, having more than 50 years of experience in normal and atypical histology, is perfectly sure of the correct identification of all the definitive larval structures. The reliance on molecular markers [*by others*] has actually given rise to misinterpretations . . . in several recent studies . . .'' (Nieuwkoop and Koster, 1995).

In the late 1950s he also studied the neural differentiation of pH-shocked newt ectoderm and endorsed the interpretation that induced ectoderm has a self-organizing capacity to differentiate local neural structures such as individual brain vesicles, despite the incoherence of the inductive sig-

0012-1606/97 $25.00

nal. He considered this self-organization capacity a major research problem for future experimental attention. His interest in neural induction continued throughout his life, especially with regard to the means by which the activating and transforming factors reach the responsive ectoderm cells to give the anteroposterior order of the neural plate. Whereas others pursued exclusive spatial interpretations such as double morphogen gradients, he pursued a largely temporal interpretation based on (1) the progressive movements of the inductive dorsal mesoderm under the ectoderm during gastrulation and (2) the changing competence of the ectoderm. With regard to movements, the prechordal plate in the lead would neuralize all ectoderm under which it passed. The chordamesoderm would follow thereafter and transform (posteriorize) whatever neuralized ectoderm it passed under, to an extent related to the duration of contact. The posterior neural plate formed posterior structures because the chordamesoderm had passed under it for the longest time, hence exposing it to transforming agent for the longest period. Anterior ectoderm near the animal pole, on the other hand, would form fore- and midbrain because it received activator only from the prechordal plate and was never reached by the chordamesoderm. Intermediate neural plate levels experienced intermediate durations of exposure to the transforming factor. Thus the plate gained its anteroposterior organization. For Nieuwkoop the spatial distribution of signals in the dorsal mesoderm was rather simple: the prospective prechordal mesoderm was the main locus of the activating agent and the chordamesoderm the main locus of the transforming agent. Signals from these tissues reached the overlying ectoderm by a vertical path, inducing the midline of the neural plate, the future floor plate. Then, according to him, signals spread laterally and anteriorly by a propagation mechanism within the plane of the neural plate.

With regard to the ectoderm's changing competence, he considered the boundaries of the neural plate as set by the cessation of the ectoderm's competence at stage 12 to respond to activating signals slowly propagated in its tissue plane and not set by the location of the low end of a morphogen gradient. Such signals, he thought, continued to pass through the ectoderm even after stage 12, despite its nonresponsiveness, and B. Albers (1987), in her published thesis work done under Pieter's direction, supported this conclusion by grafting stage 10 gastrula ectoderm into the stage 12 neural plate and showing that it was still neuralized. Nieuwkoop and Albers (1991) then showed that although the competence toward activators was over by stage 12, the competence to respond to propagated transformation signals went on until stage 16. This analysis involved transplantation of prospective forebrain regions to posterior positions in the neural plate and assessment of their extent of posteriorization. In his last experimental publication, Nieuwkoop and Koster (1995) concluded that neural induction could only start by way of a vertically transmitted activating signal, not a planar one, although planar propagated signals had a role thereafter. Whereas this remains to be analyzed further in *Xenopus*, where conflicting results obtain, such a requirement for vertical activation has been taken seriously for many years by researchers of urodele neural induction. In summary, Pieter Nieuwkoop's contributions to studies of neural induction have been major and lasting, and the temporal aspects of his proposals, uniquely emphasized by him, have still not been explored by others.

Meso-endoderm Induction

In 1969 (a,b) Nieuwkoop made his second major contribution, the discovery and description of endo-mesoderm induction in the amphibian blastula. He found this induction first in urodeles by surgically recombining vegetal hemisphere cells with animal hemisphere cells of the 2000-cell blastula, after eliminating all prospective mesoderm including the Spemann organizer. Neither the cap nor vegetal cells alone or *in situ* would differentiate mesoderm or pharyngeal endoderm. However, the recombinate made these tissues and in some cases developed an embryoid with good axial organization and a nervous system, a clear indication that the Spemann organizer had been restored. He and G. Ubbels (1972) showed by several means that it was the animal cap cells that responded to inducers and the vegetal cells that released these inducers. E. Boterenbrood and Nieuwkoop (1973) then showed that the inductive vegetal cells were of two kinds: the majority lateroventral members inducing adjacent animal cap cells to form ventral meso-endoderm, whereas the minority dorsal members induced adjacent cap cells to form dorsal meso-endoderm. The blastula vegetal hemisphere as a whole carried a dorsoventral pattern that was inductively imprinted on the cell population of the equatorial level, generating at least two regions of meso-endoderm in the marginal zone. The dorsal region of this zone was none other than the Spemann organizer, and hence the dorsal vegetal cells were ''the organizer of the organizer.'' Sudarwati and Nieuwkoop (1971) soon extended the analysis to the anuran *Xenopus*, and hence meso-endoderm induction was seen as general to amphibia, and probably to most chordates. In the 1980s the ''endo-'' part of meso-endoderm induction tended to be dropped by other researchers in the enthusiasm to study the formation of mesoderm (especially muscle) by ectoderm treated with purified protein growth factors, but Nieuwkoop had emphasized from the beginning that pharyngeal endoderm was also induced, and hence ''meso-endoderm induction'' was the appropriate term. So great has been the influence of Nieuwkoop's work on current studies of meso-endoderm inducers, regional gene expression, and organizer formation that it seems appropriate to call the doral vegetal cells the ''Nieuwkoop Center.'' This is the site of maternal components, localized by cortical rotation and needed at the blastula stage for the induction of the Spemann organizer, the source of inductive signals in the gastrula stage.

Nieuwkoop, upon finding that mesoderm and pharyngeal endoderm were derived exclusively from the animal cap ectoderm, concluded that an induction was at work and not

a regulation of an animal–vegetal double gradient as favored by Ogi, Nakamura, and their colleagues in their interpretation of simultaneous similar studies of recombinates. At first Nieuwkoop thought that ventral and dorsal vegetal cells differed quantitatively in their release of a single meso-endoderm inducer. While he was well aware that the organizer exerted mesoderm patterning effects during gastrulation, he thought that the marginal zone mesoderm gained extensive patterning even before gastrulation, due to the gradient of meso-endoderm inducers from vegetal cells, the greatest amount coming from dorsal vegetal cells (Weijer *et al.*, 1977). Later J. Slack proposed in his three-signal model that the two parts of the vegetal hemisphere differed qualitatively in the kind of inducer they released, and that the marginal zone mesoderm gained only a two part pattern by this induction; the rest built up later in gastrulation by organizer inductions. The proposals of Kimelman *et al.* (1992) added a further distinction about the meso-endoderm inducers: that a general mesoderm inducer exists in both the ventral and dorsal sectors of the blastula vegetal half, sufficient to induce a ventral type of mesoderm, whereas a competence modifier additionally exists in the dorsal sector. This modifier is without effect on its own but acts in concert with the mesoderm inducer to lead to dorsal mesoderm (rather like the transforming agent of neural induction). By this proposal, the Nieuwkoop Center would be the region where both the general inducer and the competence modifier are released and available. In summary, Nieuwkoop's discovery of meso-endoderm induction at the blastula stage, the embryo's earliest induction, has opened a fruitful interesting area of developmental biology in which many laboratories worldwide are engaged in molecular analyses of inducers and responses and in which there is an abundance of new ideas about the early steps of axis formation.

Germ Cell Induction in Urodeles

With colleagues Nieuwkoop continued these studies begun in his doctoral thesis research (Sutasurya and Nieuwkoop, 1974). Urodele germ cells are formed by ventral marginal zone cells exposed to ventral meso-endoderm inducers. This mode of formation is a surprise to *Xenopus* researchers since the eggs of anurans contain at the vegetal pole a collection of germ plasm granules remarkably like those at the posterior pole of the insect egg. In these anurans, germ cells arise only from the cell lineage harboring these granules, a compelling example of a cytoplasmic localization mechanism, with no evidence for induction. The presence of an induction process in urodeles but a localization process in anurans led Nieuwkoop to favor the notion that amphibia may be diphyletic, with the urodele branch closer to the germ cell-inducing reptile/bird/mammal branch.

Finally, in less well-known work, he undertook in the 1980s the study of turtle development (at the Institute of Technology, Bandung, Indonesia), feeling that reptilian development was a neglected area and that turtles represented a particularly unmodified order of reptiles. Following his interest in the evolution of the cleidoic amniote egg, he studied turtle egg organization, noting the soft shell, thin albumen solution, and great uptake of water as intermediate characters in the evolution of this land adaptation (Nieuwkoop and Sutasurya, 1983).

He wrote three books of lasting value to developmental biologists and comparative embryologists. These include *Primordial Germ Cells in the Chordates: Embryogenesis and Phylogenesis* (Nieuwkoop and Sutasurya, 1979) and *Primordial Germ Cells in the Invertebrates* (Nieuwkoop and Sutasurya, 1981). These grew from his lifelong studies of germ cells and his evidence for a diphyletic origin of amphibia. His third book was the *The Epigenetic Nature of Early Chordate Development* (Nieuwkoop *et al.*, 1985), in which he explored the possible universality of meso-endoderm induction in chordates and the central role of this induction in organizing the chordate body plan. He suggested that studies of meso-endoderm induction in Amphioxus ought to be done to probe the evolutionary origins of this induction. For his synthesis of amphibian development, several reviews are well worth reading (Nieuwkoop, 1973, 1977), in which he emphasizes the amphibian oocyte's two-part organization, the animal and vegetal hemispheres, and the stepwise build up of complexity in the early embryo by way of repeated and ever more local inductive interactions among ever more parts. Throughout his career he believed strongly in the importance of inductive interactions across compartment boundaries for chordate pattern formation, and this has certainly proved to be correct.

Pieter Nieuwkoop was a Professor of Zoology at the University of Utrecht from 1956 to 1984 and was the Director of the Hubrecht Laboratorium (a semigovermental institution under the supervision of the Royal Netherlands Academy of Arts and Sciences) from 1953 until 1980. While he was Director, the laboratory moved in 1964 from a city location at the University of Utrecht to a new building on the city outskirts. He assembled a group of staff searchers studying the development of frogs, urodeles, chicks, mouse, *Dictyostelium*, and *Drosophila*, by a variety of techniques. This selection reflected his very broad interests in development and made this laboratory the world's only national laboratory of developmental biology at the time. Among his doctoral students and postdoctoral colleagues are J. Faber, H. Eyal-Giladi, K. Hara, L. Sutasurya, E. Boterenbrood, R. Rao, and S. de Laat, the current Director of the Laboratory. Many researchers, including myself and Marc Kirschner, visited the laboratory for sabbatical research and discussions with Pieter and staff members and for an introduction to *Xenopus*. We all found that Pieter had an enormous store of unpublished observations and ideas and that he delighted in sharing these, in his quietly intent manner, with those who asked. Some of his broad views of, and deep interest in, chordate development can be found in an article based on an interview I had the privilege to conduct at the time of his 70th birthday (Gerhart, 1987). For many years, Pieter

participated in an international course on developmental biology and techniques offered at the laboratory. Students of many countries benefited from this introduction to the subject and contact with him and other laboratory members. It is with sorrow that we note the passing of Pieter Nieuwkoop and with appreciation that we remember his numerous contributions to our understanding of early chordate development, contributions that still vitalize our study (see the companion article in this issue by E. De Robertis on neural induction).

REFERENCES

Albers, B. (1987). Competence as the main factor determining the size of the neural plate. *Dev. Growth Differ.* **29,** 535–545.

Boterenbrood, E. C., and Nieuwkoop, P. D. (1973). The formation of mesoderm in the urodelean amphibians. V. Its regional induction by the endoderm. *Roux' Arch.* **173,** 319–332.

Gerhart, J. (1987). The epigenetic nature of chordate development: An interview with Pieter D. Nieuwkoop on the occasion of his 70th birthday. *Development* **101,** 653–657.

Kimelman, D., Christian, J. L., and Moon, R. T. (1992). Synergistic principles of development: Overlapping patterning systems in *Xenopus* mesoderm induction. *Development* **116,** 1–9.

Lamb, T. M., and Harland, R. M. (1995). Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anteroposterior neural pattern. *Development* **121,** 3627–3636.

Nieuwkoop, P. D. (1946). Experimental investigations on the origin and determination of the germ cells, and on the development of the lateral plates and germ ridges in the urodeles. *Arch. Neerland. Zool.* **8,** 1–205.

Nieuwkoop, P. D. (1969a). The formation of mesoderm in the urodelean amphibians. I. Induction by the endoderm. *Roux' Arch.* **162,** 341–373.

Nieuwkoop, P. D. (1969b). The formation of mesoderm in the urodelean amphibians. II. The origin of the dorso-vegetal polarity of the endoderm. *Roux' Arch.* **163,** 298–315.

Nieuwkoop, P. D. (1973). The organization center of the amphibian embryo: Its origin, spatial organization, and morphogenetic potential. *Adv. Morphog.* **10,** 1–39.

Nieuwkoop, P. D. (1977). Origin and establishment of embryonic polar axes in amphibian development. *Curr. Topics Dev. Biol.* **11,** 115–132.

Nieuwkoop, P. D. (1996). What are the key advantages and disadvantages of urodele species compared to anurans as a model system for experimental analysis of early development? *Int. J. Dev. Biol.* **40,** 617–619.

Nieuwkoop, P. D., and Albers, B. (1990). The role of competence in the cranio–caudal segregation of the central nervous system. *Dev. Growth Differ.* **32,** 23–31.

Nieuwkoop, P. D., Boterenbrood, E. C., Kremer, A., Bloemsma, F. F. S. N., Hoessels, E. L. M. J., Meyer, G., and Verheyen, F. J. (1952). Activation and organization of the central nervous system in amphibians. *J. Exp. Zool.* **120,** 1–108.

Nieuwkoop, P. D., and Faber, J. (1956). ''Normal Tables of *Xenopus laevis* (Daudin),'' 1st ed. North–Holland, Amsterdam.

Nieuwkoop, P. D., and Florschutz, P. A. (1950). Quelques caracteres speciaux de la gastrulation et de la neurulation de l'oeuf de *Xenopus laevis* Daud. et de quelques autres anoures. *Arch. Biol.* **61,** 113–150.

Nieuwkoop, P. D., Johnen, A. G., and Albers, B. (1985). ''The Epigenetic Nature of Early Chordate Development.'' Cambridge Univ. Press.

Nieuwkoop, P. D., and Koster, K. (1995). Vertical versus planar induction in early amphibian development. *Dev. Growth Differ.* **37,** 653–668.

Nieuwkoop, P. D., and Sutasurya, L. A. (1979). ''Primordial Germ Cells in the Chordates: Embryogenesis and Phylogenesis.'' Cambridge Univ. Press.

Nieuwkoop, P. D., and Sutasurya, L. A. (1981). ''Primordial Germ Cells in the Invertebrates.'' Cambridge Univ. Press.

Nieuwkoop, P. D., and Sutasurya, L. A. (1983). Some problems in the development and evolution of the chordates. *BSDB Symp.* **6,** 123–136.

Nieuwkoop, P. D., and Ubbels, G. A. (1972). The formation of mesoderm in the urodelean amphibians. IV. Quantitative evidence for a purely ectodermal origin of the entire mesoderm and pharyngeal endoderm. *Roux' Arch.* **169,** 185–199.

Sudarwati, S., and Nieuwkoop, P. D. (1971). Mesoderm induction in the anuran Xenopus laevis. *Roux' Arch.* **166,** 189–204.

Sutasurya, L. A., and Nieuwkoop, P. D. (1974). The induction of the primordial germ cells in the urodeles. *Roux' Arch.* **175,** 199–220.

Weijer, C. J., Nieuwkoop, P. D., and Lindenmayer, A. (1977). A diffusion model for mesoderm induction in amphibian embryos. *Acta Biotheor.* **26,** 164–180.

John Gerhart

Department of Molecular and Cell Biology
University of California, Berkeley, California 94720