How did urodele embryos come into prominence as a model system?

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Introduction

Experimentation on animal embryos actually began during the years 1880-1900. Just one century ago (1894), the German embryologist Wilhelm Roux founded the first journal devoted to experimental studies on development, the *"Archiv für Entwicklungsmechanik der Organismen,"* now *"Roux's Archives for Developmental Biology."* The study of "developmental mechanics" at that time aimed at a physiological approach to embryology. The discipline was considered to represent an independent science and no longer a mere instrument for phylogenetic studies (Maienschein, 1991). It was quickly discovered that experiments on different organisms produced conflicting results, which were integrated into controversial theories. Amphibian eggs, and more precisely frog eggs, were among the organisms first chosen by several investigators, including Roux himself. Urodele eggs and embryos were only later used in experiments, though more and more frequently. But in the 19th century, descriptive embryology of amphibians was far from being perfect at the time experimentation began. In this article, I will focus on the first studies that compared normal development of urodele and anuran amphibians. I will then attempt to show why urodeles were (or were not) used in pioneering experimental studies. Finally, I will concentrate on the rise of experimental embryology of urodeles, leading to their prominence during the period 1920-1950, prior to the progressive expansion and present dominance of the anuran *Xenopus laevis*. Table 1 summarizes the early history of urodele embryology.

Descriptive embryology of urodele amphibians at the end of the 19th century

Newt egg development had been described and nicely illustrated by Rusconi (1821), even before Prévost and Dumas (1824) provided the first description of frog egg cleavage and development. These studies were immediately followed by another report by Rusconi (1826). The significance of egg cleavage was of course not understood by these authors. The cell theory (Schwann, 1839) later allowed "cleavage spheres" to be recognized as embryonic cells. A detailed comparative study of egg cleavage in vertebrates was published by Sobotta (1896), who devoted seven pages to urodeles. Irregularities of urodele egg cleavage, as compared to that of the anuran egg, were emphasized by the author, who referred to Jordan and Eycleshymer (1894) and to the description of *Salamandra* egg cleavage by Benecke (1880) and Grönroos (1895). *At that time, newt eggs from several species had already been studied (Gasco, 1880; Grönroos, 1890; Ebner, 1893; Jordan, 1893), as had axolotl and Ambystoma eggs (Bambeke, 1880; Clarke, 1880; Houssay, 1890; Eycleshymer, 1895).* The authors cited worked in any of several European countries (Austria, Belgium, Finland, France, Germany, Italy) or in the United States. A cytological study of newt egg fertilization and cleavage was published in Berlin by Michaelis (1897), one of Hertwig's students.

Comparative studies on advanced developmental stages were then performed on urodeles, anurans and other vertebrates, with theoretical considerations. First, attempts were made to establish the origin of germ layers in amphibians for comparison to the findings in bird embryos, on which pioneering studies by Pander (1817) and especially Baer (1828) had started building a discipline of comparative embryology. Phylogenetic considerations based upon the hypothetical ancestral *gastraea* (Haeckel, 1874) later led embryologists to investigate different kinds of gastrulae in the animal kingdom. Oskar and Richard Hertwig thus published two extensive articles on the coelom theory and the origin of mesoderm in animal embryos (Hertwig and Hertwig, 1882; Hertwig, 1882).

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0214-6282/96/$03.00

Transverse sections through embryos of the common European newt *Triturus taeniatus* were extensively described and compared to those of frog and lamprey embryos. But the precise origin of the different germ layers arising from blastula cells remained controversial.

Schultze (1888) claimed that Hertwig's coelom theory did not apply to the frog embryo, since there were neither a diblastic gastrula stage nor paired mesoblastic anlagen, but the inner and intermediate germ layers formed simultaneously, and the notochord was, from the beginning, part of the mesoblastic layer. The process of amphibian gastrulation, studied mostly from histological sections, remained poorly understood until much later, when Vogt (1925, 1929) described complex cell layer movements in living gastrulae, with the help of vital dye marks. The first attempts to put marks on the surface of a young gastrula seem to have been those of Eycleshymer (1895, 1898) in Chicago. He used a segment of hair, maintained with forceps, to make very small punctures on selected areas of gastrulae from *Ambystoma* and two species of the Anurans *Acris* and *Bufo*. The small exovates moved during gastrulation and neurulation, which proved the reality of invagination processes, already mentioned at that time (Kopsch, 1895a,b) but still controversial (Brachet, 1903). The jelly envelopes of *Ambystoma* eggs could be easily removed, which made a fairly large number of operations possible. This advantage contrasted with the difficulties encountered by several authors working on newt eggs, which were frequently damaged or even exploded during extrusion from the envelopes. *That might explain, at least partly, why at first urodele eggs were not so widely used in experiments as were anuran eggs*. Meanwhile, Schwink (1888) alleged that the clearer alpine newt egg was much more convenient than the dark frog egg for clarifying problems of gastrulation. But these eggs had still to be fixed *in toto* with their envelopes still intact. And the first constriction experiments on newt eggs, which will be reported later on, were also carried out on eggs that were still surrounded by their envelopes.

The early questions of amphibian embryology

Within the limits of this article, it is unfortunately impossible to give a detailed account of the controversial results that were obtained by early investigators of normal amphibian development. Let us only mention several questions that were addressed during the period 1880-1900 and were debated for a long time (see Samassa, 1895 and Brachet, 1903): *Is the origin of archenteron walls purely "entoblastic," or does "epiblast" contribute to dorsal roof formation? Does notochord form from entoblast or from mesoblast? How does blastopore closure occur? Is there a neurenteric canal in the urodele embryo as is the case in the anuran embryo? Does the blastopore give rise directly to the definitive anus in urodeles, since the anus is formed secondarily in anurans?* Some authors found the right answers earlier than others (Houssay, 1890) but they were not confirmed for a long time. For example, Vogt (1926) definitely stated that there is no neurenteric canal in the posterior part of urodele embryos.

It must still be added that extensive observations on the development of specialized organs frequently followed the description of early stages. Houssay (1890) devoted the main part of his long article to the origin and development of the peripheral nervous system and of head morphology (metameric organization) in the axolotl.

TABLE 1

MILESTONES IN THE EARLY HISTORY OF URODELE EMBRYOLOGY

Year	Author(s)	Contribution
1821	Rusconi	First recorded description of newt egg development.
1880	Bambeke, Clarke	Description of axolotl and *Ambystoma punctatum* development.
1895a,b	Kopsch	First use of serial photographs to investigate axolotl and frog gastrulation movements.
1895, 1898	Eycleshymer	Fine exovates used as surface markers to study gastrulation
1895	Endres, Herlitzk	First demonstration that isolated parts of newt eggs can undergo normal embryonic development.
1896	Sobotta	Comparative study of urodele egg cleavage.
1897	Herlitzka	First twin embryos obtained by constriction of single newt egg at the 2-cell stage.
1901	Spemann	First article devoted to an extensive analysis of regulative vs non-regulative properties of newt eggs.
1907	Harrison	Embryonic transplantations performed to study limb innervation in Urodeles.
1911a,b	Goodale	First use of vital dye marks to study gastrulation movements in *Ambystoma* and *Spelerpes*.
1915	Harrison	First article on transplantation of rotated limb rudiments in *Ambystoma* as a means to investigate axis determination.
1916	Spemann	Transplantations at the gastrula stage: summary of current work relative to determination concepts
1919	Spemann	First mention of the concept of an "organization center" in the dorsal area of blastopore lip, as a new hypothesis.
1923	Vogt	First use of small agar blocks to imprint vital dye marks on early embryos.
1924	Spemann and Mangold	Founding experiments on the "organizer": transplantation of a second dorsal blastopore lip on the ventral side of a newt gastrula gives rise to a secondary embryo.
1929	Vogt	Maps of presumptive developmental areas in the amphibian gastrula and detailed analysis of gastrulation movements.
1931	Holtfreter	A new salt solution for the culturing of embryos and explants.

The rise of experimental studies on Urodele embryos (1895-1920)

Experiments on frog eggs were performed in Germany during the early 1880s. They dealt with effects of gravity on egg cleavage, determination of the symmetry plane of the future embryo, and artificial interspecific hybridization (e.g., studies by Born, Pflüger, and Roux). Roux (1888) studied the fate of one of the first two blastomeres after the second one had been killed with a hot needle; he obtained right or left half-embryos, i.e., results which appeared to be similar to those previously described on an Ascidian egg (Chabry, 1887; Fischer, 1992). Roux thus supported a theory of developmental mosaicism, including also the nuclear germ plasm theory proposed at that time by Weismann. Later on, Driesch (1891) showed that isolation of the first blastomeres of the sea-urchin egg gave rise to small but fully-formed larvae. From the opposite results a controversy arose between supporters of neo-preformationism (Roux, Barfurth) and of neo-epigenesis (Driesch, Hertwig).

Hertwig (1893) in Berlin tried to separate the first two blastomeres of a newt egg by means of a knotted loop of silk thread. He could not achieve a complete separation without killing the egg, but he did obtain, in addition to an unorganized mass, a small embryo developing perpendicular to the constriction in the cleavage plane, which indicated a strong tendency to regulation. In fact, Hertwig's studies were mostly devoted to an extensive series of compression experiments on frog eggs. The latter were maintained in various positions between two glass plates, which induced modified cleavage patterns, thus permitting successive cleavage nuclei to be incorporated into cytoplasmic territories different from normal. Such eggs nevertheless eventually gave rise to normal embryos. Hertwig also repeated Roux's experiments by killing one of the first two blastomeres, but he mainly obtained regulative embryos. He accordingly considered that his studies supported epigenetic concepts and could not fit with Roux's and Weismann's theories. Schultze (1894) then showed that it was possible to produce double frog embryos by rotation of an egg at the 2-cell stage. These results allowed Morgan (1895) to propose a solution to the Roux-Hertwig controversy, since he himself obtained either half-embryos or well-formed reduced embryos, according to the position that the remaining frog blastomere was obliged to adopt after killing the other one: a rotated blastomere could give rise to a small regular embryo.

At the same time, in Freiburg and in Breslau, Endres and Walter (1895) and Endres (1895b) confirmed Roux's experiment at the 2-cell stage by obtaining frog half-embryos. They adhered to Roux's conclusions, only admitting that cytoplasmic territories might be more specific than cleavage nuclei in predetermining embryonic areas. But Endres, following Ebner (1893), also tried to separate the first two blastomeres of the newt egg after constriction with a silk thread according to Hertwig's method. He was more successful than Hertwig, after destroying the remaining bridge between the blastomeres with a hot needle. He also succeeded in constricting embryos at more advanced stages, from morula to neurula. He consequently observed development of small complete embryos from constricted eggs and published his first results (Endres, 1895a). Spemann (1901) repeated and extended these experiments and mentioned that Endres died prematurely before he could exploit all the conclusions of his work.

Independently of Endres but concomitantly, Herlitzka (1895), in Florence (Italy), obtained similar results after constricting crested newt eggs with a fine hair. To obtain regular and progressive constriction, Herlitzka devised a micrometric apparatus which he described in a subsequent article (Herlitzka, 1897). His article includes a detailed bibliographical review of previously obtained results concerning development of experimentally fragmented, compressed or oriented eggs. These brilliant results with newt eggs were not continued in Italy. Chiarugi (1898) only published a short account of compression and 180° rotation experiments on *Salamandrina perspicillata* eggs. The results were similar to those of Schultze (1894), i.e., duplicitas appeared in 20% of the eggs. In Chicago, Eycleshymer (1895) too had tried compression experiments on a few *Ambystoma* eggs. Hertwig himself incited one of his foreign students to apply compression and rotation to 2-cell stage newt eggs resulting from artificial fertilization, which he used for better timing. Dramatic pigmentation changes (like those seen on frog eggs) were not observed on oriented newt eggs, but a few duplications occurred (Tonkoff, 1900).

Spemann's early studies

The successful technique of egg constriction by means of a fine hair loop was further exploited by Hans Spemann (1869-1941), then at the Zoological Institute of Wurzburg University. *His studies on the newt egg and embryo (T. taeniatus) can be considered as the real starting point of experimental embryology in urodeles* (Spemann, 1901, 1902, 1903). The 1901 article largely quotes Ebner (1893), Endres (1895a) and Herlitzka (1895, 1896, 1897), whose data Spemann reobtained and discussed (for example, observations on the cleavage pattern of isolated blastomeres). Constriction applied at the 2-cell or blastula stage gave rise either to two nearly normal embryos, with slight defects on the side corresponding to the separation plane, or to a normal embryo plus a much less organized embryonic mass. In his 1902 article Spemann investigated the consequences of various constrictions on newt gastrulae (in particular, of "frontal" separation leading to dorsal and ventral halves). The 1903 article mostly dealt with *duplicitas anterior*, originating from incomplete constriction. In summary, the differing results frequently obtained from two halves of the same egg or early embryo had to be attributed to initial lack of coincidence between the first cleavage plane and the early symmetry plane of the future embryo. Actually, the coincidence between the two planes is much less frequent in the urodele egg than in the anuran egg. Spemann came to the conclusion that lack of normal development characterized the most ventral part of the egg or of the blastula, and that regulative processes only occurred when the partial embryonic system contained at least a portion of the dorsal components of the whole egg. In the following years, he was led to look for initial organizing factors in the dorsal part of the embryo.

It would be impossible to analyze the many aspects of Spemann's studies within the scope of this article. A careful and critical analysis of Spemann's ideas and achievements throughout his career was fortunately published a few years ago by one of Spemann's former students, Viktor Hamburger (1988). An obituary article by Otto Mangold (1942) is also of interest and contains a list of Spemann's publications. In this review only a few important steps, relating to the use of the urodele embryo, are mentioned.

During the first years of the present century, Spemann mainly worked on frog embryos (1901-1912), in which he investigated eye lens formation in relation to the optic vesicle, concurrently with Lewis in the United States. But he also operated on newt embryos, sectioning the optic cup and the covering epiblast. A complete though smaller eye nevertheless formed (Spemann, 1905). In connection with this problem it should be mentioned that a fascinating regeneration phenomenon had been described a few years earlier: Wolff (1895) established that the urodele eye lens could be regenerated from the dorsal margin of the iris, not only in the larva, but also in the adult animal. "Wolffian" regeneration was to be investigated throughout the following century.

In 1908, Spemann moved to Rostock, where he remained for six years, until he was appointed as the co-director and head of the Division of Developmental Mechanics in the Kaiser Wilhelm Institute for Biology in Berlin-Dahlem. There he worked during World War I (1914-1919). It is somewhat paradoxical that the "years he spent there were among the most productive of his life, during which he moved from relative obscurity to wide recognition" (Hamburger, 1988).

Spemann designed a sophisticated technique of transplantation adapted to amphibian embryos, inventing a microburner to fashion operative instruments made of fine glass needles, hair loops, and a special pipette that was devised to take a small fragment from an embryo and to reimplant it in another one with successful healing. *But even such a technique would have been useless, had Spemann not chosen appropriate live material.* He worked with embryos of the newt *T. taeniatus*, which displayed different kinds of pigmentation, from pale grey or yellow to dark brown, allowing homoplastic grafts to be distinguished from the surrounding area. Later, he practised graft exchanges between two different species, using darker embryos of *T. taeniatus* and almost unpigmented embryos of *T. cristatus*. Such heteroplastic transplantations had already been used on *Ambystoma* species by R.G. Harrison in the United States.

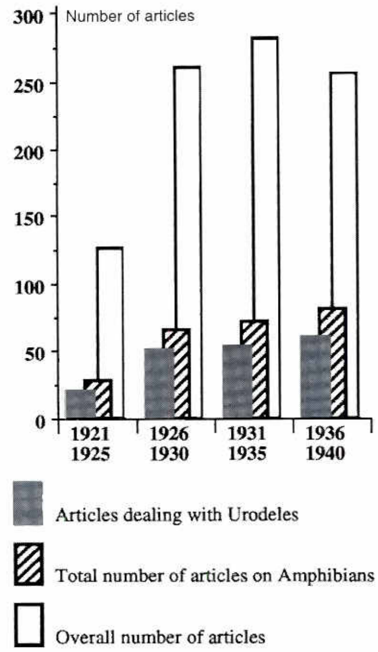

Fig. 1. Number of articles published in the *Journal of Experimental Zoology* during the period 1921-1940, divided into four 5-year periods. *For each period, the overall number of articles (white bar) includes the total number of articles on amphibians (hatched bar), itself including the number of articles dealing with urodeles (black bar). A few articles concerned both anuran and urodele amphibians; each of them was counted 0.5 only for urodeles.*

One can only admire the fact that transplantations were initially performed in water and not in an isotonic saline medium. Also considering the lack of sterile conditions, this was a very serious obstacle to obtaining long-surviving operated embryos, in spite of the hundreds of operations that were performed. This situation lasted until Holtfreter (1931) designed his famous, and carefully sterilized, salt solution.

The first preliminary account of transplantations at the gastrula stage is remarkable (Spemann, 1916). It deals mainly with the problem of determination vs non-determination, with the ability of the ectoderm to follow several possible differentiation pathways, and, more precisely, with the events that transform the young gastrula ectoderm either into neural plate or into epidermis. Detailed results were published two years later (Spemann, 1918). In this article, the concept of a "differentiation center" is applied to the dorsal part of the blastopore lip. In the spring of 1919, Spemann was appointed Director of the Zoological Institute at Freiburg in Brisgau, where he spent the rest of his life. His inaugural lecture was published in August (Spemann, 1919) and consisted of a general summary of the experiments conducted from 1916-1918, with a few additional details. Moreover, *Spemann modified the concept of "differentiation center" and coined the term "organization center" for the dorsal lip of the blastopore.* The discovery of the "organizer" was then imminent.

The results so far obtained by Spemann by means of the transplantation technique were rightly considered as a significant breakthrough in experimental embryology. *They opened the way for the triumph of the urodele embryo in Germany that was to occur during the following two decades;* it was prepared at the same time in the United States under the influence of Ross Granville Harrison (1870-1959), working on somewhat later embryonic stages.

Harrison's contributions

Harrison had been a student at Johns Hopkins University in Baltimore, where he obtained his Ph.D. in 1894. But he worked in Bonn (Germany) on three occasions between 1892 and 1900. He spent 8 years at Hopkins as an Associate Professor of Anatomy, leaving Baltimore in 1907 for Yale University, where he stayed until the end of his life. Details of his career and achievements can be found in a memorial article (Abercrombie, 1961), in Oppenheimer (1966), and in a volume assembling five previously published outstanding articles, as well as the description of developmental stages of the spotted salamander *Ambystoma punctatum*, and a list of his publications (Harrison, 1969). Herein, mention will be made of only the main features of Harrison's work in early experimental embryology of amphibians.

In Germany, Born (1894, 1897) had managed to perform grafting operations, including parabiotic union, between frog embryos, even between those of different species. Harrison (1898) was enthusiastic about the importance of these results for experimental embryology and applied the new technique himself to study growth and regeneration of the tadpole tail. He then spent several years working on axon formation, of which several conflicting theories prevailed. He first obtained *in vitro* culture of neural tissue fragments from frog embryos, demonstrating that axons grew from neurons (Harrison, 1907a). His experiments on axon and nerve origin also involved limb transplantation in the salamander embryo (Harrison, 1907b, 1908). The tissue culture method was characteristic of an analytical reductionist approach, but it did not preclude the importance of developmental cell interactions that Harrison wanted to dissect from each other. For that reason, he did not persist in tissue culture after he had solved the problem of the origin of axons. He was interested in the determination of embryonic polarity, of which he imagined molecular mechanisms at the protein level, and he began extensive research on polarity of limb rudiments in *Ambystoma* embryos. Urodele species were suitable for this work because in those amphibians the forelimbs are formed before the hatching stage, and, because of the large size of the embryos, their early rudiments could be operated through ophthalmological scissors. The first article appeared in 1915, followed by two major contributions (Harrison, 1915, 1918, 1921).

Harrison consequently stimulated work through a school of American embryologists (e.g., Burns, Detwiler, Hooker, Nicholas, Stone, and Twitty), who also participated in spreading urodele developmental biology during the following decades. Harrison himself had been one of the founders (1904) of the *Journal of Experimental Zoology*, of which he became the managing editor for the following 42 years! This prestigious journal will be referred to again in the next paragraphs.

Prominence of Urodele experimental embryology (1920-1950)

Firstly, the emergence of the organizer concept in Spemann's laboratory (1921-1924) will be considered. Following the abovementioned experiments on newt gastrulae, Spemann entrusted one of his students, Hilde Pröscholdt (who married Otto Mangold), with the task of grafting the dorsal blastopore area in various positions. As previously reported, the resulting mortality was unfortunately very high (see Hamburger, 1988 and Holtfreter, 1991, for more comments). When Pröscholdt grafted the dorsal lip on the ventral side of a host gastrula, she nevertheless obtained five advanced embryos on which a complete secondary embryo had formed on the ventral side. The histological structure of that additional embryo showed that the chorda and part of the somites were derived from the unpigmented grafted tissue, whereas the neural tube and the rest of the somites had formed mostly from pigmented cells of the host embryo. *The concept of primary induction in vertebrate development, i.e., an organizing influence spreading from the blastopore region into the ectodermal superficial layer and the lateral mesoderm, originated from those experiments (Spemann and Mangold, 1924).* Many collaborators were quick to seize upon and develop it in the following years (Hilde Mangold accidentally died at age 26, in September 1924). Some authors later criticized these pioneering results, which led to Spemann's being awarded the Nobel Prize in 1935, because of the very small number of double embryos that were obtained. It should be emphasized, however, that those results were later confirmed and extended on a much larger scale. When operative medium and sterilization had been consistently improved, half-organizers were used as grafts by Mayer (1935), and Capuron (1968) grafted blastopore lips whose nuclei had been marked with ^{3}H-thymidine (a tag that provides reliable information as late as the hatching stage).

During the early 1920s, Walther Vogt (1888-1941) devised the technique that enabled a full exploitation of the transplantation technique and that constituted a breakthrough in the analysis of cell layer movements during gastrulation and neurulation. Vital dye staining (Nile Blue, Neutral Red) had been used by Goodale (1911a,b) in the United States to mark embryonic fragments before transplantation. Vogt very ingeniously attempted to mark small areas of the whole embryo without operating on it. He succeeded in incorporating the dye in agar blocks that were applied to the surface of the embryo for a short time (1923). This permitted an extensive analysis of different kinds of cell movements during gastrulation and neurulation. Vogt was also able to generate elaborate maps, at advanced blastula and early gastrula stages, of presumptive superficial territories corresponding to future organs of the advanced embryo. Maps were drawn for several amphibian species, especially for urodeles *(Triturus, Pleurodeles)* and for an anuran *(Bombinator)*. The results were published in two highly cited articles (Vogt, 1925, 1929).

Vogt had been interested in gastrula cell behavior as early as 1913, when he was a student in Marburg. He spent the decade 1920-1930 working on amphibians, mostly on urodeles. Appointed as Professor of Histology and Embryology in Munich (1925), he moved to Zurich (Switzerland) in 1930, then returned to Munich again (1935). He prematurely died from cancer in 1941. Most of his articles were issued in the proceedings of annual meetings of German biological and medical societies and thus are not easily available. Spemann (1941) wrote an obituary, in which a complete list of Vogt's publications (and those of his students) can be consulted.

Spemann worked in Freiburg constantly from 1919 on, but several of his former students obtained appointments at different German Universities, giving a strong impulse to experimental embryology of amphibians. Most of them continued working on the organizer and on induction problems.

In 1928, Otto Mangold invited one of Spemann's former students to become an assistant at the Kaiser Wilhelm Institute in Berlin-Dahlem: Johannes Holtfreter (1901-1992) accepted the position with enthusiasm (Holtfreter, 1991). *There, during the next five years Holtfreter "made some of his greatest contributions to the burgeoning investigations of embryonic development"* (Bagnara, 1993). Those included the discovery of an isotonic salt solution (1931), which, carefully sterilized, much improved the success of embryonic surgery on a wide scale. "Holtfreter's standard solution" is still used today. He then devised the "sandwich technique" for testing the organizing or inducing properties of an isolated embryonic fragment or of any other material. By this technique, the fragment is wrapped in two sheets of urodele undetermined gastrula ectoderm, the subsequent differentiation of which was histologically analyzed. By means of these techniques, Holtfreter demonstrated (1933) that a killed organizer still kept its inductive properties, giving a strong blow to some of Spemann's initial vitalistic speculations. On the other hand, the way was opened for investigations on the chemical status of the supposed inducing factors. Holtfreter himself did not work in that direction. He was compelled to leave Germany in 1939 and emigrated to England, but, as a German citizen, he was interned in Canada until 1942. After a stay at McGill University (Montreal), he obtained a stable position in Rochester (New York) in 1946 and continued to contribute important data on embryonic cell behavior.

Holtfreter's results quickly initiated a new step in the international expansion of experimental embryology. In Great Britain, Needham and Waddington were the leaders of new groups, devoted to "chemical," then "biochemical embryology." They also extended the "organizer" concept to other vertebrates. In the Netherlands, an International Institute of Embryology, based in Utrecht, had been founded in 1916 in memory of the zoologist and embryologist A.A.W. Hubrecht (died 1915). It became for a long time a center for experimental embryology on urodeles (Bijtel, Woerdeman). *There Peter Nieuwkoop began working in the late 1940s and later discovered mesoderm induction in the axolotl blastula.* It should be added that the embryological Museum of Hubrecht Laboratory harbors a collection of original drawings and histological slides of Spemann and Mangold. In Brussels (Belgium), following the great embryologist Albert Brachet (1869-1930) but preceding his equally famous son Jean Brachet (1909-1988), two names were associated with the theory of "morphogenetic potential" in amphibians during the period 1930-1950: Albert Dalcq (1893-1973) and Jean Pasteels (1906-1991). The Belgian embryologists usually worked with anuran embryos *(Rana, Discoglossus)*, but Pasteels also made important contributions to detailing the axolotl fate map and the effects of egg rotation and inversion on the formation of additional blastopores in both axolotl and frog eggs. Later, Jean Brachet and his co-workers frequently used urodele embryos for their experiments on "biochemical embryology."

The preparation of killed heterogenous inductors by one of Holtfreter's students (Chuang, 1939) opened a long-lasting series of experiments aimed at characterizing specific inducing factors, mainly carried out in Finland (see Saxén and Toivonen, 1962 for an extensive review). *Japan also became a major country for experimental studies on the newt Cynops pyrrhogaster, especially under* *the initial guidance of Yo. K. Okada, Osamu Nakamura and Tuneo Yamada.* The latter proved to be a brilliant investigator of heterogenous regional inductors, like the Finnish authors (see Nakamura and Toivonen, 1978).

These studies unfortunately led to ultimate disappointment vis-à-vis early hopes that they would reveal the presence, and perhaps allow the identification, of *in vivo* inductors. That is because many unspecific substances were found to elicit ectoderm neuralization *in vitro*. The importance of the reacting ectoderm was highlighted by these observations, although it must be stressed that Spemann and O. Mangold themselves had already rightly considered this point.

We shall now return to the United States, where Harrison and his former collaborators actively worked on urodele amphibians during the 1920-1940 period. Harrison himself carried on his experiments on limb polarity and extended them to other organs (gills, balancer, eye, ear). Screening the articles published in the *Journal of Experimental Zoology* during the decades 1921-1940, between the World Wars, is especially informative in that respect (Fig. 1). For the period 1921-1925, only 20 articles dealt with urodeles and 8 with anurans, and 22 per cent of all articles were devoted to amphibians. A dramatic increase in published papers occurred during the following three 5-year periods: fifty articles on urodeles from 1926 to 1930, then finally 60 articles from 1936 to 1940, i.e., nearly 25 per cent of the 255 articles published during those last five years. The number of articles on anurans only rose from 16 to 22 during the following three 5-years periods. It must be emphasized that most publications on urodeles dealt with experimental embryology, while anurans were mostly used in other fields of developmental biology (mainly metamorphosis) or in physiology. During the considered 20-year period, 182 articles were devoted to urodeles and only 66 to anurans, from a total of 923 publications. *Those data illustrate the increasing importance of amphibian, and especially urodele, embryology in experimental biology.*

In Europe, the *Roux' Archiv für Entwicklungsmechanik* remained the renowned journal in the field, but, in spite of the importance of amphibian embryology in Germany, in other European countries or in Japan, urodele research did not greatly exceed the preceding levels during the decade 1931-1940. Sixty-four articles on urodeles and 21 on anurans (together constituting 31.8 per cent of all the articles published) appeared during the period 1931-35. The subsequent 1936-40 years corresponded to the triumph of Nazism in Europe: the Roux' Archiv published only 184 articles, of which still 31 per cent were devoted to amphibians and 26 per cent dealt with urodeles. It must be emphasized that the decade 1931-40 includes most of Holtfreter's fundamental contributions.

In the United States, the *Journal of Experimental Zoology* dedicated a special volume (55, 1930) to Harrison's sixtieth birthday. This volume included 11 articles on urodeles from different collaborators of the great man. Among them, Detwiler (at Harvard, later at Columbia University) was one of the most prolific contributors to the journal during the analyzed 1921-40 period, publishing his numerous experiments on neuroembryology and related topics in *Ambystoma*. A few prominent scientists also explored new biological pathways in reproductive biology, especially of urodeles. Burns (1925, 1930, 1931) developed Born's parabiosis technique (homo- or heterospecific) to investigate sexual differentiation in *Ambystoma*, as influenced by association between male and female partners, united at the embryonic tail-bud stage. R.R. Humphrey (1892-1977) similarly initiated a series of studies on sex-reversal obtained by exchange of grafts or by parabiotic association in such salamanders (Humphrey, 1929, 1936). His studies ultimately led him to obtain progeny from crosses between a neo-male (a masculinized genetic female) and a normal female. These crosses allowed Humphrey to characterize the male genetic formula as homogametic (ZZ), the female being heterogametic (ZW), in *Ambystoma* (Humphrey, 1945), a situation which was later confirmed on *Pleurodeles* in France by Gallien (1954). From 1941 to 1957, Humphrey collaborated with Gerhard Fankhauser at Princeton University to investigate abnormal sex differentiation and progeny of polyploid salamanders. Fankhauser first obtained (1939) triploid urodeles from normally fertilized oocytes, from which second polar body extrusion was prevented by a temperature shock (Fankhauser, 1945). The cold shock (1-3°C) technique had been applied to anuran eggs in France a few years earlier (Rostand, 1934) to obtain diploid gynogenetic frogs and toads from oocytes fertilized with heterologous sperm. Fankhauser had been trained in Berne (Switzerland) as one of Baltzer's students. Fritz Baltzer himself had been one of Spemann's collaborators in Freiburg in the early 1920s. There he worked on hybridization and haploidy in newts (see Fankhauser, 1962). Another of Baltzer's student was Ernst Hadorn, who remained in Switzerland. During the 1930s he worked on haploid nucleo-cytoplasmic heterospecific associations in newts before he settled at Zurich University, where he later turned to the developmental genetics of *Drosophila*. Hadorn nevertheless wrote a booklet (1961) on experimental embryology in which urodeles held a prominent position.

Conclusion

This short review was written to demonstrate how important the use of urodele embryos was in establishing experimental methods for embryological studies and, consequently, our knowledge of basic mechanisms in animal development. At the very beginning experiments on urodele embryos were designed mainly to confirm earlier trials made on anuran eggs. Then urodele eggs themselves were used for decisive breakthroughs. This situation lasted for decades. After 1960, it progressively changed, due to the increasing importance of *Xenopus laevis* in laboratories, initially because it was possible to obtain eggs easily all year long after hormone injections. In spite of its small size, the *Xenopus* egg has proved to be quite useful in experimental embryology before becoming the preferred amphibian for molecular biologists. Although the urodele embryo has receded in prominence in embryological circles, nevertheless there are still opportunities for complementary studies using both amphibian models.

Summary

Experimental studies on amphibian embryos started mainly in Germany during the last two decades of the 19th century. At first, urodele amphibians were used much less frequently than anurans. At that time, embryological studies on urodeles were mostly descriptive and comparative. In Germany, Spemann (1900) — immediately followed by Harrison in the United States — began extensive studies on the newt egg and early embryo. Those studies finally led to the prominence of urodele embryos in general experimental embryology during the period 1920-1950. Milestones of that era are described and the main researchers are indicated.

KEY WORDS: *history of embryology, experimental embryology, urodeles, amphibians*

Acknowledgments

I wish to express my sincere thanks to Professor Klaus Sander (Freiburg i. Br., Germany) who kindly sent to me copies of the reference list in Hertwig's handbook (1906) and two articles. I am much indebted to Professor Julian Smith (Toulouse) for kindly improving the English text, to Marie-José Guinaudy for designing the figure, and to Christiane Mont for preparing the typescript.

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NOTE ADDED IN PROOF. In the February 1996 issue of this journal devoted to "Developmental Biology in Germany", interested readers will find three articles on Hans Spemann, his school of embryology and the impact of his concepts on molecular embryology (*Int. J. Dev. Biol. 40:* 49-57, 59-62 and 63-68, whose authors are P.E. Fässler, V. Hamburger and H. Steinbeisser, respectively). Similarly, in *Developmental Dynamics*, three articles deal with Johannes Holtfreter, his contributions to ongoing studies of the organizer and related problems of Amphibian morphogenesis (*Dev. Dynamics 205:* 214-216, 245-256 and 257-264, 1996, whose authors are V. Hamburger, J. Gerhart and R. Keller).