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REVIEW ARTICLE

Peter G.H. Clarke · Stephanie Clarke

Nineteenth century research on naturally occurring cell death and related phenomena

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Abstract Research on naturally occurring cell death is older than current opinion gives credit. More than 100 nineteenth century publications deal with it, and we review most of these. Soon after the establishment of the cell theory by Schleiden and Schwann, Carl Vogt (1842) reported cell death in the notochord and adjacent cartilage of metamorphic toads. Subsequent landmark discoveries included the massive cell death that occurs in pupating diptera (Weismann 1864), chondrocyte death during endochondral ossification (Stieda 1872), phagocytosis associated with cell death in the muscles of metamorphic toads (Metschnikoff 1883), chromatolytic (apoptotic) cell death in ovarian follicles (Flemming 1885), the reinterpretation of "Sarkoplasten" as "Sarkolyten" in metamorphic amphibia (Mayer 1886), the programmed loss of an entire population of neurons in fish embryos (Beard 1889), the death of scattered myocytes and myofibres in mammalian muscle (Felix 1889), and the death of many motor and sensory neurons in chick embryos (Collin 1906). Other lines of nineteenth century research established concepts important for understanding cell death, notably trophic interactions between neurons and their targets, and intercellular competition.

Key words Programmed cell death · Apoptosis · Necrosis · Development · History

Introduction

In development and in adult life, large numbers of cells are known to die in many different tissues of both vertebrates and invertebrates. In some cases, whole regions or entire organs are thus eliminated. In other cases, scattered dying cells occur in developing tissues destined to survive, and in adult tissues subject to cell turnover.

P.G.H. Clarke (✉)
Institute of Anatomy, University of Lausanne, Rue du Bugnon 9, CH-1005 Lausanne, Switzerland
Fax: +41 21 692 5105

S. Clarke
Institute of Physiology, University of Lausanne, Rue du Bugnon 7, CH-1005 Lausanne, Switzerland

The importance of these phenomena is now widely recognized, and a considerable research effort is currently directed at unravelling the role of cell death, the signals that regulate it, and the mechanisms by which the cellular demolition is actually achieved (for reviews, see Bowen and Bowen 1990; Clarke 1990, 1994; Raff 1992).

The current literature gives the impression that this field of research began near the start of the twentieth century. For example, many papers cite Glücksmann (1951) for a historical review; but he only cites three papers published before 1900 (Looss 1889; Schaffer 1893, 1897). Hamburger (1992) attributes the "discovery that cell death is an integral part of certain processes in embryonic development" to E. Kallius (the mentor of M. Ernst and A. Glücksmann), mainly on the grounds that Kallius added to a paper by Glücksmann (1930) a footnote stating he had been interested in cell death since 1920. However, we show here that naturally occurring cell death was investigated throughout much of the nineteenth century, becoming a major research topic towards the end of it.

The erroneous belief in a late discovery of naturally occurring cell death is frequently linked to an explanation of why this came so late. It is argued that researchers neglected the phenomenon throughout the last half of the nineteenth century owing to a preconceived notion that ontogeny (and phylogeny) were progressive (e.g., Oppenheim 1981; Jacobson 1991). There may be an element of truth in this, because most early reports on cell death dealt with situations where ontogeny was obviously *not* progressive, namely, the metamorphosis of insects and amphibia. However, cell death during less dramatic forms of development, and in adult tissue turnover, was also clearly demonstrated in a small number of nineteenth century reports.

Our purpose is to review this old literature, although the sheer abundance of papers after 1885 has obliged us to be selective in our treatment of these latter years. The only twentieth century papers known to us that refer to significant numbers of nineteenth century reports on cell death are two works in German by Ernst (1926) and Wendler (1972), and a very recent review by Majno and Joris (1995). Unfortunately, Ernst missed almost all of the key papers, and, oddly, he refers in his text to only about 12%

of his reference list, providing negligible discussion of the nineteenth century reports. Wendler provides some useful lists of early references, but minimal discussion of them. Majno and Joris (1995) devote two pages to nineteenth century studies of *pathological* cell death, but cite only two papers relevant to naturally occurring cell death. Brief preliminary reports of part of the present survey have been produced (Clarke and Clarke 1994, 1995a,b).

Prelude to the discovery of developmental cell death

That many fetal and larval structures regress during development was widely recognized in the early years of the nineteenth century (e.g., von Baer 1828), having been shown much earlier. For example, the regression after birth of the ductus arteriosus was known possibly to Aristotle, and certainly to Galen, who described it along with the foramen ovale and its valve, commenting on their postnatal closure (Barclay et al. 1944); numerous further descriptions of the remodelling of the heart and its great vessels were published in the seventeenth and eighteenth centuries (e.g., Harvey 1628; de Haller 1758). The first account of the ductus venosus occurred in 1564, in a posthumous publication by Vesalius (Barclay et al. 1944). The numerous progressive and regressive changes that occur during tadpole metamorphosis in many parts of the body were first described in detail by Dugès (1835), but the basic idea of such changes was by then already current knowledge. The fact that mammalian fetuses transiently develop gill arches was discovered somewhat earlier by Rathke (1825).

However, although the notion of regressive changes was old, its extension to the concept of naturally occurring cell death had to await the establishment of the cell theory by Schleiden (1842) and by Schwann (1839). By then, microscopes were being manufactured in several European countries, and a few primitive microtomes had been constructed. Methods of fixation were being developed, following the introduction of chromic acid as a "hardening agent" by Jacobson in 1833 and of chromium tetroxide by Hannover 7 years later (Bracegirdle 1978).

In short, the discovery of developmental cell death became possible in about 1840, when the long-recognized fact of tissue regression was complemented by the cell theory and technical advances. Realization of the possible followed immediately.

Cell death in metamorphosis

The discovery of developmental cell death by Vogt in 1842

In 1842, and indeed throughout the next half century, histology was dominated by Germans and its lingua franca was German. Carl Vogt was born in Germany (Giessen) of Swiss (Bernese) descent and he always wrote in German, but his 130 page monograph (Vogt 1842) was based on research done while working with Jean-Louis Agassiz at the Collège de Neuchâtel in the French-speaking part of Switzerland. Vogt had been obliged to flee from Germany in 1835 for political reasons and had obtained his medical doctorate in Bern in 1839, before moving to Neuchâtel.

Translated into English, the title of Vogt's monograph is "Investigations on the developmental history of the midwife toad (*Alytes obstetricans*)." As the title suggests, Vogt covers a wide range of questions beyond our present concern. What is relevant is that, influenced by Schwann, whom he mentions, Vogt came to this research with specifically cellular questions in mind and used microscopy to examine what happened to individual cells. One of these questions was whether the well-known disappearance of the anuran notochord during climax, and its replacement by the vertebrae and by the base of the skull, was due to the transformation of the notochord cells into cartilage (as had been proposed by Rathke) or whether it was due to their destruction and replacement by new cells. He unequivocally (and, we now know, correctly: Fox 1983) supports the latter hypothesis of naturally occurring cell death, followed by the neoformation of cartilage from cells of a different origin. He does not, however, use the word "death," but refers to the cells as being "resorbed" or "destroyed" or as "disappearing." He writes:

"The role of the core of the notochord in the formation of the vertebrae is quite simply that its cells are resorbed, beginning when the proliferation of the surrounding cartilage exerts pressure on the notochord" (Vogt 1842, p 86, free translation).

A related question was what happens in the formation of cartilage adjacent to the notochord. Here, too, Vogt identified the death of cells, in this case those of the cartilage itself. He writes:

"The first anlage of the cartilaginous tissue of the base of the skull consists of a thick, dark cytoblastema... apparently originating from the destruction of the original embryonic cells.... Also, the light vesicular nuclei of the embryonic cells have disappeared; at least, I could not detect any trace of them." (Vogt 1842, p 105, free translation).

Vogt does not, however, appear to regard the occurrence of cell death as particularly important, and in his long scientific career he never went back to it. In abandoning an early interest in cell death, he set a trend that was to be followed by many eminent scientists including Weismann, Metschnikoff, Bataillon and Collin.

Vogt envisaged a role for cell death also in the *initial formation* of the notochord, involving the destruction of its primary cells to form a "secondary blastema" in which "new cells, the true cells of the notochord" subsequently develop (Vogt 1842, pp 49–50). This earlier phase of cell death probably does occur (Glücksmann 1951), but the idea of new notochordal cells forming from degenerate debris was contested only 2 years later by two other Swiss authors, Prévost and Lebert (1844)

and sounds quaint to modern ears. However, these authors do agree with Vogt about the occurrence of cell death, stating that the "simple globules" of the notochord are resorbed.

Metamorphic insects

More than 20 years then elapsed before the next major report on naturally occurring cell death. In a seminal study, August Weismann, working in Freiburg-im-Breisgau in the southern part of Germany, described at the microscopic level the embryonic (Weismann 1863, 1864b) and postembryonic (Weismann 1864a,b, 1866) development of three species of diptera. Of particular relevance in the present context are his descriptions of widespread degenerative phenomena during the metamorphic transformation of pupating diptera. To denote this he coined the term "Histolyse." He showed that most of the larval tissues were destroyed through wholesale cell death, although certain organs, including the central nervous system, were profoundly *modified by histolysis* without being destroyed. Weismann also employed the term *fatty degeneration* ("fettige Entartung" – a term already in use by pathologists such as Virchow) because the dying cells contained numerous vacuoles, assumed to be filled with lipids although in reality they were probably autolysosomes (Locke 1981; Clarke 1990). Weismann was also mistaken in that he considered the tissue to dissolve into a pulp ("Brei") from which new cells, "Körnchenkugeln," arose. Although such an idea had been rejected by Prévost and Lebert (see above) and is no longer accepted, it was not absurd in light of what was then known, but it now seems strange to see this cells-from-soup notion written by the pen of the same August Weismann who is remembered primarily for his theory of heredity based on the transmission of a physical "germ plasm," now known to be DNA (Mayr 1982).

Weismann also showed that the teguments of the head and thorax of the adult were produced largely from the imaginal disks (a term he coined, in German: *imaginale Scheiben*). These small, pale structures had been described by other authors but without their significance being understood. Even Weismann did not grasp it fully, because he failed to realize that many other structures, in addition to the teguments, notably the muscles, nerves and tracheas of the adult, were likewise generated from the imaginal disks, as was gradually clarified by Künckel (1868) and Ganin (1869, 1875, 1877).

Additional observations of cell death during insect metamorphosis (Table 1) were made in flies, ants and beetles by Ganin (1869, 1875, 1877), in blow-flies by Lowne (1870), in butterflies and bees (with emphasis on the intestines and rectal glands) by Chun (1876) and in a species of silk moth (with emphasis on the silk glands) by Helm (1876). The widespread importance of "histolysis" in the muscles, fat bodies, salivary glands and tracheas of flies, during metamorphosis, was further described and discussed in substantial detail by Viallanes (1882), who cut histological sections (rather than dissociating the tissue mechanically, as did many scientists at that time). The essential correctness of these early reports, with respect to the wholesale degeneration of most of the larval cells, has been confirmed in modern studies (e.g., Locke 1981).

Table 1 Early reports of cell death in metamorphosis

Date	*Author*	*Tissue*	*Species*
1842	C Vogt	Notochord	Midwife toad
1844	Prévost & Lebert	Notochord	Frog
1864, 66	A Weismann	Various	Various flies
1869, 75	M Ganin	Various	Various insects
1870	BT Lowne	Various	Blow-fly
1870	C Kupffer	Various	Sea-squirt
1875	A Goette	Various	*Bombinator* (toad)
1876	C Chun	Intestine, glands	Butterfly, bee
1876	FE Helm	Silk glands	Silk moth
1882	H Viallanes	Various	Fly
1883	E Metschnikoff	Muscle	*Bombinator* (toad)
1886, 87	S Mayer	Muscle	Frog
1887	D Barfurth	Various	Frog
1891	E Bataillon	Various	*Alytes, Bufo* (toads)

Lowne (1870) also mentioned the degeneration *after hatching* of muscles involved in the hatching process of blow-flies. In moths, such posthatching muscle degeneration is currently a major model system for the analysis of programmed cell death (Beaulaton and Lockshin 1977; Schwartz 1992).

Weismann and his contemporaries attempted to describe the cytological appearance of the dying cells. For example, muscles were described by Weismann (1864a,b) as first of all losing their transverse striations, after which the nucleus and contractile material were transformed into a finely granular mass that was released into the extracellular space following the rupture of the sarcolemma. Somewhat similar descriptions were given by Lowne (1870) in the blow-fly, by Ganin (1869, 1875) in various species of fly, and by Chun (1876) in a species of hawk moth, but the latter papers do not confirm the release of cellular contents into the extracellular space; nor do modern studies on naturally occurring cell death (Beaulaton and Lockshin 1977; Locke 1981; Clarke 1990).

On the grounds of rather dubious observations, Viallanes (1882) proposed that the destruction of tissues could occur by three different means. In the case of muscle, he thought there were two alternatives: one, "histolysis by degeneration," involved the loss of stainable nucleoli from the nucleus and the dissolution of contractile material from the cytoplasm, leading to cell death; the other alternative, "histolysis by regressive evolution," involved intense proliferation, followed by the dispersal (not death) of the new, smaller cells. Viallanes believed, wrongly, that the "histolysis" of the salivary glands and tracheas was entirely due to the latter means. In the case of fat bodies, he invoked a third means of destruction, involving the formation of granules in the "protoplasm"

(meaning cytoplasm), followed by their release from the cell after rupture of the cell membrane. We can find little support for these observations in modern reports on insects (Beaulaton and Lockshin 1977; Locke 1981; Schwartz 1992), but the notion that there are two or three distinct morphologies of naturally occurring cell death was subsequently proposed by several other nineteenth century authors (Demarbaix 1889; Schaffer 1893; Noetzel 1895) and is currently topical and controversial (Clarke 1990). The idea of destruction by proliferation calls to mind the recent notion that "apoptotic" cell death involves an abortive attempt by the cell to proliferate (Rubin et al. 1993).

The above-cited nineteenth century authors had little to say on the causes of the histolysis, but Kowalevsky (1885, 1887) and van Rees (1888–89), inspired by the observations of Kowalevsky's former collaborator Metschnikoff (1883) on the muscles of metamorphic toads, attributed a major role in insect metamorphosis to amoeboid phagocytic cells, since he identified fragments of muscle within these cells. Kowalevsky and van Rees believed that the phagocytes actually caused the cellular destruction, but subsequent authors, including Noetzel (1898), argued that the initial degeneration was independent of the phagocytes, which arrived only later to help clear away the debris. This latter view is currently accepted (e.g., Clarke 1990).

Weismann's last publication relating to cell death was that of 1866. He was shortly afterwards obliged to abandon histological research because of a serious eye disease, which was a factor leading to his well-known career in theoretical biology (Mayr 1982). In this later phase, he ignored the phenomenon of cell death even when it was relevant. For example, in his essay "On Regression in Nature," Weismann (1886), reviewed evidence that organs and body parts can be lost in evolution through natural selection, as in wingless birds and eyeless moles. But he made no mention of the fact, well known even then, that evolutionary regression is often due to ontogenetic regression and therefore presumably involves cell death.

Metamorphic amphibia – muscle

Even before the work of Weismann, Margo (e.g., 1862) described what seem in retrospect to have been degenerating muscle fibers in the dorsal musculature of larval and juvenile anura. The fibers in question were refringent, ovoidal bodies that were transversely striated and appeared to contain one or two nuclei. Margo appears not to have considered the possibility that these fibers were degenerating, but interpreted them as a stage in the progressive development of striated muscle fibers, and called them "Sarkoplasten" (myoblasts). Paneth (1885) made further observations on the same cells and claimed that the "nuclei" were in fact outside the muscle fibers; but this did not lead him to a fundamentally different interpretation, and he retained the term *Sarkoplasten*. Then Mayer (1886, 1887), at the German University in Prague, studied the so-called Sarkoplasten in various muscular tissues of tadpoles and young frogs and deduced correctly that they were the products of muscle degeneration; he suggested that they should therefore be called Sarkolyten. Mayer came to this conclusion with a prepared mind, because he had already, in 1881, made the probably false claim that spinal ganglion neurons constantly die and are renewed throughout adult life. However, his subsequent, and this time correct, identification of muscle cell death in metamorphosis was based on sound arguments. He argued on the grounds that the "Sarkolyten" occurred even in well-differentiated muscle and were more numerous in the regressing tail than in the growing limbs, although present even in the latter. Moreover, he thought that their very appearance was suggestive of degeneration. He also noticed that they were sometimes contained inside other cells, subsequently identified as phagocytes.

Indeed, although unmentioned in Mayer's 1886 paper, Metschnikoff (1883) had already inferred the occurrence of hystolysis from the presence of accumulations of phagocytes in regressing muscles of metamorphic toads and had noted that these sometimes contained fragments of striated (hence presumably muscular) tissue as well as the still identifiable debris of nerve fibers. Metschnikoff concluded that the histolysis was being caused by the phagocytes. The causal role of the phagocytes was hotly disputed by subsequent authors. Thus, Mayer (1887) considered that, independently of the phagocytes, the muscle fibers separate from each other and are then broken into stumps that may or may not contain nuclei, the phagocytes acting only secondarily. Looss (1889) minimized their role still further, partly on the grounds that only a tiny proportion (about 3%) of the sarkolytes appeared to him to be contained within phagocytes. Bataillon (1889, 1891, 1892) considered that a much larger proportion (about 95%) were contained in phagocytes, and he agreed with Metschnikoff that they played an active role in the histolysis, but he denied that they *initiated* it, on the grounds that they arrived too late.

There was further debate on the *origin* of the phagocytes, coming to a head at two sessions of the Société de Biologie in Paris in March 1892 (Bataillon 1892; Metschnikoff 1892b). Both Looss and Bataillon considered that the phagocytes were leukocytes that invaded from the blood, and Bataillon (1891) argued that some of them left by the same route whereas others degenerated in the muscle. In contrast, Metschnikoff (1892a,b) argued that the phagocytes were not derived from the blood (as his 1883 paper had been interpreted by several authors to imply) but by the transformation of the muscle itself into a mass of phagocytes containing the striated material of the muscle. But what do the phagocytes phagocytose, if the muscle is already transformed into phagocytes? We think that Metschnikoff's view is almost identical to that of Mayer (1887) described above, but that Metschnikoff confusingly uses the term "phagocyte" for the nucleated stumps of muscle described by Mayer. In this he presages the notion, formulated clearly a few years later by De

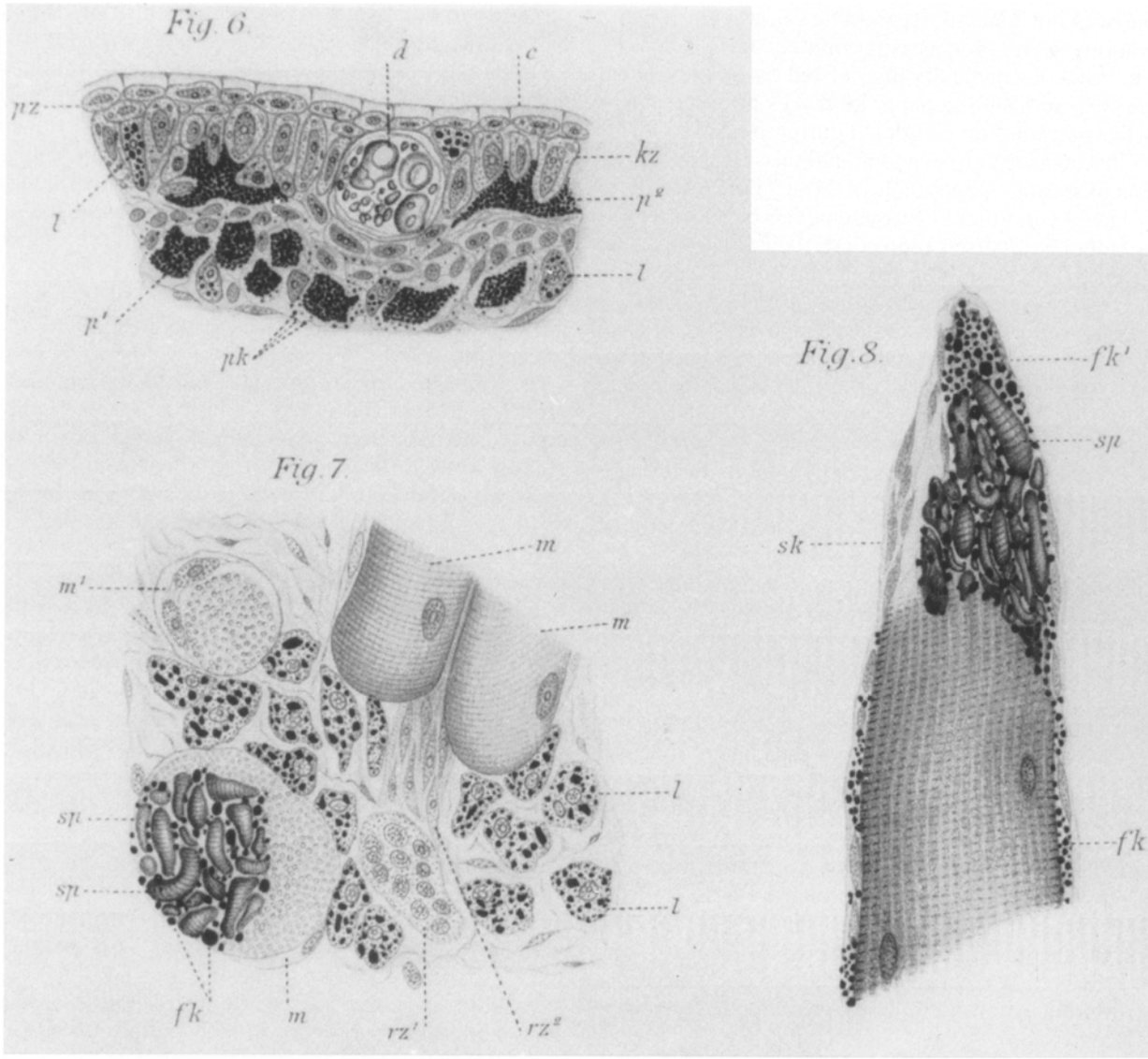

Fig. 1 From Barfurth (1887b). **His Fig. 6** Section through the skin of a regressing tail, 4th day of regression. *c* Cuticle, *pZ* flat cells of the upper layer, *kZ* club-shaped cells in the lower layer of the epidermis. Between the latter are inserted leukocytes (*l*) filled with fatty spheres and prolongations of pigment cells (p^2). p^1 Pigment cells of the cutis, *pk* free pigment granules, *l* leukocytes in the cutis, *d* gland. **His Fig. 7** Part of a transverse section through the tail of *Rana fusca*, third day of regression. *m* Muscle fibers in transverse and longitudinal sections, at m^1 withdrawal (proliferation) of the sarcolemma. *sp* The so-called "Sarcoplasten," a product of the degeneration; *fk* fatty granules, rz^1 giant cell, rz^2 pseudo-giant cell, *l* leukocytes. **His Fig. 8** Muscle fiber in longitudinal section from the tail of *Rana fusca* on the third day of regression. *sk* Nuclear proliferation in the perimysium internum, *sp* "Sarcoplasten," fk^1 fatty spheres in the "Sarcoplasten," fk^2 fatty spheres in the sarcolemma (fixed with Flemming's mixture, stained with hematoxylin)

Bruyne (1898), in the context of insect metamorphosis (in various diptera and lepidoptera), that cells can destroy themselves by *autophagocytosis* (or autophagy). In accordance with Mayer, Looss and Bataillon, De Bruyne maintained that invading phagocytes played only a secondary role (if any) in the destruction of the tissues, but were involved in clearing away the debris.

Now, more than 100 years later, although many details are still unclear, there is little doubt that Bataillon was essentially correct; phagocytes do invade from the blood, but only after the degeneration has progressed considerably owing to autodestructive mechanisms in the muscle fibers themselves (e.g., Fox 1983; Atkinson 1981). Autophagocytosis is now believed to be one of several mechanisms by which cells destroy themselves during development (Beaulaton and Lockshin 1977; Clarke 1990; Schwartz 1992).

Metamorphic amphibia – various systems

The studies of Mayer (1886, 1887) were noteworthy in that they corrected the early and well-known reports of Margo (e.g., 1862), thereby helping to bring cell death into the forefront of nineteenth century research. Moreover, they showed that cell death could occur even in tis-

sue that was not blatantly regressing. However, Mayer's observations were of necessity limited to muscle, because he used mechanically dissociated tissue rather than sections, and in his 1886 paper he makes no reference to the earlier literature on cell death during metamorphosis, in both insects (see above) and amphibia.

The pioneering monograph of Vogt (1842) seems in fact to have been quickly forgotten even by those working on tadpoles. Eberth (1866) described what appear to have been pyknotic cells in the dermis of the regressing tadpole tail and raised the possibility that he was seeing dying cells, only to reject his correct hypothesis. Then Goette (1869) studied various regressing tissues including the notochord, but he failed to cite Vogt (1842) and even argued that the disappearing notochordal cells might still be present, but invisible.

But six years later, Goette was convinced of the reality of cell death. In a major publication (Goette 1875), he described cell death in the notochord and implied that it must occur in various tissues of the regressing tail and gills and in other transforming organs, including the aorta. Among the 170 references, he included Vogt (1842), but ignored the growing literature on cell death in metamorphosing insects. Following Goette's 1875 paper, the subject of cell death in amphibia sank back into oblivion until a spate of new papers was triggered by the reports of Metschnikoff (1883) and, especially, of Mayer (1886).

Unlike Mayer, subsequent workers used embedded, sectioned material and were able to observe dying cells in a wide variety of regressing or transforming tissues and organs. Several of these histological studies concentrated on the regressing tail (Barfurth 1887a,b; Noetzel 1895), but the investigations of Looss (1889) and Bataillon (1891) dealt with other regions as well, since many different parts of the body are remodelled during metamorphosis.

Barfurth's major contribution (Barfurth 1887b) followed Mayer (1886) in referring to *die sogenannten Sarcoplasten* in the title, and gave detailed descriptions of the dying cells in the epidermis, capillaries, notochord, spinal cord, muscle and elsewhere (Fig. 1). He argued that the ultimate aim of the cell death was the liquefaction of all decayed cell material, so that it could be conveyed into lymph and blood vessels and used for building new tissues and organs. As for the mechanism initiating the cell death, he shared the assumption of many authors, going back at least to Whitney (1867), that tissue regression in metamorphic tadpoles resulted from ischemia owing to the occlusion of capillaries; Barfurth attributed this to a reduced "trophic" influence from the nervous system owing to disuse of the tail once the limbs appeared. This is now known to be untrue, as is easily shown by culture of the isolated tail (e.g., Tata 1966), a technique that was introduced long before the reports of Barfurth (Vulpian 1859). The accompanying paper (Barfurth 1887a) documented a more rapid resorption of regressing tissues in starved tadpoles, which was taken to support Barfurth's view that the products of degeneration were important as nutrition for other parts of the body.

Bataillon's main publication (Bataillon 1891) likewise documented cell degeneration in many different tadpole tissues during metamorphosis, and he came to wrong conclusions not unlike those of Barfurth concerning the underlying mechanisms. He concluded that metamorphosis is "glycemic asphyxia," meaning that the tissue regression is due to glycemia resulting from prolonged asphyxia. More adequate mechanistic theories had to await the discovery of the various thyroid and other hormones now known to initiate tissue degeneration in metamorphosis (Fox 1983).

Barfurth and Bataillon continued to be scientifically productive. Barfurth turned his interest mainly to regeneration and published, on an annual basis, 23 reviews devoted mainly to this subject in the *Ergebnisse der Anatomie und Entwickelungsgeschichte* between 1891 and 1914 (see Churchill 1991). However, in all but the first two and the very last of these reviews he also devoted several pages to "Involution," and these reviews document the abundant research on cell death in that period (Barfurth 1893–1913). Barfurth was unique, in the nineteenth century, in maintaining an active interest in regressive phenomena for more than two decades. In contrast, Bataillon completely abandoned this subject and became better known for his work on experimental parthenogenesis (Fischer and Smith 1984).

Other metamorphic species

Although most work on metamorphosis was (and still is) done on insects or amphibia, it was also shown that many tissues of metamorphosing sea squirts undergo substantial degeneration, including notably the nervous system and the muscles of the tail (Kupffer 1870). This paper provides, however, no description of the histological appearance of the dying cells.

Cell death without metamorphosis

Since transient structures were known to occur and regress even in the absence of metamorphosis (see above), it would have been logical to search there for dying cells. In particular, following Vogt's (1842) observation of dying cells in the regressing amphibian notochord, it would have been logical to look for an equivalent phenomenon in the regressing notochord of higher vertebrates, but this seems not to have been done until well into the twentieth century (reviewed in Glücksmann 1951). The first report of developmental cell death in the absence of metamorphosis (Table 2) was, in fact, in the other situation where Vogt had reported cell death, in developing cartilage.

Table 2 Early reports of cell death during nonmetamorphic development

Date	*Author*	*Tissue*	*Species*
Wholesale cell death			
1872	L Stieda	Cartilage	Various mammals
1873	Z Strelzoff	Cartilage	Birds
1879	G Wagener	Graafian follicle	Dog
1881	N Kastschenko	Cartilage	Various amphibia
1885	W Flemming	Graafian follicle	Rabbit
1889	J Beard	Rohon-Beard cells	Skate
Cell death within tissues that persist			
1889	W Felix	Muscle	Human fetus
1892	R Krösing	Muscle	Human, rabbit fetuses
1893	J Schaffer	Muscle	Human, cat, dog, eel embryos
1906–07	R Collin	Spinal cord	Chick embryo

Endochondral ossification

It is generally accepted that endochondral ossification involves the death of the differentiated chondrocytes. The existence of such cell death is much less obvious than in a regressing organ and was frequently contested throughout the last quarter of the nineteenth century (for review, see Brachet 1893), but it was correctly recognized by several authors from 1872 onwards (Stieda 1872; Strelzoff 1873; Kastchenko 1881; Leser 1888; Brachet 1893; Schaffer 1897).

The details of endochondral ossification vary with age, with bone type, and with species, but in all cases the cartilage cells swell and die, and bone is laid down by a new population of mesenchymal cells called osteoblasts (Hinchliffe and Johnson 1980). Stieda (1872) studied this process in a variety of young mammals and stated clearly that the cartilage regresses and is replaced by bony tissue formed by osteoblasts that are not derived from chondrocytes. This view was not entirely original, dating back to some unconvincing and purely theoretical arguments of Nesbitt (1736), but it had been rejected in many publications, not least in Schwann's (1839) famous book that established the cell theory, and was still controversial. Stieda's important contribution was that he provided sound experimental evidence. In particular, he described the dying cartilage cells and recognized that they were dying. He documented both the swelling of cells and their nuclei, and their subsequent collapse to take on a star-shaped morphology (Fig. 2). He argued for a separate origin of the osteoblasts on the grounds that mitoses were rare in the cartilage close to an ossification border.

Strelzoff (1873) reported similar findings only 1 year later. Kastschenko (1881) extended Stieda's conclusions to the larvae of various amphibians, confirming that the cartilage cells died and that the new osteoblasts were from a different origin. Brachet (1893), working on chick embryos, further confirmed the death of many cartilage cells, but believed some of them could survive and transform to become osteoblasts or connective tissue cells (fibroblasts). Such a transformation is not nowadays accepted.

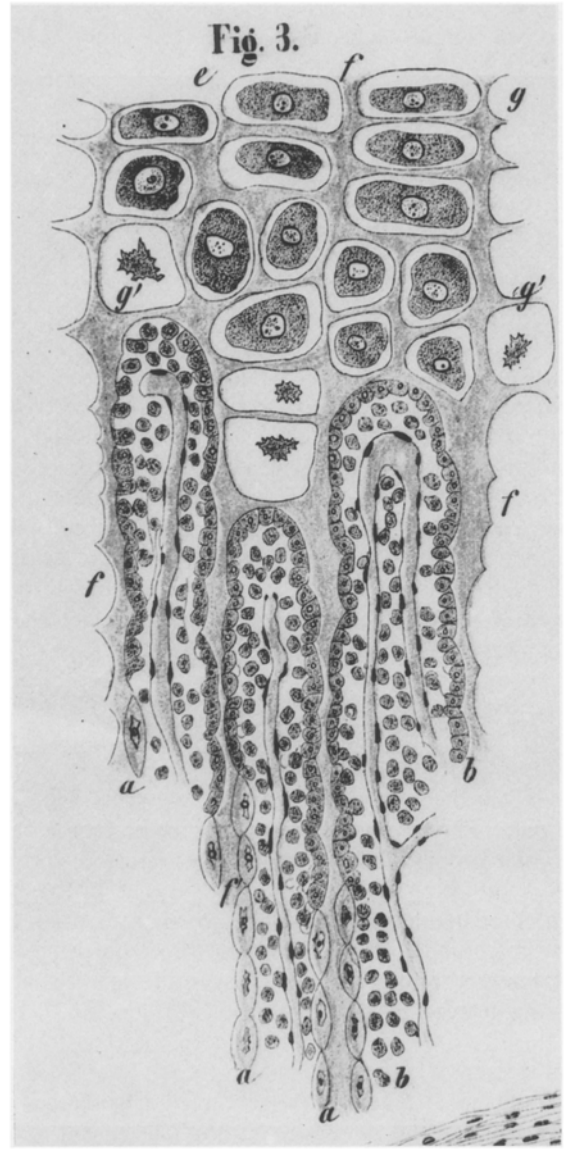

Fig. 2 From Stieda (1872). **His Fig. 3** from a longitudinal section of a newborn dog's metatarsal bone: *a* bone substance with bone corpuscles, *b* osteogenic tissue (osteoblasts), *e* cartilaginous tissue, *f* extracellular matrix of the cartilaginous tissue, *f'* remains of the extracellular matrix, *g* swollen cartilage cell, *g'* shrunken (collapsed) cartilage cell

The degeneration of cartilage in development and its remodelling in adulthood are both accompanied by the invasion of large, multinucleated phagocytes (called chondroclasts in cartilage, osteoclasts in bone), whose main role is to digest the extracellular matrix and remove debris (Silvestrini et al. 1979; Hinchliffe and Johnson 1980). Like most professional phagocytes, these "giant cells," as they were called in the nineteenth century, are short-lived. Their death in the bones of various adult mammals was suggested by Rindfleisch (1879), who thought (incorrectly) that they transformed into blobs of

Table 3 Early reports of cell death in adulthood. We exclude reports of cell death in adulthood that seem to be false (e.g., Mayer 1881; Teuscher 1890; Hammer 1895)

Date	*Author*	*Tissue or cell type*	*Species*
1879	Rindfleisch	Chondroclasts	Guinea pig
1886	Nissen	Milk gland	Dog, cat, rabbit
1889	Demarbaix	Osteoclasts	Various mammals
1890	Heidenhain	Various glands	Newt
1893	Solger	Cartilage turnover	Man, pig, stickleback

fibrin; this process was described in some detail by Demarbaix (1889), who documented two kinds of "natural degeneration," both exhibiting intense staining with methyl green (Table 3). In one kind, the nucleus became a uniform, heavily stained mass that subsequently fragmented to several small, round blobs in the cytoplasm. The other kind involved loss of cytoplasm and infolding of the nuclear membrane. Both kinds were shown by Demarbaix to be different from "cadaveric degeneration" occurring post mortem.

Ovarian follicles

Only a minority of ovarian follicles matures sufficiently to ovulate. The overwhelming majority undergoes a degenerative process known as atresia, either as primary follicles (already present in the ovary at birth) or as Graafian (vesicular) follicles. The atresia of ovarian follicles was studied by many authors throughout the nineteenth century, and it became clear in the 1860s that this could occur even in newborn girls (Pflüger 1863). However, the first reports giving details of the death of individual cells appeared much later.

The earliest degenerative changes associated with atresia occur in the membrana granulosa, and it is there that most observations of cell death were made. The first explicit statement that the cells of the granulosa die appears to have been made by Wagener (1879), but this was not of great importance given that degeneration of the granulosa was by then common knowledge. Of much greater importance was the detailed description by Flemming (1885) of the dying cells. He obtained excellent cellular preservation using a powerful fixative of his own invention that is still used and referred to as Flemming's fluid (containing chromic acid, osmium tetroxide and acetic acid), and stained the tissue with safranin or gentian violet as nuclear stains. He was thus able rather accurately to describe the dying granulosa cells in rabbit Graafian follicles as having an ill-defined nucleus containing several small, heavily stained lumps, and a pale, homogeneous cytoplasm containing what appeared to be fine fat droplets (Fig. 3, bottom). Most of these observations were confirmed in a wide variety of mammals and in selected lower vertebrates by several nineteenth century authors (e.g., Schottlaender 1893; Henneguy 1894).

Flemming argued that the cell death was not simply the result of mechanical disruption, but involved chemical changes within the cells. He coined the word *chromatolytic* to characterize this kind of cell death because the nuclear chromatin appeared to disintegrate. Chromatolytic cell death became accepted as a distinct variety of cell death at the end of the nineteenth century (e.g., Heidenhain 1890; Schottlaender 1893; Henneguy 1894), corresponding, in modern terminology, to *apoptosis* (Kerr et al. 1972). Cell death in the membrana granulosa shows the morphological and biochemical characteristics of apoptosis in pharmacologically induced follicular atresia (Hughes and Gorospe 1991). The term *chromatolysis* is currently reserved for the transient and often nonlethal loss of cytoplasmic chromatin that follows axotomy in certain kinds of neuron.

Various authors also attempted to describe the degeneration of the oocyte itself, but their descriptions seem confused owing to technical and other difficulties (Wagener 1879; van Beneden and van Bambeke 1880; Henneguy 1894).

Cell death in tissue turnover

The above reports concern the deletion of entire populations of cells, but it is now known that much naturally occurring cell death involves partial cell loss within a population whose remaining cells will persist. Such partial cell death occurs in both development (where it is sometimes referred to as *histogenetic*, following Glücksmann, 1951) and in adulthood in all cell populations subject to a turnover, proliferation being balanced by cell death (Gräper 1914; Kerr et al. 1972). Less obviously, it also occurs among developing *postmitotic* cells, notably muscle fibers and neurons.

Its first description (in the absence of metamorphosis) was in tissue turnover. Using Flemming's fixative and a hematoxylin nuclear stain, Nissen (1886) described scattered, apparently dying, cells in mammary glands of lactating dogs, rabbits and cats, and concurred with Flemming's (1885) designation of such cells as "chromatolytic." It is now known that a few mammary gland cells do die during lactation and are probably lost in the milk, but this is minor compared with the massive cell death that occurs in the regressive phase after the end of lactation, a stage that Nissen did not study. Soon afterwards, Heidenhain (1890) made similar observations of chromatolytic dying cells in various glands of adult newts. Then Solger (1893) reported scattered dying cells in cartilage of adult man, pig and stickleback.

Histogenetic cell death of postmitotic myocytes

The first example of histogenetic death of postmitotic cells (in the absence of metamorphosis) was in the development of mammalian skeletal muscle, where its occurrence has been confirmed in modern investigations. Ul-

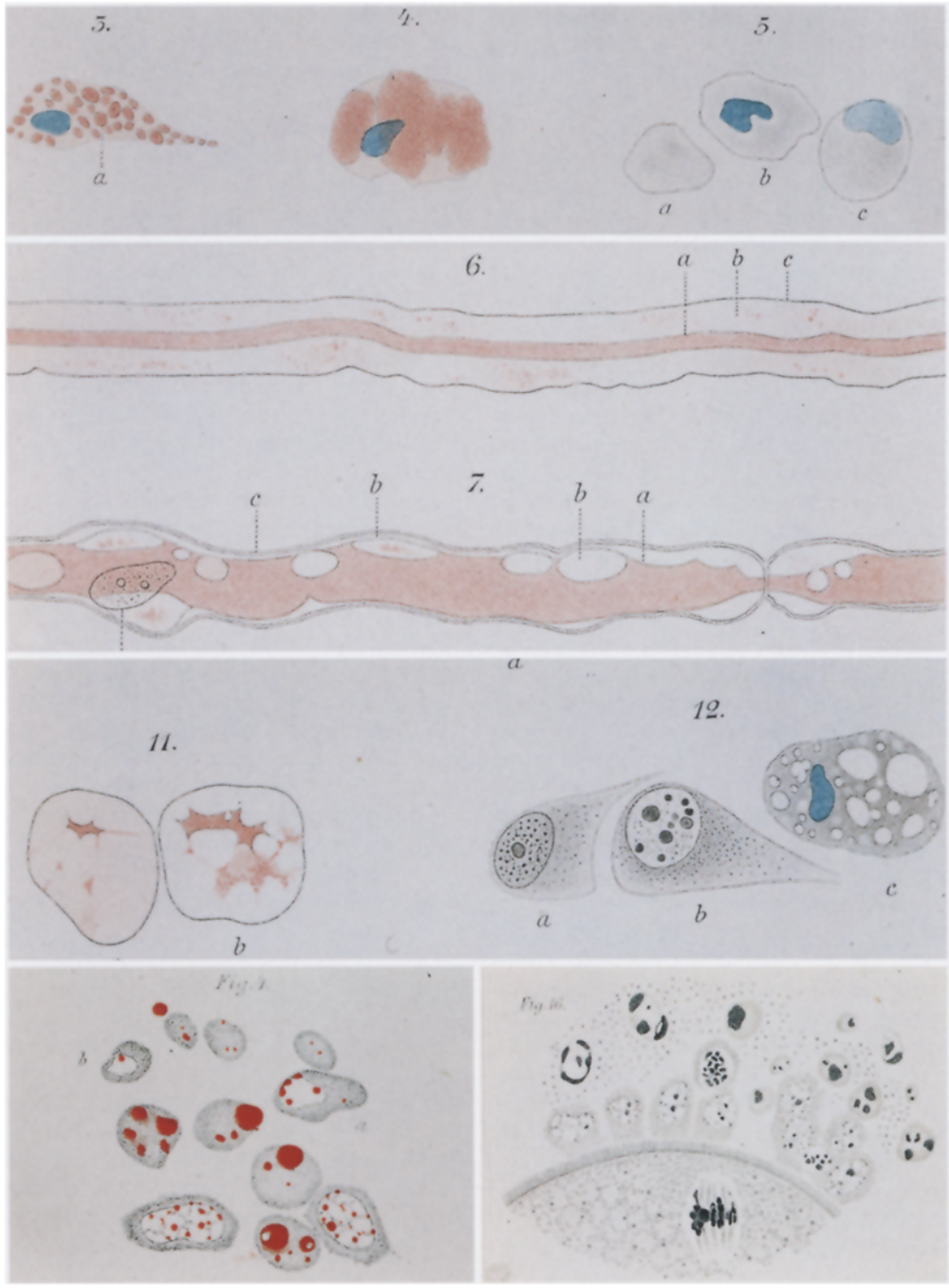

Fig. 3 *Top three frames* from metamorphic tadpoles of *Bufo variabilis* and *Rana esculanta* (Noetzel 1895). *Noetzel's 3–5* isolated cells stained with acid fuchsin (*red*) and hematoxylin-alum (*blue*) from shrunken notochord in sagittal celloidin sections through the tail, which is reduced to a third of its original length. The cell in *3* contains intensely red-stained grains and a diffusely blue nucleus. The cell in *4* contains a homogeneous, intensely red-stained mass in the more lightly stained cytoplasm. *Noetzel's 6, 7* normal nerve fibers in sagittal celloidin sections, stained as above, from tails not yet reduced (*6*) or reduced to half-length (*7*). In *6 a* axis cylinder (axon), *c* Schwann sheath, *b* space between them. In *7 a* swollen axis cylinder, *b* vacuoles in axis cylinder, *c* Schwann sheath with nucleus (*towards left*). *Noetzel's 11* transversely cut axons from a *Bufo* tail reduced to a third of its original length, in paraffin sections stained as above. *Noetzel's 12* hematoxylin-alum stained celloidin section through a spinal ganglion in a 2.5 mm tail-stump. *a* Unaltered ganglion cell, *b* ganglion cell with decay of the chromatin matrix in enlarged nuclei, *c* ganglion cell containing a very shrunken nucleus (*blue*) and riddled with numerous vacuoles. *Lower two frames* show safranin-stained sections from rabbit ovarian follicles (Flemming 1885). *Flemming's Fig. 4* epithelial cells, of which two are unchanged (those with largest nuclei), and the rest show chromatolysis, including cells *a* and *b* whose nuclei have not yet lost their outline. A lump of chromatin is visible in the extracellular space. *Flemming's Fig. 16* part of the ovule (containing a mitotic spindle), and adjacent epithelium, in which only some of the cells are drawn. Eight of these cells are unchanged, but the remainder show chromatolytic changes

trastructural studies have demonstrated the degeneration of myoblast-like cells in the deep muscles of the back in embryonic mice (Seinsch and Schweichel 1974) and of myotubes and satellite myofibers in quadriceps and other skeletal muscles of human fetuses (Webb 1972; Fidzianska and Goebel 1991). Light microscopic observations indicated cell death also in myotomes of human fetuses (Ilies 1970). The cause of the cell death is unknown, but one of the factors necessary for survival appears to be the receipt of innervation, since early destruction of motoneurons causes their target muscle to degenerate (Sohal and Holt 1980).

The earliest reports of this are those of Felix (1889), Krösing (1892) and Schaffer (1893), who identified dying cells, resembling the sarcolytes of Mayer and Barfurth, in the muscles of human and other mammalian fetuses. Felix (1889) and Bardeen (1900) both refer vaguely to supposedly similar observations in an earlier study on cow and pig embryos by Born (1873), but although Born does refer to the "Sarkoplasten" of Margo, and describes them, we can find no hint that he understood they were dying.

One must therefore credit Felix (1889) with the discovery of histogenetic cell death (in nonmetamorphic situations). Most of his observations were on accidentally aborted human fetuses. His discovery was by no means accidental, because he knew in advance of the reinterpretation of "sarkoplasts" as sarkolytes by Mayer and Barfurth. Moreover, in his introduction he refers to counts by Harting (1845) describing a postnatal reduction in the number of muscle fibers in human babies. One intriguing observation by Felix was that innervated muscle fibers were morphologically different from those that lacked innervation and that the myofibers that died were always of the latter category. His interpretation was that innervation provides "growth energy" that favors survival. This is compatible with modern theory based on the observation of muscle cell death after innervation has been prevented (Sohal and Holt 1980), but it is still uncertain whether the myocytes that die in normal development are those that have failed to be innervated. Felix did not refer to the available literature on trophic dependence (see section on Neurotrophic interactions) and appears to have been unaware of Weber's (1851) most relevant observation that muscle cells are absent in fetuses lacking the corresponding part of the spinal cord.

Neuronal death

Loss of entire populations

In metamorphosis, neurons are not spared. This fact was largely neglected in nineteenth century reports on insect metamorphosis, but was clearly stated in the context of amphibians, by Barfurth (1887b), Looss (1889) and Bataillon (1891), who were aware, for example, of the degeneration of the dorsal root ganglion cells innervating the tadpole tail.

The first clearly established case of neuronal death in the absence of metamorphosis involved what is now regarded as a special case. The cells in question were the Rohon-Beard neurons, a population of primary sensory neurons in developing lampreys, fish and amphibia, that is eliminated when the dorsal root ganglion cells take over its sensory function (Lamborghini 1987). These cells had been described by various early authors including Sigmund Freud (1877), but their neuronal death wish was uncovered by John Beard in 1889. Beard's discovery was in fact anticipated by Kleinenberg (1886), who suggested by analogy with similar cells in embryonic annelid worms that the Rohon-Beard neurons might be transient. Beard's key observations were published in an initial paper concerning mainly the development of teleosts (Beard 1889), followed by a more detailed account based on observations in a species of skate (Beard 1892). These initial reports were subsequently reviewed and supplemented in great detail (Beard 1895). Beard studied transverse sections with high- and low-power objectives. He reports that the cells shrink and "become glassy, having lost all traces of nucleus and nucleolus, and disappear" (Beard 1889). This description gives no hint of the autophagic mechanisms now known to be active in the destruction of the Rohon-Beard cells (Lamborghini et al. 1987). The notion that neurons could be transient was already implicit in earlier reports that various sensory ganglia associated with motor nerves regress (Table 4), notably the hypoglossal ganglion in selachian fish (Balfour 1878) and various mammals (Froriep 1882, 1885). However, these authors did not actually describe the cellular degeneration and did not refer to cell death.

Neuronal death as a general phenomenon

The phenomenon of *histogenetic* neuronal death is now recognized to be of much greater importance than the loss of entire neuronal populations. Indeed, it is one of the major factors shaping the nervous system in vertebrates and in at least some phyla of invertebrates. In vertebrates, 30–70% of most neuronal populations are eliminated when they are making and receiving their connec-

Table 4 Early reports of neuronal death in non-metamorphic development

Date	*Author*	*Tissue*	*Species*
1878	F Balfour[a]	Transient ganglion	Fish
1882, 85	A Froriep[a]	Transient ganglia	Mammals
1889, 92	J Beard	Rohon-Beard	Fish embryos
1895	Barfurth	Dorsal root ganglia	Mammals
1905	F Capobianco[b]	Spinal ganglia	Cat
1905	C Barbieri	Spinal cord	Amphibia
1906, 07	R Collin	Spinal cord + ganglia	Chick embryo

[a] Described regression of the ganglia but did not observe dying cells

[b] Wrongly interpreted the cell death as cell fusion with degeneration of the excess nuclei

tions. The main functions of this cell death appear to be the elimination of developmental errors and adjustment of the numbers of neurons to the size of their target territory (Clarke 1994).

The first documentation known to us of such neuronal death was by Bataillon (1891) in the context of metamorphosis. After describing the morphology of dying neurons in dorsal root ganglia, he writes: "One can see in the centers (encephalon, medulla, spinal cord) entire zones of elements showing the same pigmented degeneration... it is interesting to note that, *from one end to the other, the nervous centers are partially affected"* (our translation, his italics).

Histogenetic neuronal death in the absence of metamorphosis was not demonstrated convincingly until the reports of Collin in 1906–1907 that are discussed below. However, several previous authors came close to understanding its existence. Mayer (1881), Teuscher (1890) and Hammer (1895) all claimed to have observed degenerating nerve fibers in peripheral nerves of normal adult vertebrates, ranging from frogs to various mammals. They considered this to betoken a normal turnover. This is now considered not to occur and it seems likely that these authors were misled by the rapid post-mortem degeneration of axons that is now known to take place in the absence of rapid fixation.

Intriguingly, Mayer (1881; the same Mayer who later reinterpreted the "Sarkolyten") and Hammer (1895) also reported degenerating fibers in *young* animals; it is difficult to know whether they were observing a genuine phenomenon of degeneration in development, or whether they were merely being misled as in their observations on adults. Somewhat more convincingly, Barfurth, in his annual review of 1895 (p. 377), claims to have observed degenerating ganglion cells in the Gasserian and spinal ganglia of young mammals and states that he will report on this elsewhere. This inspires more confidence, because Barfurth clearly understood that he was dealing with a developmental phenomenon, and he observed the dying perikarya, not just their axons. However, we have been unable to find the promised report despite scanning the reference lists of all Barfurth's annual reviews as well as checking the rather comprehensive "Namenregister" of the *Jahresberichte der Anatomie und Entwicklungsgeschichte* for 1892–1901.

Thus, the first unequivocal descriptions of histogenetic neuronal death are those of Collin (1906, 1906–1907, 1907); they deal with the death of motoneurons in chick embryos, with brief mention also of neuronal death in the spinal ganglia. Collin reports the timing of the motoneuronal death (embryonic days 5–11, which is correct) and the fact that spinal cord *interneurons* are *not* subject to "necrobiosis," as has been confirmed only recently (McKay and Oppenheim 1991). Collin describes in precise detail the morphology of the dying neurons in iron-haematoxylin-stained sections, including the clumping of chromatin into a few dark balls (Fig. 4). He also deduces the ejection of some of these balls from the nucleus into the cytoplasm (as confirmed recently: Clarke and Hornung 1989) or their complete ejection from the cell and persistence in the extracellular space. Small, dark remnants do indeed sometimes occur in the extracellular space, but they are membrane bound "apoptotic bodies" (Kerr et al. 1972).

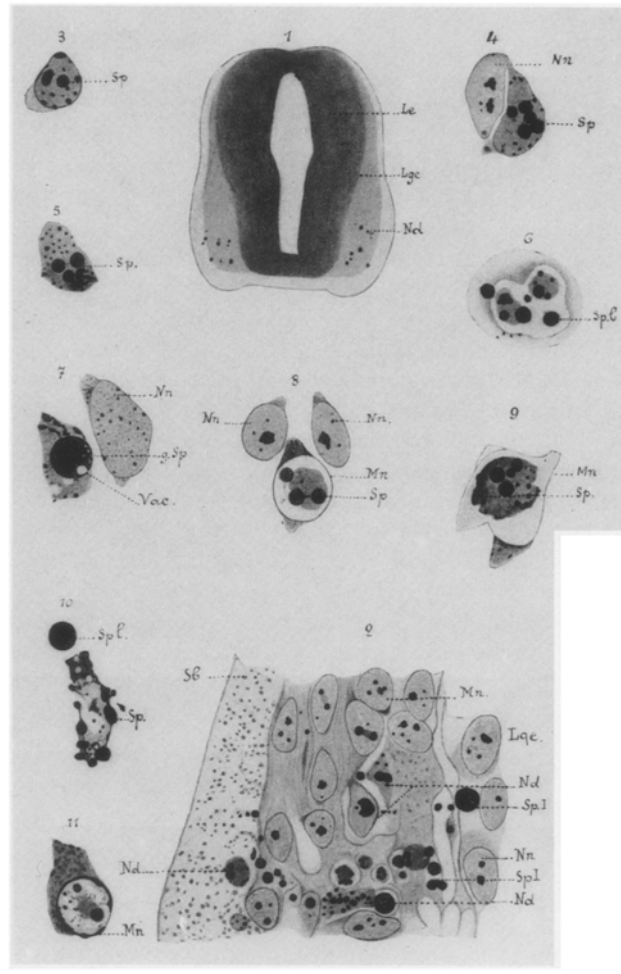

Fig. 4 Death of chick spinal motoneurons (from Collin 1906–1907; also in Collin 1907). *1* Embryo of 102 h; note the localization of degenerating neuroblasts (*Nd*) to the ventral horn. *2* From transverse section at 143 h. *3–11* Details of chromatolysis, with maximal magnification (*Le* ependymal plate, *Lge* external grey plate, *Nd* degenerating neuroblast, *Nn* normal neuroblast, *Sb* white matter, *Mn* nuclear membrane, *Sp* intranuclear chromatic balls, *Spl* free chromatic balls, *vac* vacuole; the terminology is Collin's, translated)

Hamburger (1992) dismisses the work of Collin as being "of no historical interest" because "he did not recognize its significance." Hamburger refers only to Collin's full paper (1906–1907), pointing out that only one brief chapter of it was devoted to neuronal death. However, he fails to draw attention to several indices showing that Collin did at least glimpse the significance of his observations. First, it was precisely the subject of neuronal death that Collin singled out for his presentation at a meeting (held in his own University, of Nancy) of the Société biologique de Paris (Collin 1906). Second, Collin realized the numerical importance of the phenome-

non, because he mentions the report of Capobianco (1905) that the spinal ganglia of the cat foetus contain 3 times as many neuroblasts as at birth. Capobianco, influenced by the reticular (syncytial) theory, believed that the reduction in number was due to cellular fusion, followed by degeneration of the excess nuclei. Collin attributes the threefold reduction to neuronal death. Third, Collin raises the possibility that his observations may betoken a general phenomenon characteristic of all vertebrates. In this context, he cites unpublished observations of his supervisor, Prof. A. Nicolas, concerning degeneration in the spinal ganglia of rabbit embryos and emphasizes the distinction between the new observations on higher vertebrates and earlier reports restricted to neuronal death in metamorphosing species, in which context he cites Barbieri (1905). Collin even argues that overproduction of cells followed by degeneration of the excess is a characteristic of all tissues (Collin 1906–1907, page 202), although he cites hardly any of the nineteenth century literature on cell death.

Yet Collin never again worked on neuronal death, devoting most of his career to neuroendocrinology. Near the end of his career, he published a monograph on the nervous system containing more than 80 pages devoted to its development, but with no mention of neuronal death (Collin 1944).

Cellular interdependence and competition in relation to cell death

It is now generally recognized that developing cells depend for their survival on signals arising from other cells and that competition for survival signals is a major cause of cell death (Raff 1992; Clarke 1994). The molecules that mediate the survival signals are sometimes called "trophic," especially in the context of the nervous system. Ironically, the notions of *trophic interaction* and *intercellular competition* were current in the nineteenth century, but they were never to our knowledge discussed in relation to the wealth of existing knowledge concerning cell death.

Neurotrophic interactions

The terms *trophic* or *neurotrophic* indicate the long-term influence of an axon on its target cell or vice versa, and these trophic interactions were a major object of study in the nineteenth century. We therefore focus on them, despite occasional early reports indicating other survival-mediating cellular interactions, as for example, a report of "fatty cell degeneration" occurring during atrophy of the prostate gland following castration (Athanasow 1898). At that time, many authors in fact attributed such prostatic atrophy (wrongly) to trophic (i.e., neural) dependence (reviewed in Athanasow 1898).

The Greek *trophé* means "nourishment," although trophic interactions are now known to be much more specific than their derivation suggests, involving the binding of various trophic molecules to specific receptors on the pre- or postsynaptic membrane (Clarke 1991). The word "trophic" was in fact first used in a different sense, by Waller in 1851, to describe the dependence of the axon on its parent cell body (Jacobson 1991), but it soon began to be used in the present sense of intercellular influence.

Although trophic influences are now known to be bidirectional (anterograde and retrograde), nineteenth century usage reserved the term for anterograde effects. In fact, the nutritional influence of nerves on their target tissues was recognized already at the end of the eighteenth century and was distinguished from their roles in sensation and movement (for review: Jacobson 1993). Throughout the middle of the nineteenth century, there was widespread belief in a distinct class of "trophic nerves" containing fibers with special trophic functions (e.g., Samuel 1860). All organs were believed to be maintained by special nutritional factors secreted by these nerves, and Jean-Martin Charcot (e.g., 1877) put his considerable authority and hundreds of pages of print behind the view that disruption of the trophic function of these nerves was responsible for various peripheral problems, such as ulcers, in patients with central nervous pathology. However, the views of Charcot were disputed, notably by Brown-Séquard (for review: Levine 1992), and it was subsequently shown that the autonomic nervous system, in which these fibers were supposed to run, could be removed without producing the disturbances described by Charcot. Following this, and the discovery of hormones as an alternative class of messengers, the idea of trophic nerves fell into disrepute and is now largely forgotten, although Wyburn-Mason (1950) devoted more than 1000 pages to defending it.

While the concept of *special* trophic nerves is abandoned, the nineteenth century also produced a wealth of evidence for the current notion that *all* neurons exert trophic influences, on which the survival and differentiation of their target cells depend. Examples include the dependence of limb regeneration on its innervating nerve in newts (Todd 1823), the absence of muscle cells in mammalian fetuses lacking the corresponding part of the spinal cord (Weber 1851), the morphological dependence of the vertebrate taste bud on its sensory innervation (Vintschgau and Honigschmied 1876), and the dependence of central neurons on their afferents in birds and mammals, especially when young (Panizza 1855; Baginsky 1886). All these claims are corroborated by modern evidence (Sohal and Holt 1980; Oppenheim 1981; Clarke 1991). As mentioned above, Barfurth (1887b) believed wrongly that tissue regression during metamorphosis was due to changes in trophic influence.

Although nineteenth century usage restricted the term "trophic" to anterograde effects, the equivalent retrograde phenomenon was also demonstrated. Thus, in mammals, axotomy was known to cause the shrinkage and/or degeneration of the upstream neuron, especially at younger ages. This in itself would not have been proof of

a retrograde trophic interaction between cells, because it could have been attributed to the direct damage caused by the axotomy. However, conclusive evidence was available from the demonstration by von Gudden (1870, 1880) that a lesion of the limbic cortex led to degeneration not only in the anterior nucleus of the thalamus (which projects directly to the limbic cortex), but secondarily in the medial mammillary nucleus (which projects to the anterior thalamic nucleus but not to the cortex), and then still further upstream in the ventral tegmental nucleus. Similar results were soon obtained by others in various neural systems; for example, retrograde transneuronal degeneration of retinal ganglion cells was described following lesions of the visual cortex (Ganser 1882; von Monakow 1889).

Competition

It is now known that neurons compete for the molecules mediating the above survival signals (Clarke 1994). The notion of competition was uppermost in many minds in the last half of the nineteenth century owing to the publication of Darwin's *The Origin of Species* in 1859. Only 10 years later, T.H. Huxley (1869) affirmed that the "molecules" within each organism "are competing with each other for opportunity to exist and multiply." Similarly, Lewes (1877) argues that just as competition between populations of organisms is inevitable, since they would otherwise multiply to such an extent that their food requirements could not be met, so competition must occur between the tissues and organs within a given organism. However, Lewes did not discuss development and did not extend his argument to the cellular level. Then Wilhelm Roux published an entire book devoted to this theme (Roux 1881), including a section devoted to "The Battle of the Cells." Roux argued that the cells with the greatest "irritability" would be the most successful in obtaining trophic support, but although he refers extensively to the literature concerning trophic interactions and takes for granted the notion that cells which lose their battle will die, he cites no direct evidence that they

Table 5 Citations in nineteenth century papers reporting naturally occurring cell death (*0* not cited, *1* vague reference without explicit citation, or citation of another relevant publication by same author, *2* precise citation but little discussion, *3* substantial discussion)

Cited work	Insect metamorphosis									Amphibian metamorphosis							Other			
	Weismann (1864a, b)	Ganin (1869)	Lowne (1870)	Ganin (1875)	Chun (1876)	Helm (1876)	Viallanes (1882)	Kowalevsky (1887)	Van Rees (1888–1889)	Vogt (1842)	Goette (1875)	Metschnikoff (1883)	Mayer (1886)	Barfurth (1887b)	Looss (1889)	Bataillon (1891)	Stieda (1872)	Flemming (1885)	Beard (1889, 1892, 1895)	Felix (1889)
Citing work																				
Insect metamorphosis																				
Lowne (1870)	3	0	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–
Chun (1876)	3	0	0	–	–	–	–	–	–	0	0	–	–	–	–	–	0	–	–	–
Helm (1876)	2	0	0	0	1	–	–	–	–	0	0	–	–	–	–	–	0	–	–	–
Viallanes (1882)	3	0	0	3	3	0	–	–	–	0	0	–	–	–	–	–	0	–	–	–
Kowalevsky (1887)	3	0	0	2	0	0	3	–	–	0	0	3	0	3	–	–	0	0	–	–
Van Rees (1888–1889)	3	0	0	3	0	0	3	1	–	0	0	0	0	0	–	–	0	0	–	–
Noetzel (1898)	2	0	0	0	0	0	0	3	3	0	0	2	0	2	2	2	0	0	0	0
De Bruyne (1898)	3	3	0	0	0	0	3	3	3	0	0	3	3	3	3	3	0	0	0	0
Amphibian metamorphosis																				
Goette (1875)	0	0	0	–	–	–	–	–	–	3	–	–	–	–	–	–	0	–	–	–
Metschnikoff (1883)	0	0	0	0	0	0	0	–	–	0	0	–	–	–	–	–	0	–	–	–
Mayer (1886)	0	0	0	0	0	0	0	–	–	0	0	0	–	–	–	–	0	0	–	–
Barfurth (1887b)	0	0	0	0	0	0	0	–	–	0	2	3	3	–	–	–	0	1	–	–
Looss (1889)	2	0	0	2	1	2	2	1	0	0	2	3	2	3	–	–	0	0	–	–
Bataillon (1891)	1	1	0	1	0	0	2	2	0	0	3	3	3	3	3	–	0	0	–	–
Noetzel (1895)	0	0	0	0	0	0	0	0	0	0	3	3	2	3	3	3	0	0	0	0
Other																				
Stieda (1872)	0	0	0	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–
Flemming (1885)	0	0	0	0	0	0	0	–	–	0	0	0	–	–	–	–	0	–	–	–
Beard (1889, 1892, 1895)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	–	0	0	0	–
Felix (1889)	0	0	0	0	0	0	0	2	0	0	0	0	3	3	–	–	0	0	0	–
Brachet (1893)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Schaffer (1893)	2	0	0	0	0	0	0	3	0	0	0	3	3	3	3	3	0	0	0	3
Bardeen (1900)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	3


● 1842-1875
■ 1876-1890
▲ 1891-1906
Stieda 1872 ● Dorpat
Beard 1889 ■ Edinburgh
GREAT BRITAIN
Flemming 1885 ■ Kiel
Lowne 1870 ● London
GERMANY
Ganin 1869 ● Warsaw
RUSSIA
Helm 1868 ● Leipzig
Barfurth 1887 ■ Bonn
Chun 1876 ■ Frankfurt
■ Prague
Mayer 1886
Felix 1889 ■ Würzburg
Metchnikoff 1892 ▲ Paris
▲ Nantes
Collin 1906
Goette 1875 Strassburg ●
Weismann 1864 Freiburg ●
■ Tübingen
Froriep 1882
Schaffer 1893 ▲ Vienna
FRANCE
Strelzoff 1873 ● Zürich
Vogt 1842 ● Neuchâtel
Metschnikoff 1883 ■ Odessa
Kowalevsky 1885 †
AUSTRIA-HUNGARY
Bataillon 1891 ▲ Lyon
Barbieri 1905 ▲ Bologna
ITALY
Capobianco 1905 ▲ Naples

actually do so and fails to discuss development. This may be because in 1881 the available literature on cell death in development was almost exclusively devoted to metamorphosis. Eleven years after Roux, Metchnikoff (1892c) wrote a semi-popular article on "The struggle for existence between the different parts of the organism," replacing the notion of competition by the attack by macrophages against weakened cells of other kinds. However, current opinion favors competition as the main mechanism.

Overview

Who influenced whom?

From a historical point of view, it is interesting to know not merely who made which observation, but to trace the influences that prepared minds and hands for discovery.

One line of evidence on this point is the acknowledgement by the authors themselves of the previous publications to which they were indebted, as expressed by citation (Table 5). Several points emerge from examining this table. First, Vogt's (1842) description of cell death was ignored by everybody except Goette (1875), apart from a brief mention by Prévost and Lebert (1842; not shown in Table 5). Second, Weismann's (1864a,b) detailed analysis of histolysis during insect metamorphosis was cited in all the listed publications on this subject, being reviewed at length in most cases, and was referred to in a few other papers as well. However, neither of these authors was cited by Stieda (1872), Flemming (1885), Metschnikoff (1883), Mayer (1886) or Beard (1889, 1892, 1895), and none of the latter cited each other. It would therefore seem that naturally occurring cell death was "discovered" on at least seven different occasions – in fact more, since Table 5 is very limited, ignoring several additional authors who "discovered" cell death without acknowledging previous influence.

The causes of this apparent independence of discoveries of cell death are unknown but are probably multiple. Linguistic difficulties cannot have played a major role, because German served as lingua franca among histologists. Six of the above seven "independent" discoveries were published in German, only the last (Beard 1889, 1892, 1895) being in another language (English). Moreover, even Beard understood German, and contributed to meetings of the German Anatomical Society. Subject boundaries certainly played a greater role in the "independent" citing pattern. Weismann (1864a,b) was cited by all those who worked on insects, and although Mayer's (1886) short report on amphibians fails to cite Goette (1875) and Metschnikoff (1883), subsequent papers on amphibian metamorphosis reliably cite both as well as Mayer. Stieda (1872) was reliably cited by subsequent students of bone development, as was Flemming (1885) by subsequent students of follicular atresia. That is not, however, to imply that rigid subject boundaries isolated the authors, most of whom had wide-ranging interests. For example, between 1892 and 1901, Beard published at least 30 papers, of which only seven concerned the Rohon-Beard cells, others dealing with at least 11 different subjects including "A Supposed Law of Metazoan Development," the pronephros of *Lepidosteus osseus*, reproduction in animals and plants, the yolk sac, the span of gestation, the functions of the thymus, and the morphological continuity of germ cells. Most of the seven authors in question were similarly eclectic. Physical remoteness appears also to have contributed, because although many of the very early reports stemmed from southern Germany (Fig. 5), the seven in question were from widely scattered locations (Neuchâtel, Freiburg, Dorpat, Kiel, Odessa, Prague and Edinburgh), of which only Freiburg is in southern Germany.

The failure of most authors (except Goette) to cite Vogt (1842) may be a special case. Early authors such as Weismann probably failed to notice Vogt's description of cell death, because it was buried in a 130-page monograph devoted mainly to other aspects of toad development. Later authors sometimes referred to Goette (1875) for a review of the early literature and thereby cited Vogt indirectly.

However, while several of the above factors probably contributed to the "citation independence," we think an additional cause is to be found in the way cell death was considered before the mid-1880s.

How cell death was viewed in the nineteenth century

We have emphasized in this review that cell death was studied throughout much of the nineteenth century, but that should not be taken to imply a uniform level of interest in it from Vogt's (1842) publication onwards. Neither Vogt nor his contemporaries attached great importance to his description of cell death, which is understandable at a time so soon after the establishment of the cell theory, and it was not until the years following Weismann's (1864a,b; 1866) publications that "histolysis" began to attract significant interest. However, for the next 20 years this interest was largely focused on insect meta-

Fig. 5 Locations where naturally occurring cell death was investigated. The dates refer to the first relevant paper published by a given author as a result of research done at the city indicated. The criteria for selecting authors is the importance of their research on cell death, but we have been obliged to exclude van Rees (1888–1889; Freiburg) for lack of space, and Looss and Viallanes because their cities are not specified in their papers. However, from remarks in Viallanes's paper it appears that his research was initiated in Würtzburg under the direction of P. Storr and A. von Koelliker, before being continued at an unspecified location – possibly Paris. It is clear that most of the research was done in southern Germany and in the adjacent territory. The borders shown are valid for the period 1871–1914. The (then) German-speaking Russian town of Dorpat (now Tartu, in Latvia) is shown 50 miles south of its true location in order to bring it onto the map. Although Barfurth published his 1887 paper from Bonn, he moved shortly afterwards to Dorpat, from where he published several of his annual reviews before moving again to Rostock in 1896

morphosis. In research on vertebrates, the situation was different. For example, although Stieda (1872) clearly described cell death in bone, this was only one aspect of what he had to report, and he did not single it out as his main discovery. Even Metschnikoff (1883) was not primarily interested in cell death, but in phagocytosis. In short, outside the field of insect research, cell death was not generally recognized as an important phenomenon in its own right until the mid-1880s. This may explain the failure of Stieda, Metschnikoff and others to acknowledge previous descriptions of cell death.

A change came with Mayer's (1886, 1887) reinterpretation of the "Sarkoplasten" of Margo and Paneth as "Sarkolyten." These entities had attracted wide interest precisely because they were thought *not* to be dying, but proliferating. The understanding that the well-known "Sarkoplasten" were in fact dying cells helped to bring research on cell death to the center of interest, where it remained throughout the last 15 years of the century.

Nothing new under the sun

It is striking how many topics currently under investigation were already being studied in the nineteenth century. In most cases, the original findings were forgotten, only to be rediscovered with better tools about 80 years later.

For example, the well-known morphological distinction between *apoptosis* and *necrosis* has roots going back long before the definition of these terms by Kerr et al. in 1972. Apoptosis was identified as a distinct type in the nineteenth century. The term "chromatolysis" was used exclusively in situations where apoptosis is now considered to occur, and its morphological description matches apoptosis (Flemming 1885; Nissen 1886; Heidenhain 1890; Schottlaender 1893; Henneguy 1894). Moreover, the histology textbook of Prenant et al. (1904) devotes a whole chapter to summarizing nineteenth century views on "cell degeneration and death" and makes the point that some morphologies of cell death occurring in pathology resemble those occurring naturally, whereas others do not. This is close to the view of Kerr et al. (1972), who held that cell death in severe pathology is necrotic, whereas mild pathological cell death is apoptotic, resembling natural cell death.

Modern contrary claims that several types of cell death occur even in normal development (e.g., Clarke 1990) were likewise preceded by various nineteenth century morphological typologies (Viallanes 1882; Demarbaix 1889; Schaffer 1893; Noetzel 1895). The drawings (Fig. 3) of Noetzel (1895) anticipate the classification of neuronal death as "nuclear type" (i.e., apoptosis) or "cytoplasmic type," and Metchnikoff's (1892a) description of muscle-derived cells phagocytozing their own debris anticipates the "autophagic" kind of cell death (for review, see Clarke 1990).

As mentioned above, another nineteenth century topic that has been revived in recent decades is that of neurotrophic influences on cell survival. Yet another is the debate over whether phagocytes mediate cell death or merely clear away the debris. Despite long acceptance of the latter view, there is recent evidence that macrophages are necessary for cell death to occur in some situations (Lang and Bishop 1993).

Where next will swing the slow pendulum of scientific attention, restoring to daylight the forgotten insights of Weismann's contemporaries? It warns us that today's novelty has ancient roots and that roots are destined for burial.

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References

Athanasow P (1898) Recherches histologiques sur l'atrophie de la prostate consécutive à la castration à la vasectomie et à l'injection sclérogène. J Anat Physiol (Paris) 34:137–186

Atkinson BG (1981) Biological basis of tissue regression and synthesis. In: Gilbert LI, Frieden E (eds) Metamorphosis, 2nd edn. Plenum Press, New York, pp 397–444

Baer KE von (1828) Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion. Bornträger, Königsberg

Baginsky B (1886) Über den Ursprung und den centralen Verlauf des Nervus acusticus des Kaninchens. Virchows Arch Pathol Anat 105:28–46

Balfour FM (1878) A monograph on the development of elasmobranch fishes. Macmillan, London

Barbieri C (1905) Ricerche sullo sviluppo del midollo spinale negli Anfibi. Arch Zool Ital 2:79–105

Barclay AE, Franklin KJ, Prichard MML (1944) The foetal circulation and cardiovascular system and the changes they undergo at birth. Blackwell, Oxford

Bardeen CR (1900) The development of the musculature of the body wall in the pig, including its histogenesis and its relations to the myotomes and to the skeletal and nervous apparatus. Johns Hopkins Hosp Rep 9:367–399

Barfurth D (1887a) Der Hunger als förderndes Princip in der Natur. Arch Mikrosk Anat 29:28–34

Barfurth D (1887b) Die Rückbildung des Froschlarvenschwanzes und die sogenannten Sarcoplasten. Arch Mikrosk Anat 29:35–60

Barfurth D (1893–1913) Regeneration und Involution. Ergeb Anat Entwicklungsgesch 3:132–209, 4:458–498, 5:329–438, 6:392–438, 7:486–529, 8:626–694, 9:327–414, 10:547–598, 11:517–582, 12:444–544, 13:368–487, 14:451–602, 15: 410–529, 16:323–451, 17:337–430, 18:288–352, 19: 1035–1105, 20:356–469, 20:1086–1172, 21:162–243

Bataillon E (1889) Recherches expérimentales sur la métamorphose des Anoures. CR Acad Sci 109:682–684

Bataillon E (1891) Recherches anatomiques et expérimentales sur la métamorphose des Amphibiens anoures. Ann Univ Lyon 2: 1–123

Bataillon E (1892) A propos du dernier travail de M. Metschnikoff: sur l'atrophie des muscles pendant la transformation des Batraciens. CR Soc Biol (Paris) 4:185–187

Beard J (1889) On the early development of *Lepidosteus osseus* – preliminary notice. Proc R Soc Lond 46:108–118

Beard J (1892) The transient ganglion cells and their nerves in Raja batis. Anat Anz 7:191–206

Beard J (1895) The history of a transient nervous apparatus in certain ichthyopsida. Zool Jahrb Abt Anat Ontog Thiere 9: 319–416

Beaulaton J, Lockshin RA (1977) Ultrastructural study of the normal degeneration of the intersegmental muscles of *Antheraea*

polyphemus and *Manduca sexta* (Insecta, Lepidoptera) with particular reference to autophagy. J Morphol 154:39–58
Beneden E van, Bambeke C van (1880) Contribution à la connaissance de l'ovaire du *vespertilo murinus* et du *rhinolophus ferrum-equinum*. Arch Biol 1:475–550
Born G (1873) Beiträge zur Entwicklungsgeschichte der quergestreiften willkürlichen Muskeln der Säugethiere. Inaugural dissertation, Friedrich-Wilhelms-Universität, Berlin
Bowen ID, Bowen SM (1990) Programmed cell death in tumours and tissues. Chapman & Hall, London New York
Bracegirdle B (1978) A history of microtechnique. Heinemann, London
Brachet A (1893) Etude sur la résorption du cartilage et le développement des os longs chez les oiseaux. Int Monatschr Anat Physiol 10:391–417
Capobianco F (1905) Recherches ultérieures sur la genèse des cellules nerveuses. Arch Ital Biol 44:187–200
Charcot JM (1877) Leçons sur les maladies du système nerveux faites à la Salpétrière, 3rd edn vol 1, and 2nd edn vol 2. Delahaye, Paris
Chun C (1876) Ueber den Bau, die Entwickelung und physiologische Bedeutung der Rectaldrüsen bei den Insekten. Abh Senckenb Naturforsch Ges 10:27–52
Churchill FB (1991) Regeneration, 1885–1901. In: Dinsmore CE (ed) A history of regeneration research. Cambridge University Press, Cambridge, pp 113–131
Clarke PGH (1990) Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol 181:195–213
Clarke PGH (1991) The roles of trophic communication in biological neural networks. Concepts Neurosci 2:201–219
Clarke PGH (1994) Neuronal death in the development of the vertebrate central nervous system. Semin Neurosci 6:291–297
Clarke PGH, Hornung J-P (1989) Changes in the nuclei of dying neurons as studied with thymidine autoradiography. J Comp Neurol 283:438–449
Clarke E, O'Malley CD (1968) The human brain and spinal cord. University of California Press, Berkeley Los Angeles
Clarke S, Clarke PGH (1994) Programmed cell death: a Swiss discovery. Experientia 50:A1
Clarke PGH, Clarke S (1995a) Nineteenth century research on cell death in development. J Physiol 487:205P
Clarke PGH, Clarke S (1995b) Historic apoptosis. Nature 378:230
Collin R (1906) Histolyse de certains neuroblastes au cours du développement du tube nerveux chez le poulet. CR Soc Biol (Paris) 60:1080–1081
Collin R (1906–07) Recherches cytologiques sur le développement de la cellule nerveuse. Névraxe 8:181–309
Collin RG (1907) Recherches cytologiques sur le développement de la cellule nerveuse. Thèse pour obtenir le grade de Docteur en Médecine, Université de Nancy, Nancy
Collin R (1944) L'organisation nerveuse. Michel, Paris
Demarbaix H (1889) Division et dégénérescence des cellules géantes de la moelle des os. La Cellule 5:27–57
De Bruyne C (1898) Recherches au sujet de l'intervention de la Phagocytose dans le développement des Invertébrés. Arch Biol 15:181–300
Dugès A (1835) Recherches sur l'ostéologie et la myologie des batraciens à leurs différens ages. In: Mémoires présentés par divers savans à l'Académie royale des Sciences le l'Institut de France; sciences mathématiques et physiques 6:1–216
Eberth CJ (1866) Zur Entwicklung der Gewebe im Schwanze der Froschlarven. Arch Mikrosk Anat 2:490–503
Ernst M (1926) Ueber Untergang von Zellen während der normalen Entwicklung bei Wirbeltieren. Z Anat Entwicklungsgesch 79:228–262
Felix W (1889) Ueber Wachsthum der quergestreiften Muskulatur nach Beobachtungen am Menschen. Z Wiss Zool 48:224–259
Fidzianska A, Goebel HH (1991) Human ontogenesis 3. Cell death in fetal muscle. Acta Neuropathol 81:572–577
Fischer JL, Smith J (1984) French embryology and the "mechanics of development" from 1887 to 1910: L. Chabry, Y. Delage & E. Bataillon. Pubbl Stn Zoll Napoli [II] 6:25–39
Flemming W (1885) Ueber die Bildung von Richtungsfiguren in Säugethiereiern beim Untergang Graaf'scher Follikel. Arch Anat Physiol (Jahrgang 1885):221–244
Fox H (1983) Amphibian morphogenesis. Humana Press, Clifton
Freud S (1877) Über den Ursprung der hinteren Nervenwurzeln im Rückenmark von Ammocoetes (Petromyzon Planeri). Sitzungsber Akad Wiss Math-Naturwiss Klasse 75, II Abt:15–27
Froriep A (1882) Ueber ein Ganglion des Hypoglossus und Wirbelanlagen in der Occipitalregion. Arch Anat Physiol Anat Abt:279–302
Froriep A (1885) Ueber Anlagen von Sinnesorganen am Facialis, Glossopharyngeus und Vagus, über die genetische Stellung des Vagus zum Hypoglossus, und über die Herkunft der Zungenmusculatur. Arch Anat Physiol Anat Abt:1–55
Ganin M (1869) Beiträge zur Erkenntniss der Entwickelungsgeschichte bei den Insecten. Z Wiss Zool 19:381–451
Ganin M (1875) Observations on the post-embryonic development of insects (in Russian). Warsaw. Reviewed, and extensive passages translated, by Viallanes (1882) and van Rees (1888–89)
Ganin MS (1877) Verbal contribution summarized in pages 386–389 and 412–417 of meeting report by Hoyer: Protocolle der Sitzungen der Section für Zoologie und vergleichende Anatomie der V. Versammlung russischer Naturforscher und Aertzte in Warschau im September 1876. Z Wiss Zool 28: 385–418
Ganser S (1882) Über die periphere und zentrale Anordnung der Sehnervenfasern und über das Corpus bigeminum anterius. Arch Psychiatr Nervenkr 13:341–381
Glücksmann A (1930) Ueber die Bedeutung von Zellvorgängen für die Formbildung epithelialer Organe. Z Anat Entwicklungsgesch 93:35–92
Glücksmann A (1951) Cell deaths in normal vertebrate ontogeny. Biol Rev Camb Philos Soc 26:59–86
Goette A (1869) Untersuchungen über die Entwickelung des bombinator igneus. Arch Mikrosk Anat 5:90–125
Goette A (1875) Die Entwicklungsgeschichte der Unke (Bombinator igneus). Voss, Leipzig
Gräper L (1914) Eine neue Anschauung über physiologische Zellausschaltung. Arch Zellforsch 12:373–394
Gudden B von (1870) Experimentaluntersuchungen über das peripherische und centrale Nervensystem. Arch Psychiatr Nervenkr 2:693–723
Gudden B von (1880) Beitrag zur Kenntniss des Corpus mammillare und der sogenannten Schenkel des Fornix. Arch Psychiatr Nervenkr 11:428–452
Haller A de (1758) Sur la formation du coeur dans le poulet. Bousquet, Lausanne, 2 vols
Hamburger V (1992) History of the discovery of neuronal death in embryos. J Neurobiol 23:1116–1123
Hammer (1895) Ueber Degeneration im normalen peripheren Nerven. Arch Mikrosk Anat 45:145–157
Harting (1845) Recherches micrométriques. Utrecht. Cited in Felix (1889)
Harvey W (1628) Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus. Francofurti, Sumptibus Gulielmi Fitzeri
Heidenhain M (1890) Beiträge zur Kenntnis der Topographie und Histologie der Kloake und ihrer drüsigen Adnexa bei den einheimischen Tritonen. Arch Mikrosk Anat 35:173–274
Helm FE (1876) Ueber die Spinndrüsen der Lepidopteren. Z Wiss Zool 26:434–469
Henneguy L-F (1894) Recherches sur l'atrésie des follicules de Graaf chez les mammifères et quelques autres vertébrés. J Anat Physiol (Paris) 30:1–39
Hinchliffe JR, Johnson DR (1980) The development of the vertebrate limb. Oxford University Press, Oxford New York
Hughes FM Jr, Gorospe WC (1991) Biochemical identification of apoptosis (programmed cell death) in granulosa cells: evidence for a potential mechanism underlying follicular atresia. Endocrinology 129:2415–2422
Huxley TH (1869) The genealogy of animals. In: Critiques and addresses of T.H. Huxley (1873). Macmillan, London. Cited in Oppenheim (1981)

Ilies A (1970) La topographie et la dynamique des zones nécrotiques normales chez l'embryon humain de 11–30 mm CR. IV. Les zones nécrotiques des arcs branchiaux, des extrémités, et des ébauches musculaires. Etude histologique et histochimique. Rev Roum Embryol Cytol Ser Embryol 7:117–130

Jacobson M (1991) Developmental neurobiology, 3rd edn. Plenum, New York London

Jacobson M (1993) Foundations of neuroscience. Plenum, New York London

Kastschenko N (1881) Ueber die Genese und Architectur der Batrachierknochen. Arch Mikrosk Anat 19:1–52

Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257

Kleinenberg N (1886) Die Entstehung des Annelids aus der Larve von Lopadorhynchus. Z Wiss Zool 44:1–227. Cited in Beard 1895

Kowalevsky A (1885) Beiträge zur nachembryonalen Entwicklung der Musciden. Zool Anz 8:98–103, 123–128, 153–157

Kowalevsky A (1887) Beiträge zur Kenntnis der nachembryonalen Entwicklung der Musciden. Z Wiss Zool 45:542–594

Krösing R (1892) Ueber die Rückbildung und Entwickelung der quergestreiften Muskelfasern. Virchow's Arch Pathol Anat 128:445–484

Künckel J (1868) Recherches sur l'organisation et le développement des Diptères du genre Volucelle. CR Acad Sci (Paris) 68: 1231–1234

Kupffer C (1870) Die Stammverwandtschaft zwischen Ascidien und Wirbelthieren. Arch Mikrosk Anat 6:115–172

Lamborghini JE (1987) Disappearance of Rohon-Beard neurons from the spinal cord of larval *Xenopus laevis*. J Comp Neurol 264:47–55

Lang RA, Bishop JM (1993) Macrophages are required for cell death and tissue remodeling in the developing mouse eye. Cell 74:453–462

Leser E (1888) Ueber histologische Vorgänge an der Ossificationsgrenze, mit besonderer Berücksichtigung des Verhaltens der Knorpelzellen. Arch Mikrosk Anat 32:213–222

Levine JM (1992) Historical perspective: the neurotrophic theory of skin ulceration. J Am Geriatr Soc 40:1281–1283

Lewes GH (1877) The physical basis of mind. Trübner, London

Locke M (1981) Cell structure during insect metamorphosis. In: Gilbert LI, Frieden E (eds) Metamorphosis, 2nd edn. Plenum, New York

Looss A (1889) Ueber Degenerations-Erscheinungen im Thierreich, besonders über die Reduction des Froschlarvenschwanzes und die im Verlaufe derselben auftretenden histolytischen Processe. Preisschr de Fürstl Jablonowski'schen Ges, Leipzig

Lowne BT (1870) The anatomy and physiology of the blow-fly. Voorst, London

Majno G, Joris I (1995) Apoptosis, oncosis and necrosis: an overview of cell death. Am J Pathol 146:3–15

Margo T (1862) Neue Untersuchungen über die Entwickelung, das Wachsthum, die Neubildung und den feineren Bau der Muskelfasern. Denkschr Akad Wiss 20, II Abt:1–74

Mayer S (1881) Über Vorgänge der Degeneration und Regeneration im unversehrten peripheren Nervensystem. Z Heilkunde 2 (Cited in Rohde 1898)

Mayer S (1886) Die sogenannten Sarkoplasten. Anat Anz 1: 231–235

Mayer S (1887) Einige Bemerkungen zur Lehre von der Rückbildung quergestreifter Muskelfasern. Z Heilkunde 8:177–190

Mayr E (1982) The growth of biological thought. Belknap Press, Cambridge, Mass

McKay SE, Oppenheim RW (1991) Lack of evidence for cell death among avian spinal cord interneurons during normal development and following removal of targets and afferents. J Neurobiol 22:721–733 and 883–885

Metschnikoff E (1883) Untersuchungen über die mesodermalen Phagocyten einiger Wirbeltiere. Biol Zentralbl 3:560–565

Metchnikoff E (1892a) La phagocytose musculaire. I. Atrophie des muscles pendant la transformation des batraciens. Ann Inst Pasteur 6:1–12

Metchnikoff E (1892b) Reponse à la critique de M. Bataillon au sujet de l'atrophie musculaire chez les têtards. CR Soc Biol (Paris) 4:235–237

Metchnikoff E (1892c) La lutte pour l'existence entre les diverses parties de l'organisme. Rev Sci 50:321–326

Monakow C von (1889) Experimentelle und pathologisch-anatomische Untersuchungen über die optischen Centren und Bahnen. Arch Psychiatr Nervenkr 20:714–789

Nesbitt R (1736) Human osteogeny explained in two lectures. London. Cited in Stieda (1872)

Nissen F (1886) Ueber das Verhalten der Kerne in den Milchdrüsenzellen bei der Absonderung. Arch Mikrosk Anat 26: 337–342

Noetzel W (1895) Die Rückbildung der Gewebe im Schwanz der Froschlarve. Arch Mikrosk Anat 45:475–512

Noetzel W (1898) Zur Kenntniss der Histolyse. Arch Pathol Anat 151:7–22

Oppenheim RW (1981) Neuronal cell death and some related regressive phenomena during neurogenesis: a selective historical review and progress report. In: Cowan WM (ed) Studies in developmental neurobiology: essays in honor of Viktor Hamburger. Oxford University Press, Oxford New York, pp 74–133

Paneth J (1885) Die Entwicklung von quergerstreiften Muskelfasern aus Sarcoplasten. Sitzungsber Wiener Akad Wiss Math-Naturwiss Klasse 92, Abt III:236–269

Panizza B (1855) Osservazioni sul nervo ottico. G Ital Ist Lombardo Sci Lett Art 7:237–252 (for English translation see Clarke and O'Malley 1968)

Pflüger F (1863) Ueber die Eierstöcke der Säugethiere und des Menschen. Leipzig

Prenant A, Bonin P, Maillard L (1904) Traité d'Histologie. Schleicher, Paris

Prévost, Lebert (1844) Sur la formation des organes de la circulation et du sang dans les batraciens. Ann Sci Nat, 3e série 1: 200–225

Raff MC (1992) Social controls on cell survival and cell death. Nature 356:397–400

Rathke H (1825) Kiemen bey Säugethieren. Isis, 747–749. Cited in Mayr E (1982)

Rees J van (1888–1889) Beiträge zur Kenntniss der inneren Metamorphose von *Musca vomitoria*. Zool Jahrb Abt Anat Ontog Thiere 3:1–134

Rindfleisch GE (1879) Ueber Knochenmark und Blutbildung. Arch Mikrosk Anat 17:21–42

Rohde (1898) Die Ganglienzelle. Z Wiss Zool 64:697–727

Roux W (1881) Der Kampf der Theile im Organismus. Engelmann, Leipzig

Rubin LL, Philpott KL, Brooks SF (1993) The cell cycle and cell death. Curr Biol 3:391–394

Samuel S (1860) Die tropischen Nerven. Wigand, Leipzig

Schaffer J (1893) Beiträge zur Histologie und Histogenese der quergestreiften Muskelfasern des Menschen und einiger Wirbelthiere. Sitzungsber Akad Wiss Math-Naturwiss Klasse 102, II Abt:7–147

Schaffer J (1897) Bemerkungen über die Histologie und Histogenese des Knorpels der Cyclostomen. Arch Mikrosk Anat 50: 170–188

Schleiden MJ (1842) Grundzüge der wissenschaftlichen Botanik. Engelmann, Leipzig

Schottlaender J (1893) Ueber den Graaf'schen Follikel, seine Entstehung beim Menschen und seine Schicksale bei Mensch und Säugethieren. Arch Mikrosk Anat 41:219–294

Schwann T (1839) Mikroskopische Untersuchungen über die Uebereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen. Sander, Berlin

Schwartz LM (1992) Insect muscle as a model for programmed cell death. J Neurobiol 23:1312–1326

Seinsch W, Schweichel J-U (1974) Physiologic cell necroses during the early development of muscles of the back in embryonic mice. Z Anat Entwicklungsgesch 145:101–112

Silvestrini G, Ricordi ME, Bonucci E (1979) Resorption of uncalcified cartilage in the diaphysis of the chick embryo tibia. Cell Tissue Res 196:221–235

Sohal GS, Holt RK (1980) Role of innervation on the embryonic development of skeletal muscle. Cell Tissue Res 210:383–393

Solger B (1893) Ueber Rückbildungserscheinungen im Gewebe des hyalinen Knorpels. Arch Mikrosk Anat 42:648–661

Stieda L (1872) Die Bildung des Knochengewebes. Festschrift des Naturforschervereins zu Riga zur Feier des fünfzigjahrigen Bestehens der Gesellschaft practischer Aertze zu Riga. Engelmann, Leipzig

Strelzoff Z (1873) Zur Lehre von der Knochenentwicklung. Med Zentralblatt 18 (cited in Brachet 1893)

Tata JR (1966) Requirement for RNA and protein synthesis for induced regression of the tadpole tail in organ culture. Dev Biol 13:77–94

Teuscher P (1890) Ueber Degeneration am normalen peripheren Nerven. Arch Mikrosk Anat 36:579–602

Todd TJ (1823) On the process of reproduction of the members of the aquatic salamander. Q J Sci Lit Arts 16:84–96

Viallanes H (1882) Recherches sur l'histologie des insectes et sur les phénomènes histologiques qui accompagnent le développement post-embryonnaire de ces animaux. Ann Sci Nat Zool 14:1–348

Vintschgau M von, Honigschmied J (1876) Nervus glossopharyngeus und Schmeckbecker. Arch Ges Physiol 14:443–448

Vogt C (1842) Untersuchungen über die Entwicklungsgeschichte der Geburtshelferkroete (*Alytes obstetricans*). Jent und Gassmann, Solothurn

Vulpian A (1859) Note sur les phénomènes de développement qui se manifestent dans la queue des très-jeunes embryons de grenouille, après qu'on l'a separée du corps par une section transversale. CR Acad Sci 48:807–811

Wagener GR (1879) Bemerkungen über den Eierstock und den gelben Körper. Arch Anat Physiol (Jahrgang 1879):175–200

Webb JN (1972) The development of human skeletal muscle with particular reference to muscle cell death. J Pathol 106:221–228

Weber EH (1851) Ueber die Abhängigkeit der Entstehung der animalischen Muskeln von den animalischen Nerven, erläutert durch eine von ihm und Eduard Weber untersuchte Missbildung. Arch Anat Physiol Wiss Medizin (Jahrgang 1851): 548–566

Weismann A (1863) Die Entwickelung der Dipteren im Ei nach Beobachtungen an Chironomus spec., Musca vomitoria and Pulex canis. Z Wiss Zool 13:107–220. Reprinted as first part (pp 1–100) of Weismann 1864b

Weismann A (1864a) Die nachembryonale Entwicklung der Musciden nach Beobachtungen an Musca vomitoria und Sarcophaga carnaria. Z Wiss Zool 14:187–336. Reprinted as second part (pp 101–263) of Weismann 1864b

Weismann A (1864b) Die Entwicklung der Dipteren. Engelmann, Leipzig. Also published in two parts in Weismann 1863, 1864a

Weismann A (1866) Die Metamorphose der Corethra Plumicornis. Engelmann, Leipzig

Weismann A (1886) Über den Rückschritt in der Natur. Akad Verlags von JCB Mohr, Freiburg i B

Wendler D (1972) Der embryo-fetale Zelltod während der Normogenese und im Experiment. Acta Hist Leopoldina 8. Barth, Leipzig

Whitney WU (1867) On the changes which accompany the metamorphosis of the tadpole, in reference especially to the respiratory and sanguiferous systems. Trans R Microsc Soc 15: 43–55

Wyburn-Mason R (1950) Trophic nerves. Krimpton, London