

“Cellular death in morphogenesis of the avian wing” (1962), by John W. Saunders Jr., et al.

In the early 1960s, John W. Saunders Jr., Mary T. Gasseling, and Lilyan C. Saunders in the US investigated how cells die in the developing limbs of chick embryos. They studied when and where in developing limbs many cells die, and they studied the functions of cell death in wing development. At a time when only a few developmental biologists studied cell death, or apoptosis, Saunders and his colleagues showed that researchers could use embryological experiments to uncover the causal mechanisms of apoptosis. The researchers published many of their results in the 1962 paper “Cellular death in morphogenesis of the avian wing.”

John Saunders had been studying chick embryonic development since the mid 1940s, but in early 1960s through the 1970s he focused on the phenomena of cell death in tissue sections from chick wings stained by vital dyes. Vital dyes, such as the Nile Blue dye Saunders used, stain tissues without killing the cells. Biologists used the dyes to study the dynamics of cells. When Saunders thinly sectioned wing tissues from chick embryos and then dyed those section, he found many stained cells in both the posterior and anterior edges of the wing buds. Initially he identified these as pigment cells, however Saunders observed that these cells were shrinking and degenerating, and thus condensing the Nile Blue dye to small areas of cell debris.

In the early 1960s, Saunders, Saunders, and Gesseling, working at Marquette University in Milwaukee, Wisconsin, speculated that cell death played an important role in the normal development of embryos. They hypothesized that the function of cell death in developing embryos is to eliminate transient biological structures no longer needed for development. To identify the areas in a developing chick embryo where cells died during the course of normal development, the experimenters stained chick embryos at various stages of development by adding vital dyes through a small hole on the membrane and shell. The small hole was opened through a technique called fenestration. The embryos of different developmental stages were fixed, sectioned, analyzed, and for a few cases, reconstructed into three-dimensional models to demonstrate the distributions of cell death in different stages. By observing the patterns on the stained tissues, the scientists traced the typical development of tissue segments.

These three scientists observed that there were several zones of dead cells at different stages of limb development in chicks. They used the Hamburger-Hamilton schema of the forty-six chronological stages of normal chick embryo development. During stages seventeen through twenty-four, the general wing buds took shape from a thickening of the mesenchyme and the growth of the ectoderm, forming paddle-like structures, which the researchers called the paddle stage. After stage twenty-four the shape for the wing emerged, a process the researchers called the contour formation stage. They observed that the posterior part of the junction between the wing and the body contained many cells that degenerated during the late paddle stage and early contour formation stage. They identified small areas condensed with Nile Blue in the posterior part of the wing-body junction and called this region the posterior necrotic zone, or PNZ. Using similar methods, the researchers also examined cell death in the digits and metacarpals of developing chick wings. They showed that the pattern of cell death fit well with the topographical arrangements of those bones. For example, at stage thirty-one, many cells died between the first and second digits of the chick's developing wing.

After they observed the patterns by which cells died in developing chick wing buds, the scientists manipulated the developing tissues to investigate possible mechanisms of cell death in the growing wings. Their inquiries followed a 1951 hypothesis of Alfred Glücksmann's, who had worked in the UK and had conceived of cell death as a process in development of transitional developmental

structures. Saunders, Saunders, and Gesseling investigated how a chick's wing would develop if they removed its PNZ completely. In the one hundred thirty-three experiments in which the researchers extirpated the whole PNZ from chick embryos between stages seventeen and twenty-three, the scientists observed that cell didn't die in most of the cases. Those embryos, however, still formed normal wings. In the article, the researchers concluded that cell death per se was not necessary for normal development, as surgically eliminating the PNZ substitute for normal process of cell death.

The experimenters then investigated whether the environment of the limb bud or something intrinsic to PNZ cells regulates the timing of cell death in PNZs. They compared the changes in cell death after grafting tissues to embryos at different developmental stages. They excised PNZs and grafted them to the dorsal side of wing buds of the same embryos or of other embryos at the same developmental stage. They observed that, although the PNZs excised from stages seventeen to twenty-one seldom showed signs of cell death when transplanted to other locations, the grafts from stages twenty-two to twenty-three displayed typical degeneration at stage twenty-four. Additionally they observed that if they allowed the embryos to continue to develop, the donors of the PNZ grafts developed normally, but the recipients of the grafts developed extra skeletal structures or feathers on their forearms.

Saunders, Saunders, and Gesseling also grafted segments that had PNZs to chick somites. In these experiments, the scientists observed that almost all the PNZ cells from stages seventeen to twenty-three transplanted to the somites degenerated. The researchers argued that a program for cellular death, which they called the death clock, initiated at stage seventeen. However, the death program became irrevocable from the influences of their environment only after stage twenty-one.

Finally, Saunders and his colleagues investigated whether or not the environment of developing embryos could alter the timing of cell death in PNZs. By transplanting a PNZ from one embryo to another embryo in a different developmental stage, the experimenters showed that the environments couldn't alter the timing at which cells die in PNZs. For example, when PNZs obtained from stage seventeen were grafted to the posterior region of body-wing junction of the hosts in stage twenty-one, none of the grafts showed signs of degeneration when the hosts reached stage twenty-four. The grafts nevertheless degenerated later when the donor reached stage twenty-four. The experimenters concluded that the timing of cell death was immutable by the heterochrony between the developments of the hosts and the grafts.

In the discussion section of their 1962 paper, the experimenters compared their results with other theories about cell death in development. They noted that the process of cell death eliminates excessive structures, but they argued that cellular death was unlikely to causally affect the morphogenesis of cells. Instead, they suggested that cell death accompanied other morphogenetic events. This view differed from many other developmental biologists' theories at the time. For his work in studying cell death and chick limb development, John Saunders received the Edwin Grant Conklin Medal in 1996.

Sources

1. Fallon, John F. "How Serendipity Shaped a Life: An Interview with John W. Saunders, Jr." *International Journal of Developmental Biology* 46 (2002): 853-61. <http://www.ijdb.ehu.es/web/paper/12455620/how-serendipity-shaped-a-life-an-interview-with-john-w-saunders> (Accessed March 6, 2014).
2. Glücksmann, Alfred. "Cell Deaths in Normal Vertebrate Ontogeny." *Biological Reviews of the Cambridge Philosophy Society* 26 (1951): 59-86.
3. Hamberger, Viktor and Howard L. Hamilton. "A series of normal stages in the development of the chick embryo." *Journal of Morphology* 88 (1951): 49-92.
4. Lockshin, Richard A., and Zahra Zakeri. "Programmed Cell Death and Apoptosis: Origins of the Theory." *Nature Reviews Molecular Cell Biology* 2 (2001): 545-50.
5. Saunders, John W. "The proximo-distal sequence of origin of wing parts and the role of the ectoderm." *The Anatomical Record* 99 (1947): 567

6. Saunders, John W. Jr., "Death in Embryonic Systems." *Science* 154 (1966): 604-12.
7. Saunders, John W. Jr., John F. Fallon. "Cell Death in Morphogenesis." In *Major Problems in Developmental Biology*, ed. Michael Locke. Academic Press (1966): 289-314.
8. Saunders, John W., Mary T. Gasseling, and J.M. Cairns. "Effects of implantation site on the development of an implant in the chick embryo." *Nature* 175 (1955): 673-4
9. Saunders, John W. Jr., Mary T. Gasseling, and Lilyan C. Saunders. "Cellular Death in Morphogenesis of the Avian Wing." *Developmental Biology* 5 (1962): 147-78.