

# “Programmed Cell Death–II. Endocrine Potentiation of the Breakdown of the Intersegmental Muscles of Silkmoths,” by Richard A. Lockshin and Carroll M. Williams (1964)

Richard A. Lockshin’s 1963 PhD dissertation on cell death in insect metamorphosis was conducted under the supervision of Harvard insect physiologist Carroll M. Williams. Lockshin and Williams used this doctoral research as the basis for five articles, with the main title “Programmed Cell Death,” that were published between 1964 and 1965 in the *Journal of Insect Physiology*. These articles examine the cytological processes, neuronal and endocrinal controls, and the influence of drugs on the mechanism of cell death observed in pupal muscle structures of the American silkworm. Those muscle structures disappeared right after the completion of adult development. Several scientists have credited this series of articles as introducing the now standard term “programmed cell death”. Among the five articles “II. Endocrine Potentiation of the Breakdown of the Intersegmental Muscles of Silkmoths” (abbreviated hereafter as “Endocrine Potentiation”), was published first and has been cited the most often. The article suggests that the endocrinal conditions at the beginning of the adult development are necessary, but not sufficient, for precisely scheduling three weeks later the cell death activities in the pupal intersegmental muscles of American silkworms. The research was among the first to attempt to pinpoint the role of hormones in regulating cell death, a process integral to development.

In “Endocrine Potentiation,” Lockshin and Williams inquired about the endocrine conditions at the beginning of the adult development that may control the degeneration of the intersegmental muscles toward the end. In order to investigate this question, the two researchers designed experiments to alter the endocrinal conditions in developing moths, and observed the effects on cell death.

At the end of diapause and the beginning of adult development, silkworms secrete ecdysone in the absence of juvenile hormone to initiate the rapid disintegration of most larval organs. However, certain intersegmental muscles of the abdomen survive until the completion of moth development, after which the remaining pupal muscles break down and disappear. The breakdown of intersegmental muscles raised much interest because unlike other pupal structures, the degeneration was delayed by about three weeks.

Lockshin and Williams injected different amounts of juvenile hormone into silkworm pupae at the onset of adult development. When relatively small amounts of juvenile hormones were added, after three weeks the fully developed moths had no or partially degenerated intersegmental muscles. When the authors injected large amounts of juvenile hormones, however, the silkworms retained all of the pupal intersegmental muscle structures. The effect of juvenile hormones in inhibiting muscle degeneration also seemed to diminish as the authors postponed the time of injection. The authors argued that the hormonal conditions at the beginning of the diapause initiated the cell deaths that eventually lead to pupal muscle breakdown.

Then Lockshin and Williams asked whether the endocrinal signals throughout the adult development would influence the timing of the degeneration of the pupal intersegmental muscles. By using a surgical technique called parabiosis to connect the blood between different individuals, Lockshin and Williams managed to fuse the circulation from pupae of different species that had different timings for adult development, in order to make them share the same endocrinal conditions. For example, the species *Hyalophora cecropia* needs 21 days for adult development at 25 degrees centi-

grade, while *Samia cythia* requires only 17 days, with the pupal intersegmental muscles degenerating earlier. When *cecropia* and *cythia* pupae were made to share the same blood, the results showed that they kept the schedules of the intersegmental muscle degeneration characteristic of their own species. The authors concluded that although the endocrinal conditions initiated a program for degeneration of intersegmental muscles, the endocrinal conditions did not determine the exact timing for the degeneration.

After additional experiments to test temperature's influence on the schedule of pupal intersegmental muscle degeneration, Lockshin and Williams summarized their research. The authors reviewed the role of the endocrine system in controlling the timing of cell death and within the larger scheme of moth development. They noted that it was important that the intersegmental abdominal muscles remained active during adult development because the muscular contractions ultimately helped with the emergence of the moth's wings. As such, the authors argued that the intersegmental muscle cells likely evolved to respond to the endocrinal signal to initiate a cell death program that only manifested itself later. As joining the circulation systems of different species of moths did not alter the timings of intersegmental muscle death, the authors concluded that the eventual trigger for cell death was not endocrinal in nature. Therefore the authors conclude that the role of ecdysone and juvenile hormone serve as hormonal regulators of pupal intersegmental muscle cell death as a function of Potentiation. The potential for cell death is mediated through hormonal signals, so that other factors can synergize and trigger its manifestation about three weeks later.

Although "Endocrine Potentiation" was not the first article to review cell death in insect development, it introduced techniques such as parabiosis to insect endocrinology, to help researchers better understand the mechanisms of cell death. The article and the series "Programmed Cell Death" recorded the 1960s efforts to study cell death. Those articles also marked the start of a long academic career for Richard Lockshin, who focused on cell death and became an expert in apoptosis and eventually helped to found the International Cell Death Society in 1995.

## Sources

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