

"The Development of the Pronephros during the Embryonic and Early Larval Life of the Catfish (*Ictalurus punctatus*)" (1932), by Rachel L. Carson

Rachel L. Carson studied biology at Johns Hopkins University in Maryland and graduated in 1933 with an MA upon the completion of her thesis, "The Development of the Pronephros during the Embryonic and Early Larval Life of the Catfish (*Ictalurus punctatus*)."

The research that Carson conducted for this thesis project grounded many of the claims and observations she presented in her 1962 book, *Silent Spring*. This book focused on the environmental dangers of using pesticides against insects and plants deemed invasive, and it received attention from the US government, to such an extent that Carson testified before US congress in Washington D.C., and US President John F. Kennedy appointed a commission to validate her claims.

Linda Lear, Paul Brooks, and William Souder all wrote biographies of Carson, and each suggest that Carson faced several problems trying to complete her Master's thesis. She attempted experiments on several reptiles, including examination of the sensory organs of the pit viper snake. She also tried to examine the embryonic development of a species of squirrel, but this research halted because she could not get the squirrels to breed.

In 1931 Carson's advisor Rheinart Parker Cowles, a scientist studying marine biology of the Chesapeake Bay, which is surrounded by Maryland and Virginia, suggested that Carson investigate urinary tract morphogenesis in fishes. Morphogenesis is a developmental process in which the cells of an embryo differentiate, and enable tissues and organs to form. Carson examined the pronephros, sometimes called the anterior kidney, of ray-fish (which are teleosts). The pronephros is an early excretory organ in vertebrate embryos, identified in the early 1800s. The pronephros derives from the mesoderm, which is one of three primary germ layers of the early embryo, which gives rise to bone, cartilage, muscle, connective tissue, including that of the dermis. From the mesoderm also develop vascular, reproductive, excretory and urogenital systems, and the mesoderm contributes to the formation of some glands. In most early vertebrate embryos, a longitudinal excretory tubule, the nephric duct, runs the length of the body and attaches posteriorly to the cloaca, a cavity that the urinary tract empties into. The long tubing of the nephric duct carries waste materials from the blood for excretion. At the anterior end of the nephric duct, near where the developing heart and stomach will be, there are several smaller tubules that collect urine, which extend into the head region of the embryo and drain into the nephric duct. These small, paired tubules collectively make up the pronephros.

In 1931, researchers had not described this organ's function. To Carson and others, the pronephros appeared to help early fish embryos survive, but then disappeared in development, only to reappear later. Carson used channel catfish embryos to document the development of the pronephros. She studied catfish because she could get them easily, as the fish was commonly reared by fish hatcheries and Carson's advisor had connections with fish hatchery managers.

Carson's thesis is divided into an introduction, historical summary, methods, discussion, and summary. She states in her introduction that most adult teleosts have a structure called the head-kidney located in front of the swim bladder, which is a sac filled with gas that provides a buoyant center for fish. This is the location in which the pronephros is located before it disappears. In her research, Carson studied the relationship between the renal pronephros and the non-renal head kidney. To determine their relationship, she worked to see if the head kidney derives from the embryonic

pronephros or if the pronephros completely disappears and the head kidney is of independent origin, and arises in the space vacated by the pronephros.

Carson's historical summary provides a variety of hypotheses about the function of the pronephros and whether or not it remained functional but modified in certain fish and not in others. She draws upon the work of Francis Maitland Balfour, who had founded the Cambridge Morphology Laboratory at University of Cambridge, UK in the late nineteenth century. Balfour had reported in 1882 that in many teleosts, the pronephros transforms into a mass of lymphatic tissue several weeks after fertilization. In other teleosts, however, Balfour said that the pronephros sometimes shifts forward and transforms into a mesonephros, a later-stage vertebrate kidney. Carson discusses results by other zoologists that supported or questioned Balfour's conclusions about the pronephros.

At the end of the historical summary, Carson identifies three routes by which a pronephros can develop. One route is that the tubules that make up the pronephros dissolve as the embryo matures. Another possibility is that the pronephros remains the only kidney that a fish has, and a third option is that the pronephros does not disappear, but works in conjunction with the mesonephros. Carson traces the debate about the transformation of the pronephros in the literature, which spanned greater than half a century, and argues that embryological data, which might offer a solution, was lacking.

To begin her descriptive analysis of the pronephros, Carson made a series of embryological cross-sections of catfish embryos, which she obtained from Oklahoma's State Fish Hatchery. Carson followed protocol in which she collected, preserved specimens, and then sliced them to see inside them. Hatchery employees collected the embryos at twenty-four hour intervals during the first eleven days of development and preserved the embryos in glycerin and formalin. They shipped the entire series to Carson at Johns Hopkins University in Baltimore, Maryland. Carson stained the embryos with eosin, a fluorescent red dye, to help keep track of the embryos' orientation, and then she imbedded them in paraffin. Next, she sectioned the embryos and affixed them to slides to observe them through a microscope. Before examining the embryo sections with a microscope, Carson stained the slides with Heidenhain's iron-hematoxylin, which stains mitotic structure such as chromosomes, chromatin, nuclei, and muscle fibers, and Mayer's haem-alum, a blue stain that stains nuclei.

The excretory organs of vertebrates appear early in development to dispel embryonic wastes. Carson examined embryonic stages of the catfish from Day 2 through Day 11 of normal catfish development. Carson drew sketches and took photographs of each embryo day or stage, and she described the structural changes that she observed. She inserted these visual representations in the results section at the end of her thesis.

During Day 2 of catfish embryologic development, Carson says that the mesoderm shows a high degree of differentiation. The embryo is attached to a large yolk mass and the gut has yet to form. The pronephric tubules, one on each side of the embryo, start to form as the epithelium begins to curve around the tubular space. A fixed slide evaluated on the same day, but nearing Day 3 of development, shows the pronephric tubule complete, surrounded by epithelial cells and completely separate from the splanchnocoel. The splanchnocoel is an early embryonic cavity from which develop several visceral cavities, including the abdominal cavity.

When Carson observed the Day 3 catfish embryos slides, the embryos were coiled around the yolk sac, almost forming a complete ring. The anterior end of the pronephros had grown significantly since Day 2. She writes that the Day 4 embryo sections show catfish excretory organs that appear similar to that of a typical teleost pronephros. The epithelium lining the pronephric tubules change from a cuboidal shape to a more flattened, thin epithelium, and she saw capillaries innervating the epithelium. The capillaries are embedded in glomerular tissue, which looks like a cluster of grapes pushing into the pronephros.

Carson notes that catfish embryos at Day 5 have a large glomerulus of tissue and blood vessels taking up almost all of the pronephros. She documents the appearance of the urinary bladder, stating that by Day 5, the bladder is clearly defined. On Day 6, the embryos show significant changes in the region of the pronephros. The pronephric tubules are surrounded by closely packed cells not present the day before. Because these cells suddenly appeared on Day 6, Carson was unable to

demonstrate the tissue's origin. She suggests that these cells make up lymphoid tissue, a tissue described by Balfour in 1882 that invades and takes over the pronephros kidney. Carson surmises that the tissue is not suggestive of true lymphoid tissue, so she calls it pseudolymphoid tissue.

Also on Day 6, Carson documents the presence of a long space between the pronephros and the mesonephros. This region occupies an area that develops into the teleost air bladder. Carson concludes that the early embryonic development of the catfish is so rapid, that a series of embryos, only a few hours apart, would be necessary to accurately document the minute details of mesonephros development.

On Day 7 of development, the embryo has escaped from surrounding egg membranes and is now a larval fish, with an identifiable air bladder. The growth of the pronephros stalls, except for the growth of more pseudolymphoid tissue. At this point, Carson focuses in on the Malpighian glomeruli of the mesonephros. The Malpighian glomeruli help filter blood within the kidney. On Day 8, the pronephros is heavily innervated with veins and capillaries, along with pseudolymphoid tissue.

Carson combined her embryo descriptions for Days 9, 10, and 11 because she saw few changes in the embryos during those days. She said that the most noticeable change to the pronephros occurs on Day 10 when the structure reduces in size, a remnant of its former self. The last slides available for Carson study were for Day 11. She describes how pseudolymphoid tissue crowds out other tissues and blood vessels from within the pronephros, while rapidly developing structures, most notably the air bladder and the cervical vertebrae, encroach on the outside of the pronephros. The posterior mesonephros, meanwhile, remains free from internal and external obstacles and increases in size and complexity. Carson concludes in the results section that later-stage embryos are necessary to continue documenting the atrophy of the pronephros.

Carson's discussion section summarizes the developmental changes that she observed. Her observation of the emergence of the pseudolymphoid tissue aligned with Balfour's work, although it didn't indicate any conclusions about the origin of the pseudolymphoid tissue. Carson showed that in the channel catfish, the pronephros and the head kidney are the same structure and that a functional pronephros appears to dissolve away.

Carson's laboratory work with catfish embryos examined the embryonic development of the pronephros, but she did not speculate on the function of the pronephros. Nonetheless, biographers argue that Carson's background in developmental biology helped to indicate to her the role of pesticides in developmental mishaps. To Carson supporters, however, her Master's thesis credentialed her as a scientist.

Sources

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