Alexis Carrel's Immortal Chick Heart Tissue Cultures (1912-1946) [1]

By: Jiang, Lijing Keywords: Cell death [2] Cell theory [3] Apoptosis [4]

In an effort to develop tissue culture techniques for long-term tissue cultivation, French surgeon and biologist Alexis Carrel [6], and his associates, produced and maintained a series of chick [7] heart tissue cultures at the Rockefeller Institute [8] in New York City. From 1912 to 1946, this series of chick [7] heart tissue cultures remained alive and dividing. As the duration of this culture greatly exceeded the normal chick [7] life span, the cells were deemed immortal. Although further experiments in the 1960s challenged that conclusion, the publicity surrounding the immortal chick [7] heart tissue significantly influenced the concept of cell immortality [9] and cellular aging [10] from the 1920s through the 1960s. Carrel's experiment convinced many biologists to accept immortality as an intrinsic property of all cells, not just the cell line through which genetic material is passed to offspring, called the germ line [11]. Consequently, the phenomenon of cellular aging [10] was regarded not as an intrinsic characteristic, but was attributed to external factors such as the accumulation of waste products within the cell.

The basic tissue culture techniques used to generate the immortal chick [7] heart tissue were first described in Carrel's paper "The Permanent Life of Tissues outside of the Organism," published in 1912. In his experiment, Carrel adopted Ross Granville Harrison's hanging drop method [12] for culturing chick [7] heart tissues. Small fragments of embryonic chick [7] heart tissue were placed on a clear cover that sits on top of a slide, called a coverslip, in a drop of liquid composed of chick [7] blood plasma and water. After the medium made of blood plasma and water had coagulated, the coverslip was inverted over a slide. The whole slide was then incubated at 39 degrees Celcius. After the culture grew for two to three days, the tissue was passaged, wherein a fragment of the tissue was removed, washed, and put into new medium so that the growth could continue and a secondary tissue culture could be generated. The chick [7] heart tissues in this early stage of *in vitro* [13] culturing continued to pulsate. Before Carrel submitted his paper for publication, the chick [7] heart tissue had been growing and pulsating for about three months, from 17 January to 16 March 1912. In his paper, "On the Permanent Life of Tissues outside of the Organism," Carrel hypothesized that the lifespan of cultured tissues could be lengthened indefinitely and tissues should intrinsically be able to maintain permanent life *in vitro* [13] under ideal culturing methods.

The responsibility of maintaining the chick [7] heart tissue culture was transferred to Carrel's associate, Albert Ebeling [14], in June of 1912. Although some of the cultures became contaminated, Ebeling managed to preserve one line of cell cultures. In a paper published in 1913, "The Permanent Life of Connective Tissue outside of the Organism," Ebeling listed the sub-culturing and tissue growth conditions in detail, including records of the 129 passages, or times at which the cultured cells were segregated into new medium for sub-culturing, that Carrel, Ebeling, and assistants had performed from 17 January 1912 to 15 January 1913. This record shows that in March of 1912, the growth rate of the tissues increased following the inclusion of a chick [7] embryo extract into the culture medium [15]. After this finding, the chick [7] embryo extract became a standard component of the medium used by Carrel's group. Although the cells' growths accelerated in March, the pulsations began to slow, and eventually ceased by April of 1912. By the time the 1913 paper was published, the chick [7] heart tissue cells had been proliferating *in vitro* [13] for more than a year.

Carrel and Ebeling subsequently published a series of scientific papers reporting on the progress of the chick [7] heart tissue cultures in *The Journal of Experimental Medicine*. Carrel published two papers in 1913 and 1914, when the cultures were growing for sixteen and twenty-eight months, respectively. In these papers, Carrel compared the chick [7] heart cells to microorganisms and colonies of minute aquatic organisms, called infusoria, which could divide indefinitely. He also discussed questions about the effects of the composition of the medium on the growth rate, modifications growing cells made to the medium, and methods for measuring the volume of tissues in the culture. In a paper published in 1919, Ebeling gave additional descriptions of the chick [7] heart tissue cultures when they had been maintained for seven years, and the growth of cells had become even more rapid. In another paper published in 1922, Ebeling pointed out that if the whole volume of cells generated by the chick [7] heart tissue culture could be maintained, then the mass would have been larger than the sun.

In addition to scientific papers, articles in newspapers and magazines covered the immortal chick [7] heart tissue cultures. In 1921, an article in *The World* by Alessandro Fabbri engaged his audience with an account of how large the volume of the cells cultured could have been, telling readers that it would have been like a "rooster … big enough today to cross the Atlantic in a stride," and "so monstrous that when perched on this mundane sphere, the World, it would look like a weathercock." Three years later, the *New York Tribune* published an article to celebrate the twelfth birthday of the culture.

In 1939 Carrel retired from the Rockefeller Institute [8], and returned to France, leaving the chick [7] heart tissue cultures to Ebeling's care. Ebeling, with Carrel's two technicians, soon left the Rockefeller Institute [8] and took posts at the Lederle

Laboratories of the American Cyanamid Company, in Pearl River, New York. There they used the chick [7] heart tissue cultures to test the toxicity of drugs and germicides. The last batch of chick [7] heart tissue cultures was discarded in 1946, two years after Carrel's death, and following thirty-four years of sustained proliferation.

By the 1960s the immortal chick [7] heart tissue culture was seen as sound evidence supporting Carrel's hypothesis about the immortality of cells. In 1961, when Leonard Hayflick [16] performed a series of experiments that demonstrated a finite lifespan for human cells grown *in vitro* [13], Carrel's immortality hypothesis was called into question. The failure of several attempts to culture normal chick [7] somatic cells for longer than a few months further exposed a problem with Carrel's hypothesis. Some now argue that there must have been some experimental errors in Carrel's immortal chick [7] heart cultures. Scientists and historians of science have tried to reassess Carrel's immortal chick [7] heart tissue culture experiment, attempting to account for what could have been different in Carrel's methods that led to the extreme long life of his famous immortal chick [7] heart cultures.

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Subject

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