1910 to 1923 - Carrel and the early days of tissue culture

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Carrel was already very talented surgeon when he was recruited to the Rockefeller Institute in 1906. He had won acclaim for developing the first successful technique for suturing together blood vessels in way which prevented blood clots. He was awarded the Nobel Prize for Physiology and Medicine for this breakthrough in 1912. (Click here to access the Nobel Foundation site that has his biography, Nobel Lecture and banquet speech online.) This technique allowed him to perform organ transplantation in animals. This interest in organ transplantation also stimulated his thinking of growing organs outside of the body.

In early 1910 Montrose Burrows, another Rockefeller researcher, was sent by Simon Flexner, the head of the recently opened Rockefeller Institute in New York City, to Yale to learn what he could from Ross Harrison about his new tissue culture technique. While there, he adapted Harrison's hanging drop method to work with warm blooded tissues by switching from using clotted frog lymph to chicken plasma clots. When he returned a few months later to work in Carrel's lab at the Rockefeller Institute he had already successfully cultivated chick embryo tissues in vitro.

Alexis Carrel
1873-1944
Photo courtesy of the Library of Congress

With Burrows help Carrel's lab was soon culturing both embryonic and adult tissues from dog, cat, chicken, rat and guinea pig using freshly collected plasma from the same source as the tissue being cultured. By the end of 1910 they had successfully used this "plasmatic media" to culture chicken, rat, dog and human tumors. Most of these were cultured in hanging drop slides although some were cultured on large black glass plates (2). They also learned to subculture these explants once or twice by carefully cutting up successful explants into smaller pieces and transferring them to fresh clots.

Carrel's lab also began experimenting with other media including diluting the plasma with a variety of saline solutions and using serum. Work progressed quickly and soon they could subculture their cultures by carefully cutting them into small pieces and placing in to fresh plasma clots. They had developed the first cell lines and could keep them growing for up to several months (3).

Carrel's famous chicken heart cultures

By adding chick embryo extract as a medium supplement they were able to subculture these early cell lines indefinitely. In fact, one cell line, derived from explanted chick embryo heart fragments in January, 1912, was successfully subcultured hundreds of times (4, 5). After surviving initial contamination outbreaks that almost wiped them out, they were taken over by Arthur Ebling, a researcher working with Carrel (6). He followed Carrel's rigorous

Did you know?
Actor and comedian Bill Cosby recorded a skit entitled "Chicken Heart" in 1966 that appears to be strongly influenced by Carrel's famous heart

ethods at the Rockefeller Institute to subculture and maintained this cell line until 1946 when the cultures were finally terminated. These cultures were widely publicized and its birthday was celebrated yearly in the New York World Telegram. The cultures outlived Carrel by almost two years! The ability to maintain this strain for so long is a tribute to the Carrel lab's rigorous aseptic technique.

culture. He describes an accident in a laboratory in New York City where a chicken heart culture is accidentally knocked onto the floor along with nutrient solution. By feeding on the nutrient solution it grows rapidly and, in typical Cosby fashion, reaches monstrous proportions consuming everything in its path.

Wonderfulness *recorded by Bill Cosby; Warner Brothers release 1966*

Carrel's D-Flasks

Carrel's lab continued to experiment with ways of improving culture techniques, especially culture vessels. Finally, in 1923 he developed the first practical cell culture flask. These were called D-flasks; a D-3.5 flask had a diameter of 3.5cm (Figure 1). These flasks allowed the plasma clots to be submerged in a much larger volume of medium than in a hanging drop culture. As a result, where hanging drop slide cultures could not be fed and had to be subculture every two to three days, the new flask cultures were easy to feed and could be maintained for weeks without subculturing (7).

By the mid 1920s the pioneering tissue culture techniques developed and refined in Carrel's laboratory had become the methods used for most cell culture research in laboratories around the world. Very little changed in culture technology for the next 25 years until the development of quantitative measuring techniques, defined media and the use of trypsin for dissociating tissues and subculturing the resulting cell lines finally allowed the technology to rapidly expand in the 1950s.

Was Carrel good for tissue culture?

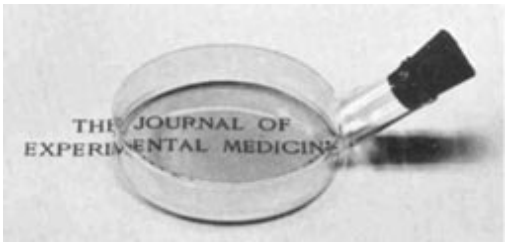

Figure 1. One of Carrel's D-flasks which were manufactured from PYREX glass.

Much of Carrel's success over the years, especially in avoiding the contamination problems that plagued many others who tried to use this new tool, was due to his rigorous aseptic techniques, careful laboratory design and layout, strict lab rules and surgical experience. (Remember this was before any antibiotics were available to take care of lapses in a surgeon's aseptic technique.) Cell culture today is still a difficult tool for many to master and back then conditions were extraordinarily difficult. Everything, from obtaining plasma and embryo extracts to culture vessels and pipets, was do-it-yourself endeavor. Sterilizing filters were primitive, laminar flow hoods did not exist and phase contrast microscopy would not available until the late 1940s. Carrel's success in tissue culture and his ability to attract widespread publicity made him the focus of this new developing branch of science. As a result, many researchers came to work in his laboratories during the 33 years he spent at the Rockefeller Institute, including Albert Fischer, who went on to work at the Kaiser Wilhelm Institute in Germany applying biochemical methods to tissue culture, Wilton B. Earle who went on to develop the excellent cell culture facilities at the National Cancer Institute and Raymond C. Parker whose team at Connaught Medical Research Laboratories in Toronto developed the culture techniques (the Toronto Method) for mass producing the polio virus for the first successful polio vaccine in the1954. They all had outstanding careers based, in part, on what they learned from Alexis Carrel and his team at the Rockefeller Institute.

However, many other researchers were turned off by all of Carrel's elaborate and complicated rules and techniques, believing that his style of cell culture was not practical. Other researchers were turned off by the publicity that always seemed to surround Carrel. His long association with Charles Lindbergh (1930s), many newspaper and magazine articles and his popular book, **Man, the Unknown** (1935), crossed an invisible boundary for some who felt that scientists should stick to science and avoid the limelight. Jan Witkowski (8) has done a thorough job examining the controversy surrounding Carrel's contributions to cell culture. He concludes: "Tissue culture was a technique fraught with frustrations, but Carrel persisted in attempting to develop and improve it. That he was able to grow cells without antibiotics for many years was in itself a considerable technical feat and by his example he demonstrated to his contemporaries that tissue culture was a method of

practical value for experimental studies. It is ironical that the methods by which he achieved his success may have deterred others from following his example".

Suggested Readings

1. Biography of Alexis Carrel From NOTABLE TWENTIETH-CENTURY SCIENTISTS, edited by Emily J. McMurray. Gale Group, 1995. http://www.pbs.org/wnet/redgold/innovators/bio_carrel.html
2. Carrel, A. and Burrow, M. T. (1911) Cultivation of tissues in vitro and its technique. J. Exp. Med. 13:387-396. This article with photos can be downloaded free from the archives at www.jem.org.
3. Carrel, A. and Burrow, M. T. (1911) An addition to the technique of the cultivation of tissues in vitro J. Exp. Med.14:244-247. This article with photos can be downloaded free from the archives at www.jem.org.
4. Carrel, A. (1912) On the permanent life of tissues outside of the organism. J. Exp. Med. 15: 516-528. This article with photos can be downloaded free from the archives at www.jem.org.
5. Carrel, A. (1913) Contributions to the study of the mechanism of the growth of connective tissue J. Exp. Med. 18:287-298. This article with photos can be downloaded free from the archives at www.jem.org.
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8. Witkowski, J. A. (1979) Alexis Carrel and the mysticism of tissue culture. Medical History.;23:279-296.
9. Online Carrel biography from Who Named It? http://www.whonamedit.com/doctor.cfm/445.html
10. Malinin, T. I., Surgery and life: the extraordinary career of Alexis Carrel, New York: Harcourt Brace Jovanovich, 1979.