Date for a Heart Cell

WITHOUT the steady, rhythmic beating of our hearts, we die. Thanks to Livermore research, scientists now know that a significant number of cells in the human heart, which may continue to beat for nearly a century, are regenerated over the course of our lives. The finding that the adult human heart retains the capacity to generate new cells could lead to regenerative therapies for heart diseases.

In 2004, scientists at Sweden's Karolinska Institutet in Stockholm began work to establish definitively whether cardiomyocytes, a type of cell in the human heart muscle, are generated later in life. To measure the age of these cells, they sought out Livermore's Bruce Buchholz and his team at the Center for Accelerator Mass Spectrometry (CAMS). The team had earlier pioneered a method for dating plaques in brain tissue samples from Alzheimer's victims. The heart cell collaboration ultimately included not only researchers from Karolinska and Livermore but also other scientists in Sweden and France.

Isolating DNA

As described in the box on p. 16, CAMS researchers have developed unique capabilities for applying carbon-14 dating to biological research. Almost every project has required developing new processes for preparing samples, and the heart cell project was no different.

While brain plaques are fairly simple structures, heart cells are full of proteins that are continually being produced and degraded. "The Karolinska researchers decided that determining the true age of a cell depended on measuring the carbon-14 in its DNA," says Buchholz. "DNA is the one thing that is formed at the moment of a cell's birth and remains unusually stable throughout its life."

Karolinska and Livermore researchers developed a method for isolating DNA from the cardiomyocyte cell. "That work took us a couple of years," says Buchholz. "Each cell has just small amounts of DNA. Existing kits for DNA separation were not adequate because they tended to contaminate the DNA with minute quantities of specious carbon such as petroleum-derived products." Ultimately, Karolinska personnel used fluorescent-activated flow cytometry, a cell-sorting technique earlier invented at Livermore, to separate the nuclei of cells. Solubility chemistry was used to isolate the DNA from the rest of the nucleus.

Using the "Bomb Pulse"

Work then moved to Livermore where multiple DNA samples from 14 cadavers were carbon dated. For AMS, DNA samples are freeze-dried, then burned (or "combusted") to carbon dioxide,

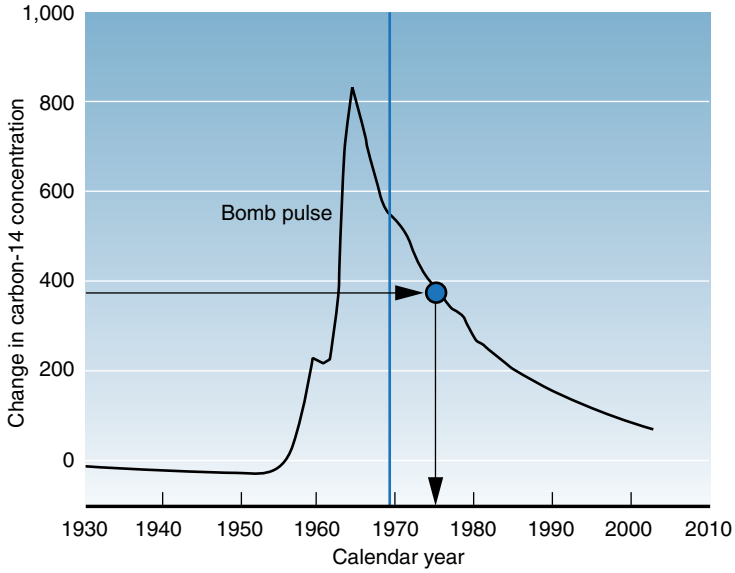
The Old Meets the New

Carbon-14 dating prompts most people to think of objects that are thousands of years old—petrified wood, archeological artifacts, or ancient glaciers. Heart cells from a 20-year-old human would seem far too young to be dated using carbon-14.

Livermore's Center for Accelerator Mass Spectrometry (CAMS) has refined the process of carbon-14 dating to a precision seen nowhere else, allowing researchers to accurately measure extremely small samples and very low doses of carbon-14 and other isotopes in various materials. CAMS researchers can thus examine not only ancient bones and DNA that naturally contain carbon-14 but also other biological materials that have been "tagged" with extremely small amounts of hydrogen-3 (tritium) and other long-lived radioisotopes.

Carbon-14 dating works because every carbon-containing molecule on Earth mirrors the level of carbon-14 in the atmosphere at the time that molecule was created: in glacial ice, tree rings, mammoth bones, and DNA. CAMS uses a huge accelerator as part of its sophisticated method for counting the very rare carbon-14 atoms among the more common carbon-12 and carbon-13. Instruments can detect one carbon-14 atom among up to a quadrillion atoms of carbon-12.

The National Institutes of Health has named CAMS a National Resource for Biomedical Accelerator Mass Spectrometry. This designation makes the facility available to biomedical scientists whose research requires measurements of very low levels of carbon-14 or hydrogen-3. AMS has been used to study human metabolism of vitamins and other substances and to identify areas of the body that absorb drugs and toxic compounds. (See related News Brief on p. 2.)



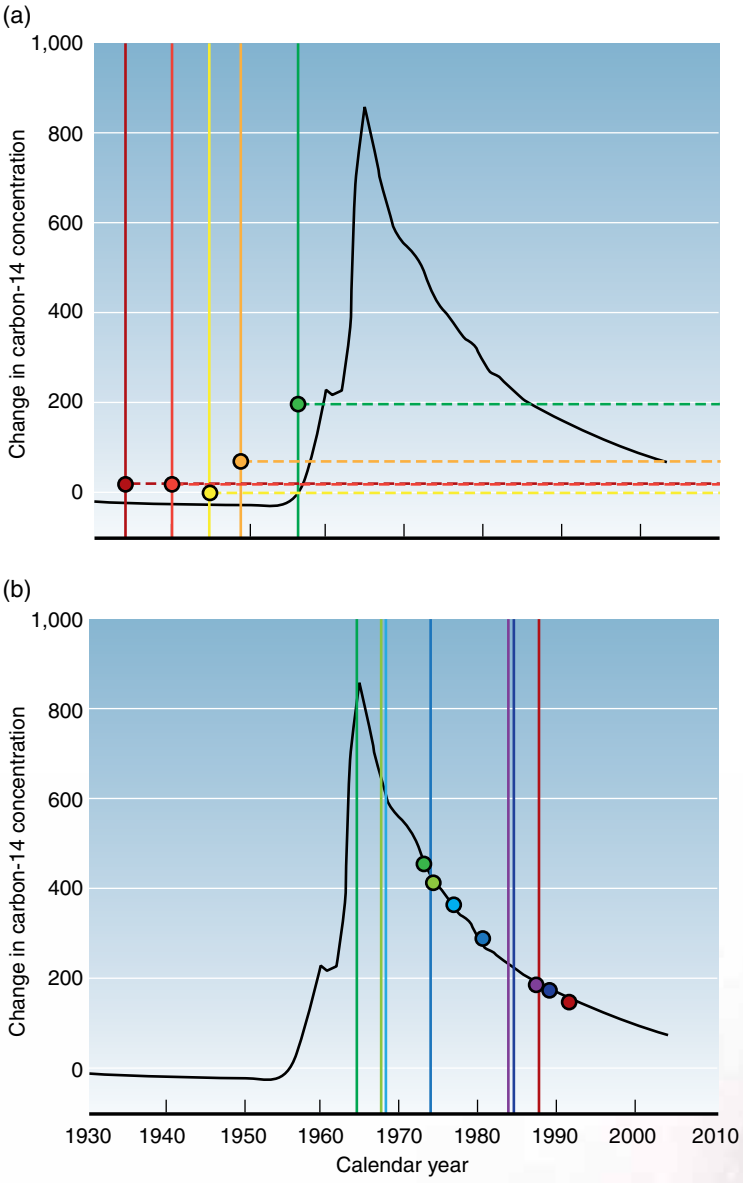
This graph shows how researchers determine the age of cardiomyocyte heart cells using carbon-14 dating. The curve is the atmospheric concentration levels of carbon-14 since 1930, and the blue vertical bar indicates the date of an individual's birth. The measured cellular carbon-14 concentration (horizontal arrow) is compared to established atmospheric carbon-14 concentration over time. The time at which the cellular and atmospheric concentrations correspond (data point) is the inferred birth date (vertical arrow) for the cells tested.

and lastly reduced to tiny graphite pellets. AMS was performed "blind," that is, the identity of each sample was unknown.

Carbon dating was first performed on general heart muscle cells, including cardiomyocytes and other cell types from the left ventricle. That was the easier task. Later, after the team worked out the technique for isolating cardiomyocyte DNA alone, they dated those cells in 12 of the cadavers. Carbon-14 dating is based on using the known decay rate of carbon-14 for long-dead samples. For this project, the dating process took advantage of an atmospheric carbon-14 phenomenon called the "bomb pulse."

Carbon-14 concentrations in the atmosphere remained relatively stable until the Cold War, when aboveground testing of nuclear weapons increased the amount of atmospheric carbon-14. Plants incorporate carbon-14 into their cells, animals eat the plants, and people eat both, absorbing the isotope into their own cells at the same level as is in the atmosphere. Aboveground testing, which started the pulse of carbon-14, began in 1955 and ceased with the 1963 Limited Test Ban Treaty. Although atmospheric nuclear testing took place in only a few locations, carbon-14 was rapidly equalized in the atmosphere around the world following each test. Since 1963, carbon-14 has diffused from the atmosphere into Earth's biosphere.

CAMS researchers found that in the five subjects born before the onset of the nuclear bomb tests, the carbon-14 concentrations in cardiomyocyte genomic DNA were higher than the prebomb atmospheric concentrations, demonstrating DNA synthesis



(a) The carbon-14 concentrations in cardiomyocyte DNA from individuals born before the time of the "bomb pulse" correspond to time points after the birth of all individuals. Vertical bars are years of test subjects' birth, and the corresponding colored data points indicate the change in the carbon-14 value. (b) Carbon-14 concentrations in cardiomyocyte DNA from individuals born after the time of nuclear bomb tests also indicate postnatal synthesis of cardiomyocytes.

after 1955. Similarly, in the seven subjects born near or after the time of the nuclear bomb tests, the carbon-14 concentrations in cardiomyocyte DNA corresponded to the atmospheric concentrations several years after their birth, indicating postnatal cardiomyocyte DNA synthesis. (See the figure at left.) The ages at death for the individuals studied ranged from 20 to 73 with dates of birth from 1987 to 1933.

The researchers found that by analyzing individuals born at different times before 1955, they could establish the age at which DNA synthesis occurs and whether it continues beyond that age. They found that cardiomyocytes are renewed at a rate of 1 percent a year up to the age of 20 years. The rate gradually decreases to less than half a percent per year by old age.

"Creation of the bomb pulse was an unintentional side effect of aboveground nuclear testing, but it has proved to be highly useful for research in many fields, including climate change, carbon turnover, and forest and animal populations," says Buchholz. "The carbon-14 dating technique is allowing us to measure reality rather than an artificial system such as a cell culture."

—Katie Walter

Key Words: carbon-14 dating, cardiomyocyte, Center for Accelerator Mass Spectrometry (CAMS), heart-cell regeneration.

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