A New World of Biomedical Research

The Center for Accelerator Mass Spectrometry

Approximately 95% of all biomedical accelerator mass spectrometry currently under way in the world is being performed at Livermore's Center for Accelerator Mass Spectrometry.

YOU are scheduled for major surgery and have been asked to come to the doctor's office a few days prior to surgery to have some preparatory tests done. One such test that is currently under development may revolutionize surgery and followup treatment. It will determine your metabolism, allowing doctors to personalize your treatment. If your body metabolizes substances quickly, you will need more anesthesia during surgery and higher dosages of medications afterward. A person who metabolizes more slowly will need less anesthesia and smaller doses of medication perhaps at less frequent intervals.

For the test, you will first inhale a small dose of the anesthesia or take a bit of the proposed medication. Then you will breathe into a bag that contains antibody molecules that have been "tagged" with carbon-14 (^{14}C), making them mildly radioactive. The antigens in your breath will react with the antibodies in the bag. A highly sensitive process called accelerator mass spectrometry (AMS) will analyze the contents of the bag, searching for the enzymes that govern metabolism. Those few radioactive molecules will have attached themselves to the enzymes, and the AMS process will count them. AMS (described in the box on p. 6) is so sensitive that it can detect just one ^{14}C nucleus among a quadrillion stable ones. The presence of more enzymes indicates that you are a fast metabolizer, while the numbers are different for a person with a slower metabolism.

With these highly sensitive breath tests, therapies of all kinds—from dosages for individual prescription drugs to complex chemotherapy treatments—can be tailored to fit the needs of a particular individual.

Breath tests are already being used by some doctors to test for hepatitis B and for the bacteria that cause certain ulcers, but AMS is not being used. AMS will make these and similar tests much more effective, allowing doctors and patients to know even earlier whether an infection is present.

The remarkable sensitivity of AMS opens the way to a host of other new diagnostic tests as well: assays for early detection of various disorders, tests that determine the efficacy of therapeutic regimens, studies of how the body handles various nutrients and vitamins, and assessment of the effects of environmental substances and toxins. When combined with such imaging technologies as magnetic resonance imaging, accelerator mass spectrometry will be able to assess changes in tissues, hormone levels, and metabolites in real time.

One of the Few

Lawrence Livermore's Center for Accelerator Mass Spectrometry is one of the few AMS facilities in the world working on biomedical and pharmaceutical applications. Since 1990, Livermore has been developing tests that can measure the effects of extremely small amounts of chemical substances, from suspected toxins to new drugs to dietary nutrients. Early testing with AMS used laboratory animals and this work continues. But the goal is to use AMS to study the effects of these substances on humans.

For example, to study a new drug using AMS, scientists modify just a few molecules of the drug to include a detectable atom such as ^{14}C. The amount of radioactivity in the drug dose is less than a person absorbs during a day on Earth from natural sources of radiation such as cosmic rays. Using a radioactive isotope such as ^{14}C as a "tracer" is not new. What is new is the high sensitivity of AMS, which allows the use of much smaller drug doses and consequently less ^{14}C—from a thousand to a million times less than is used in studies that do not use accelerator mass spectrometry.

Using AMS to count ^{14}C nuclei, researchers can follow the movement of the ^{14}C-tagged drug through the body, identifying how long it remains there, how much and when it is excreted, how much is absorbed, and what organs it affects. How does this work? Carbon-14 is a naturally occurring radioactive isotope that can easily be incorporated into a drug or nutrient before a human ingests it. Counting ^{14}C atoms in urine samples will tell researchers how much of the chemical was digested and how long the ^{14}C-tagged drug was in the body before being excreted. Similar studies may be done with samples of blood or saliva. Studies over time can determine drug absorption and excretion and what the drug's effects are.

The tiny drug dose in this kind of study contrasts with the large quantities typically given to laboratory animals to determine dose–response relationships. Data from tests of potential carcinogens, toxins, and other compounds will serve as the basis for potency calculations and risk assessments relevant to humans, few of which exist today.

Accelerator mass spectrometry was developed in the mid-1970s and was first applied to ^{14}C counting for archaeologic radiocarbon dating. In 1989, Lawrence Livermore researchers pioneered the use of AMS in ^{14}C measurements for biomedical applications. Today, Livermore holds three patents for AMS applications to bioresearch.

The center at Livermore was originally designed to diagnose the fission products of atomic tests, to monitor the spread of nuclear weapons to other countries by detecting telltale radioisotopes in air, water, and soil samples, and to use isotopic tracers to study climate and geologic records. Work recently began on assessing the effects of low-level exposure to chemical weapons.

The center's scope also includes archeology, biodosimetry, atmospheric studies, paleoclimatology, combustion processes, and material science as well as biomedical research.[1] Today it is among the largest of about 40 AMS facilities in the world. It processes more samples (about 20,000 per year) and, perhaps more importantly, measures more different kinds of isotopes than any other AMS facility. Operating 24 hours a day, the center performs about 25% of all AMS analyses in the world.

Studies of the effects of chemical substances on human subjects are few and far between, but several now under way at Livermore are looking at the metabolism and effects of various chemicals, including vitamins, calcium,

Accelerator Mass Spectrometry

Mass spectrometry has been used since early in this century to study the chemical makeup of substances. A sample of a substance is put into a mass spectrometer, which ionizes it and looks at the motion of the ions in an electromagnetic field to sort them by their mass-to-charge ratios. The basic principle is that isotopes of different masses move differently in a given electromagnetic field.

An accelerator was first used as a mass spectrometer in 1939 by Luis Alvarez and Robert Cornog of the University of California at Berkeley. To answer what at the time was a knotty nuclear physics question, they used a cyclotron to demonstrate that helium-3 was stable and was not hydrogen-3 (tritium), which is not stable. Accelerators continued to be used for nuclear physics, but it was not until the mid-1970s that they began to be used for mass spectrometry. The impetus then was to improve and expand radiocarbon dating. Van de Graaff accelerators were used to count carbon-14 (^{14}C) for archaeologic and geologic dating studies.

Accelerator mass spectrometry (AMS) quickly became the preferred method for radiocarbon dating because it was so much quicker than the traditional method of scintillation counting, which counts the number of ^{14}C atoms that decay over time. The half-life of ^{14}C is short enough (5,730 years) that counting decayed atoms is feasible, but it is time-consuming and requires a relatively large sample. Other radioactive isotopes have half-lives as long as 16 million years and thus have such slow decay rates that huge samples and impossibly long counting times are required. The high sensitivity of AMS meant that these rare isotopes could be measured for the first time.

Before a sample ever reaches the AMS unit, it must be reduced to a solid form that is thermally and electrically conductive. All samples are carefully prepared to avoid contamination. They are reduced to a homogeneous state from which the final sample material is prepared. Carbon samples, for instance, are reduced to graphite. Usually just a milligram of material is needed for analysis. If the sample is too small, bulking agents are carefully measured and added to the sample.

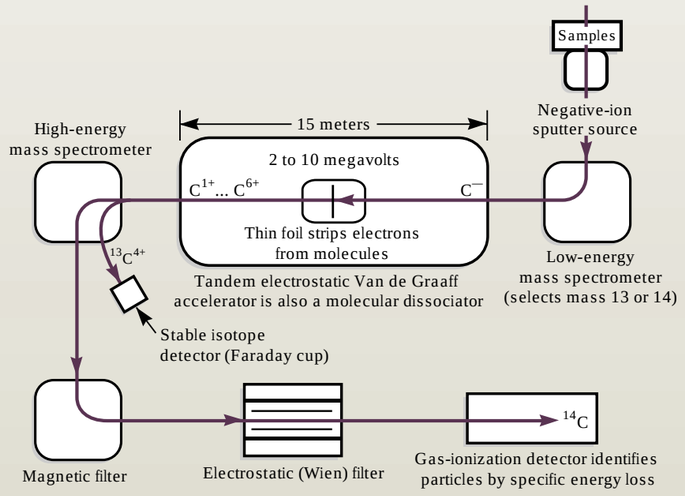
As shown in the figure below, the AMS unit comprises several parts, all of which are controlled by computer. At the ion source, the sample is bombarded by cesium ions that add an extra electron, forming negative elemental or molecular ions. The ions then pass through a low-energy mass spectrometer that selects for the desired atomic mass. In the tandem Van de Graaff accelerator, a second acceleration of millions of volts is applied, and the atoms and molecules smash through a thin carbon foil or gas, which strips them of at least four electrons. Here, all molecular species are destroyed. Without the high energies in the accelerator, the very tight carbon–hydrogen bonds could not be undone. The ions continue their acceleration toward a magnetic quadrupole lens that focuses the desired isotope and charge state to a high-energy mass spectrometer.

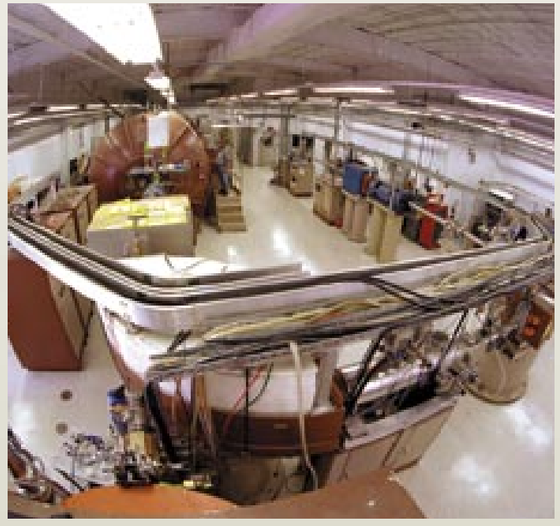
The rare isotope being examined is always measured as a ratio of a stable, more abundant (but not too abundant) isotope, e.g., ^{14}C as a ratio of ^{13}C, which acts as an internal standard and provides a clear signature to differentiate the rare isotope from the background. Their ratio is shown as $^{14}C/^{13}C$. In the high-energy mass spectrometer, the abundant isotope is removed from the ion beam and counted in the Faraday cup. Additional interfering ions are removed by the magnetic filter before the remaining ions finally slow to a stop in the gas ionization detector. The charge of individual ions can be determined from how the ions slow down. For example, carbon-14 slows down more slowly than nitrogen-14, so those ions of the same mass can be distinguished from one another. Once the charges are determined, the detector can tell to which element each ion belongs and counts the desired isotope as a ratio of the more abundant isotope.

The two "tricks" that make AMS work are the molecular dissociation process that occurs in the accelerator and the charge detection at the end. The resulting sensitivity is typically a million times greater than that of conventional mass spectrometry. AMS can detect one ^{14}C ion in a quadrillion (10^{15}) other ions.

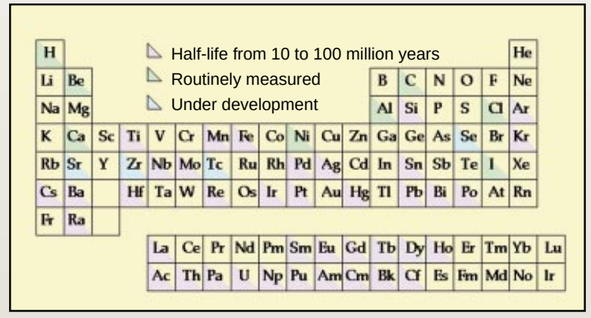
For ^{14}C dating, precision with accelerator mass spectrometry is typically within 0.5 to 1%, which corresponds to an uncertainty of plus or minus 40 years in a radiocarbon date. For other isotopes, precision generally ranges from 1 to 5% depending on the application.

In biological studies, AMS is used today primarily for counting ^{14}C because carbon is present in most molecules of biological interest and also because ^{14}C is relatively rare in the biosphere. Increasingly, however, other isotopes are being studied. The periodic table below presents the range of long-lived isotopes that are being used or have potential to be used in AMS studies.





Atomic mass spectrometry units can be arranged in many ways, but all incorporate bends with magnets that help separate ion masses in space. Ions of different masses travel around corners differently. The symmetry of Livermore's U shape makes the unit more accurate than units in other configurations, tightly focusing the beam of the desired ion mass.



The periodic table of accelerator mass spectrometry. Elements with isotopes whose half-lives are between 10 years and 100 million years are not easily detected by decay counting and are rare in nature.

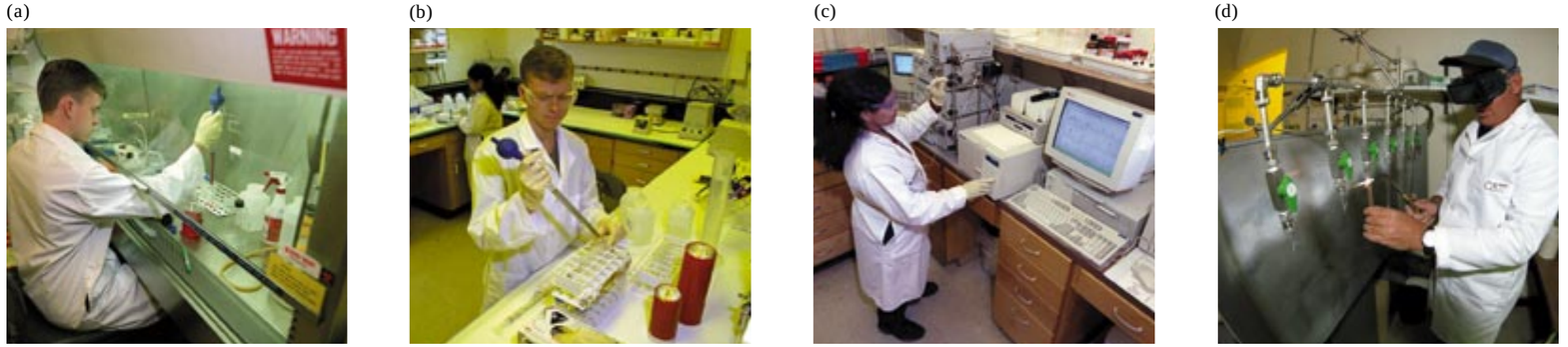


Figure 1. Preparing biomedical samples for accelerator mass spectrometry (AMS) requires many steps in an ultraclean environment to prevent contamination and maximize sensitivity. Many of the sample preparation techniques for biomedical applications of AMS were developed at Livermore. (a) Chad Risner prepares cell cultures for use in biomedical AMS experiments and then (b) uses columns to purify DNA from those cultures prior to AMS analysis. (c) For another application, Chitra Mani uses high-performance liquid chromatography (HPLC) to separate metabolites from the urine of animals to which carbon-14 benzene has been administered. Finally, (d) Kurt Haack seals the AMS samples collected from the DNA isolation procedure or HPLC analysis in quartz tubes as part of the process that turns the samples into graphite. Once the graphitization process is complete, the samples are ready to be loaded in a sample holder for their spin through the AMS system.

and several suspected human carcinogens. This kind of research represents a world of new biological research possibilities that will lead to major improvements in our everyday lives. According to Jay Davis, Associate Director of Earth and Environmental Sciences and the first director of the Center for Accelerator Mass Spectrometry, "AMS in archeology and the geosciences is a mature field. But applying AMS to bioresearch is relatively new. Ninety-five percent of all biomedical work with AMS is being done at Livermore. With our expertise, we can provide the technology that will enable these applications to find more widespread use. We hope to see these processes commercialized so that pharmaceutical and chemical companies can use AMS on a routine basis. They will be able to test—using realistic, low-level doses—drugs, pesticides, and other chemicals to learn how they affect our health."

A New Direction

The bulk of the center's work is radiocarbon dating for archeology and the geosciences, but a growing fraction of its sample analysis is related to biomedical research.

The arrival in late March 1997 of Caroline Holloway as the center's director signified the new direction that the center is taking. Holloway is a biochemist who had worked for many years in advanced technology development at the National Institutes of Health in Bethesda, Maryland. Why did she leave NIH to "hang out with a bunch of physicists"? As an NIH representative, she had visited Livermore in 1994 and was impressed with the evolution of AMS into biology being led by Livermore. When the directorship became available, Holloway says, "I jumped at the opportunity. Without question, the future of AMS in biology is now, and the future is happening at Livermore."

The center's emerging emphasis on biomedical applications has been preceded by years of experiments, many of which were performed primarily to prove the significance of AMS for biomedical research.

In 1990, the first biomedical experiment using AMS was performed at Livermore. It measured the effects on rat DNA of a suspected carcinogen, 2-amino-3,8-dimethyl-imidazo[4,5-f]-quinoxaline, known as MeIQx. MeIQx results from cooking meat and may be partly responsible for the observed frequency of gastrointestinal-tract cancer in the U.S.[2] Adducts, which are the defects formed by the covalent binding of certain chemicals to DNA, are routinely monitored as biomarkers of carcinogen exposure. These DNA adducts can result in chromosomal rearrangements, mutations, cell death, cancer, and birth defects.

Livermore gave low doses of synthesized MeIQx with a single ^{14}C atom in each molecule to rats. With AMS, they achieved a detection limit of one adduct in a trillion (10^{12}) nucleotides, a tenfold improvement over assays using other methods of detection.

Another early experiment looked at the effects of the highly toxic chemical dioxin, which was shown not to bind directly to DNA. The significance of this experiment and the one with MeIQx was not merely that AMS can be used to study genotoxicity at low levels but that accelerator mass spectrometry had potential value as a screening tool for genotoxicity of drugs or other industrial chemicals. Similar preliminary studies were performed to develop a methodology for conducting experiments on pharmacokinetics (how drugs move through an organism after being swallowed or injected) using relevant human exposure levels.

With all of this early work, Livermore scientists were defining not only how AMS could be used for biomedical research but also how best to do it. Process development continues today as Livermore "pushes the envelope" for accelerator mass spectrometry in biology. Every new experiment requires the development of new procedures and protocols and sometimes new instruments for such important work as sample preparation. A spin through the AMS machine takes just a few minutes, but preparing the sample beforehand takes much longer (Figure 1). Maximizing sensitivity requires new, ultraclean lab techniques to prevent sample contamination. Thus, the technology of biomedical AMS research is being developed along with new experimental applications.

Studies in Humans

A team of scientists that included collaborators from Livermore is using AMS to study the effects of MeIQx in humans. The purpose of this work is to quantitatively compare humans and animal models for human risk assessment. Experiments with animals typically use doses designed to induce tumors, and extrapolating from those numbers to the kind of doses that humans might encounter is not easy, in part because of the differences in physiology and metabolism between humans and animals.

At York University in England, five volunteers previously diagnosed with operable colon cancer and scheduled for colon surgery were administered ^{14}C-tagged MeIQx 4 to 6 hours before surgery. Tissue removed during surgery and not needed for pathology was analyzed for ^{14}C content by AMS. Urine samples were collected for 24 hours after administration of the dose and were analyzed for metabolism of MeIQx.

Test results suggest that the concentration of MeIQx in the human colon was highly variable among the five subjects. There also appear to be differences between normal and tumor tissues of the same volunteers. Among the volunteers, there seemed to be individual variations in MeIQx absorption and processing, which suggests differences in DNA adduct levels.

Even with all these variations, DNA adduct levels in humans appears three to four times higher than adduct levels in mice and rats. This study suggests strongly that MeIQx affects humans more than it does mice and rats and supports using AMS to pursue additional human biomedical risk assessments.

Calcium and Bone Health

AMS is also changing the study of the effects of vitamins and minerals in our daily diet. We know that they are needed for healthy living, but their effects are virtually unknown, other than that illnesses may result from their absence in our diet. AMS is finally allowing researchers to trace such nutrients in the human body.

A study of the dietary nutrient calcium is, for example, under way at Livermore. Studies of the skeleton and osteoporosis are a high priority, given the one million osteoporotic fractures that occur in the U.S. every year and their attendant $10-billion annual cost. Calcium is the key nutrient for bone health, so understanding calcium metabolism is critical (Figure 2). But until the advent of accelerator mass spectrometry, measuring calcium kinetics in bone directly was difficult. With decay-counting methods, only the short-lived radioisotopes of calcium can be used to trace the movement of calcium through the body. Very large amounts of the tracer

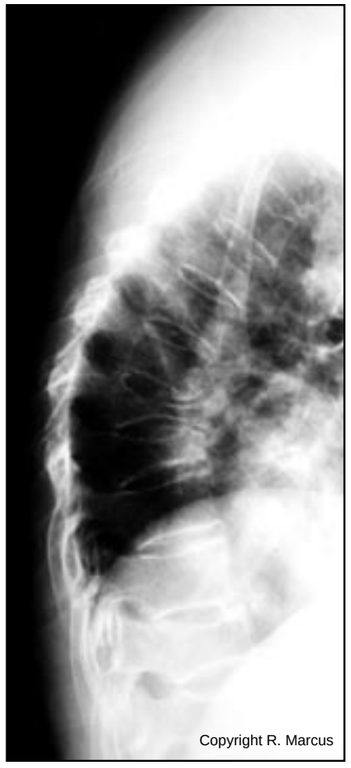


Figure 2. Sideview x ray of severely osteoporotic spine showing effects of prolonged calcium depletion.

must be used because ingested and absorbed calcium is only slowly resorbed by the skeleton. For any of the tracers to show up in urine, which is where calcium appears that has been lost from bone, the patient must ingest a dangerously high radiation dose. An alternative testing method is the use of stable isotopes as tracers, but they are extremely expensive.

What scientists do know about calcium metabolism comes from assays of blood and urine samples that look at byproducts related to calcium. These indirect data are useful for indicating trends in large populations. But they cannot be used for information on an individual because of the indirect nature of the tests.

With AMS, longer-lived isotopes can easily be studied. Because of AMS's sensitivity, only minute quantities are needed, so the patient receives a radiologically benign dose. Calcium-41 (^{41}Ca), with a 100,000-year half-life, fills the bill neatly for bone studies.

If calcium with a ^{41}Ca tracer is given to a patient, the skeleton will become "tagged" with ^{41}Ca. The ^{41}Ca in subsequent urine samples will indicate how much ^{41}Ca is being lost from the skeleton. Scientists from Livermore, the University of California at Berkeley and Davis, Stanford University, Creighton University, and the National Institutes of Health are currently testing data from initial ^{41}Ca experiments against the results of conventional calcium studies.

Calcium-41 shows promise as an effective tool in testing drugs that may prevent osteoporosis. Some drugs are designed to "seal" the bone to reduce calcium resorption. When treatment with such drugs begins, calcium loss will slow, and the amount of ^{41}Ca in urine samples will be reduced (Figure 3). Various therapeutic dosages and formulations can easily be studied and compared.

With ^{41}Ca and AMS, the kinetics of calcium in the human skeleton can for the first time be studied directly, enabling studies of fundamental bone processes and providing indicators of an individual's bone health.

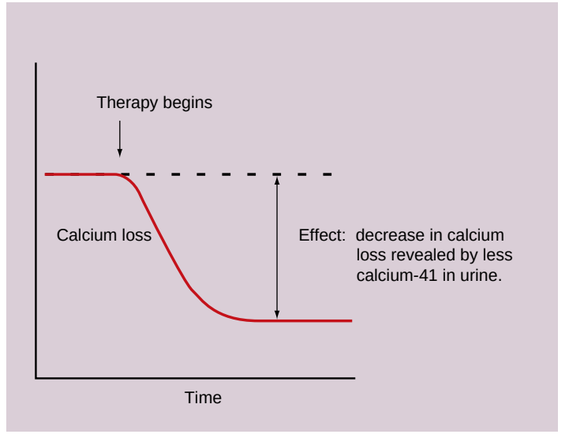


Figure 3. Graph showing drop in calcium resorption after drug treatment for osteoporosis begins.

The Future of AMS

Elemental trace nutrients are also yielding to the new developments in accelerator mass spectrometry at Livermore. Among those under investigation at the center are nickel, selenium, and iodine. The very long-lived isotopes of these elements that are detected through AMS will eventually be traced in humans at levels that present no discernible chemical or radiological danger.

More tests of drugs using human subjects and the tiny doses that AMS can measure will expand the base of information on metabolism, efficacy, and toxicity. The time period for testing drugs can be shorter, which will decrease the cost of bringing new drugs to market. Definitive human data will give users a larger margin of safety than they have today. As the database on human metabolism grows, scientists will come ever closer to being able to tailor and individualize therapeutic treatments.

According to Holloway, "Perhaps even more exciting is the relationship of accelerator mass spectrometry to the human genome. When the Human Genome Project is completed, some scientists envision a 'periodic table' for biology that comprises not 100-plus elements but 100,000 genes. Armed with knowledge of the complete human genome, scientists will begin to understand each protein that a cell makes as a result of instructions from a single gene."

Proteins are the life substances of the cell, carrying out the energy functions, synthesizing life components, and serving as the regulatory agents that control the traffic of molecules in the cell. AMS can contribute to understanding those cellular proteins that are made in only small amounts or that are present for only short periods of time because they are cell-signaling agents. For such studies, AMS scientists will collaborate across scientific disciplines and technologies with scientists using genomic tools, protein separation techniques, and other molecular speciation and separation methodologies.

Says Holloway, "Work on the human genome is just one of several new opportunities for the future growth of accelerator mass spectrometry in basic biomedical research."

—Katie Walter

Key Words: accelerator mass spectroscopy, biomedical research, calcium, Human Genome Project, human subjects, MeIQx, osteoporosis, radiocarbon dating, tandem Van de Graaff accelerator, toxicology.

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About the Scientist

CAROLINE HOLLOWAY has been director of the Center for Accelerator Mass Spectrometry since March 1997. She was previously with the National Institutes of Health, most recently as acting director of Biomedical Technology at NIH's National Center for Research Resources in Bethesda, Maryland. Holloway is a biochemist interested in lipids and biomembranes. She has conducted biomedical research at E. I. Du Pont de Nemours, the University of Virginia School of Medicine, and the Duke University School of Medicine. Holloway received her B.S. in 1959 from City College of New York and her Ph.D. in biochemistry in 1964 from Duke University, after which she completed postdoctoral research at Shell Agricultural Chemicals in England. She has published more than 25 scholarly articles and papers.