Biomedical Research Benefits

It's no fluke. An experiment that can track 35 micrograms of folic acid through a human for 200 days is routine for Livermore's Center for Accelerator Mass Spectrometry.

Members of Livermore's core biological AMS team stand in front of the new, small spectrometer (also seen in the larger photo) dedicated to biological studies using carbon-14. From left: John Knezovich, Bruce Buchholz, Graham Bench, Ken Turteltaub, John Vogel, Ted Ognibene, and Mark Roberts. Missing from the group is Karen Dingley.

THE short history of accelerator mass spectrometry (AMS) for biomedical research at Livermore has been sweet indeed. Just 10 years ago, Livermore scientists first used AMS to determine how low doses of a suspected carcinogen affect the DNA of mice. A remarkably sensitive measuring technique, AMS can seek out one carbon-14 isotope from among a quadrillion other carbon atoms. It achieved a tenfold improvement in detecting damaged DNA over the best methods then available, thus enabling studies to be conducted directly with humans. Ever since, Livermore has been expanding the development of AMS for biomedical and pharmaceutical applications and is today a recognized leader in the field. (See box on p. 14 for information on how AMS works.)

Livermore researchers are continuing to study the effects of carcinogens on humans and animals. Perhaps not surprisingly, they have discovered that humans and animals metabolize these substances differently, with resulting differences in the way DNA is affected. AMS is being used in collaborations with researchers from around the world to begin to solve many challenges in biomedicine—from examining the way we metabolize vitamins to developing a new cancer diagnostic test. AMS has even proved invaluable for learning how pesticides move through ant colonies from workers to the queen.

When Livermore proposed that the National Institutes of Health (NIH) establish a National Research Resource for AMS at Livermore, University of California (UC) and Department of Energy scientists provided key testimony to demonstrate the value of AMS. Along with all the other evidence, their testimony must have been persuasive. Last November,

from Counting Small

Lawrence Livermore joined an elite group of research facilities when NIH awarded the five-year grant. There are other NIH Research Resource facilities for various types of mass spectrometry, but this is the first for AMS. An NIH review of Livermore's proposal had this to say:

> *An overwhelming case was made for the need for an AMS resource and a number of outstanding collaborative projects have already been initiated. . . . At the present time, the LLNL Resource is clearly the most advanced site in the U.S. to explore the use of AMS in biomedical research.*

Chemist John Knezovich, director of Livermore's Center for Accelerator Mass Spectrometry (CAMS), is pleased with progress to date. "CAMS is unique in this country in concentrating on biological AMS. Beyond integrating the Laboratory's scientific expertise in both AMS and biomedical research, CAMS provides a major facility that is enabling research projects from all over the world."

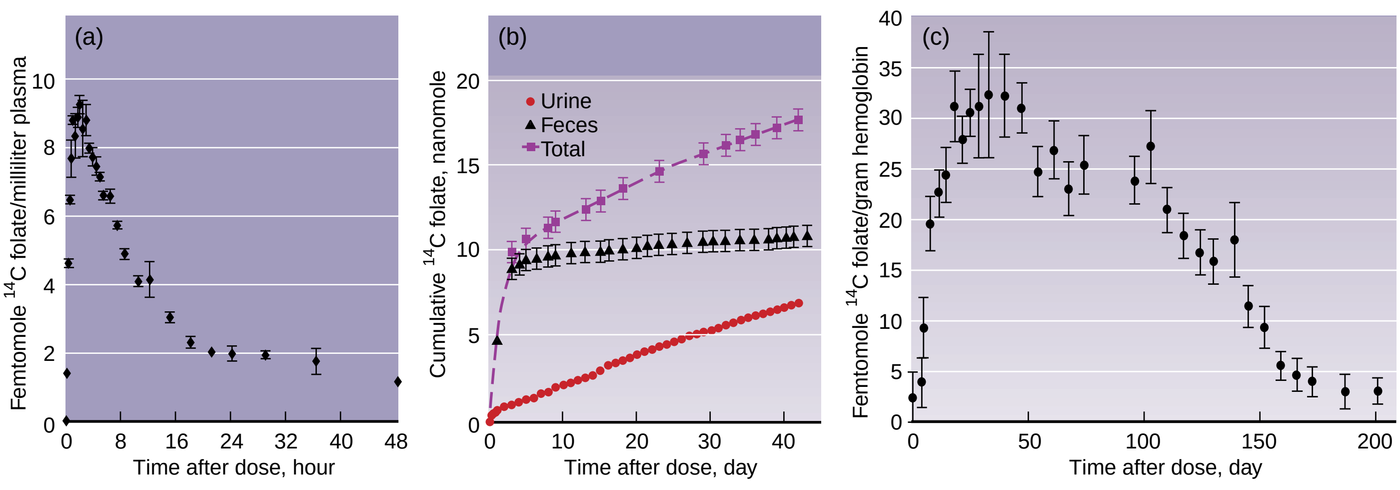
He adds, "A big plus is that in addition to the large, multipurpose AMS machine that we've had for years, a much smaller one will soon come on line that is dedicated to biological studies using carbon-14. And, we have begun to use yet another new spectrometer for biological samples that have been tagged with tritium, a new tracer element for AMS. On top of that, we have added a heavy-isotope line to our large AMS machine for studies of plutonium." (See box on p. 17 for more information on AMS equipment at Livermore.)

An important aspect of Livermore's work to date has been in establishing AMS as a routine biomedical research tool. An AMS experiment no longer requires a large and expensive staff of physicists and technicians. Livermore scientists have led the technological advances necessary to make AMS a more effective, dependable, and economic tool for the biomedical, pharmaceutical, chemical, and clinical communities (see also *S&TR*, November 1997, pp. 4–11).

Established in 1989, CAMS was designed to diagnose the fission products of atomic tests; monitor the spread of nuclear weapons to other countries by detecting telltale radioisotopes in air, water, and soil samples; and use isotopic tracers to study climate and geologic records. It still does all these things today—and much, much more.

Sensitivity Is the Key

So what is the value of AMS to you and me? AMS is an ideal method for tracing the passage of chemicals through humans without disturbing normal metabolic processes. Perhaps researchers want to know how the human body metabolizes a drug or vitamin. The molecules of the substance are manipulated slightly to "tag" them with a radioactive isotope, typically carbon-14, though other radioisotopes may be used as well. Rare radioisotopes of elements found in organic materials are used as tracers



A single dose of carbon-14-tagged folic acid was traced for 200 days. (a) The tagged folic acid appears very quickly in plasma (the liquid part of blood) and tapers off in about two days. (b) The amounts of tagged folate being eliminated in feces and urine were followed for 40 days. (c) Folate begins to be incorporated into hemoglobin at day 5. (Hemoglobin is the iron-containing, oxygen-carrying molecule in red blood cells.) The level of folate in hemoglobin peaks at about the 30th day and disappears only after 200 days.

because they can be incorporated into biomolecules and because they are present naturally at low levels, so tagged molecules can be detected easily.

A collection of human subjects swallows or otherwise ingests the substance. Then, using AMS to measure the number of carbon-14 atoms in samples of urine, feces, saliva, or blood over the next hours, days, and weeks, researchers can trace how much of the substance is absorbed, how it travels through the body, what organs it affects, how much of it is lost through excretion, and so on. The first experiment to trace the vitamin folic acid in a human followed a single dose of just 35 micrograms for a remarkable 200 days.

Information this detailed has never before been available using healthy human subjects. Less sensitive measuring techniques,

Accelerator Mass Spectrometry at Livermore

Livermore's Center for Accelerator Mass Spectrometry (CAMS) is home to the most versatile and productive AMS facility in the world. AMS is an exceptionally sensitive technique for measuring concentrations of isotopes in small samples, typically less than 1 milligram, and the relative abundance of isotopes at low levels. It can, for example, find one carbon-14 isotope among a quadrillion other carbon atoms.

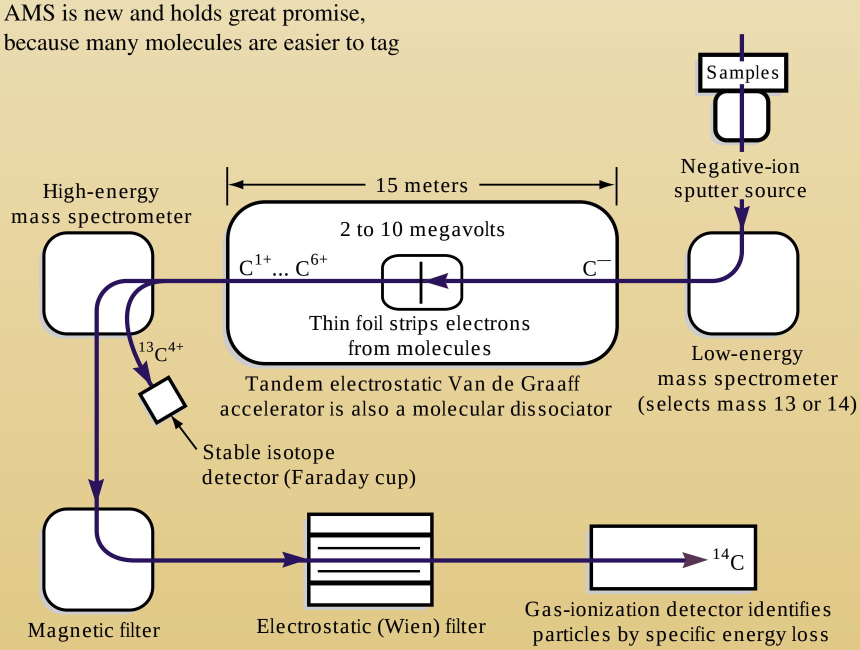
Mass spectrometry has been used since early in the 1900s to study the chemical makeup of substances. A sample is put into a mass spectrometer, which ionizes it and analyzes the motion of the various ions in an electric field to sort them out by their mass-to-charge ratios. The basic principle is that isotopes of different masses move differently in a given electromagnetic field.

In accelerator mass spectrometry, the same principle applies but the process is different. Negative ions made in an ion source are accelerated in a field of millions of volts. The accelerated ions smash through a thin carbon foil or gas that destroys all molecular species. After passing through a high-energy mass spectrometer and various filters, the ions finally slow to a stop in a gas ionization detector. (See the layout at right of the large AMS machine at Livermore.) The identity 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 a more abundant isotope—carbon-14 as a ratio of carbon-13, for example.

The two "tricks" that make AMS work so well are the molecular dissociation process that occurs in the accelerator and the ion detection at the end. The resulting sensitivity is typically a million times greater than that of conventional isotopic detection.

For biological studies, AMS has been used primarily for counting carbon-14 because carbon is present in most molecules of biological interest and carbon-14 is relatively rare in the biosphere. Tritium (hydrogen-3) has also been used extensively as a tracer in biological research. The use of tritium in AMS is new and holds great promise, because many molecules are easier to tag with tritium than with carbon-14. Other isotopes are measured by AMS as well, including plutonium-239, calcium-41, beryllium-10, chlorine-36, and iodine-129.

All over the world, AMS is still used primarily to count carbon-14 in archaeological and geologic samples for dating purposes. In the 1980s, it replaced the traditional method of scintillation counting for precise radiocarbon dating, which was time-consuming and required relatively large samples. Livermore performs radiocarbon dating and many other forms of AMS 24 hours a day, 7 days a week for its own research and collaborations as well as for others on a fee-for-service basis.



such as scintillation counting, require ingesting large doses of both the chemical being studied and the radioisotope, something few people want to do. Sometimes, such studies are done with volunteers. Usually, however, scientists have used animals for their research. Then considerable extrapolating has been required: from large doses to small doses and from laboratory animals to humans.

Now researchers can use much smaller, more realistic doses on human subjects. They can measure the true effects of a typical dose of, say, vitamin A or aspirin. AMS, the only method that can trace these low doses over such long time periods, has been described as the most significant new tool for nutritional studies since the 1930s.

And the amount of radioactivity taken in with the chemical being studied is less than one would encounter during a single day's exposure to background ionizing radiation from walking around in the sunshine. (Cosmic rays contain a small amount of radioactivity that we are exposed to every day.) An airplane flight exposes us to far more ionizing radiation than participation in one of these experiments.

Biochemist Ken Turteltaub, one of the developers of AMS for biological applications, is sold on the process. He says, "With accelerator mass spectrometry, we can address problems that cannot be solved otherwise."

Grant Recognizes Achievement

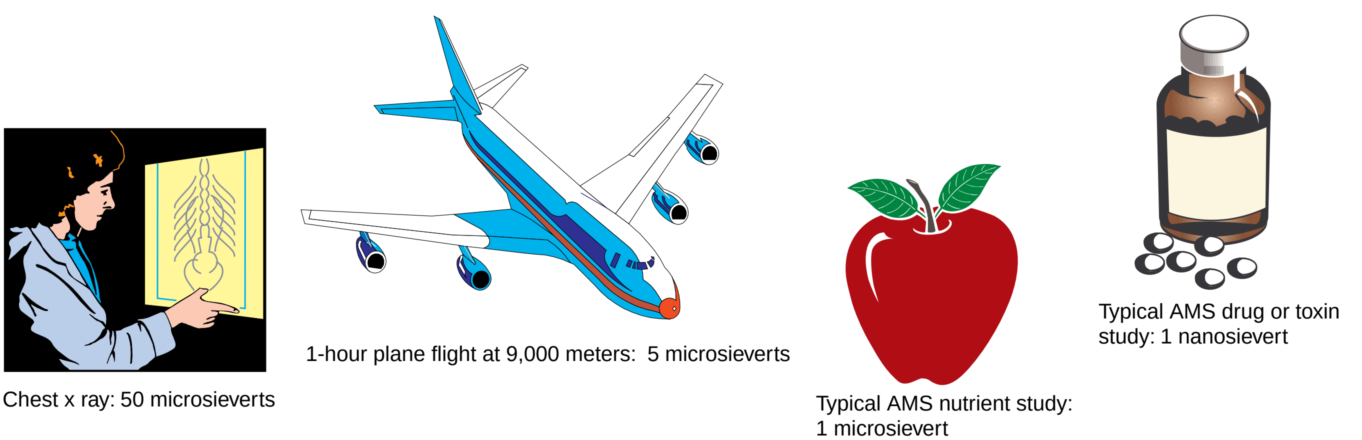
The Livermore team spent five years building and demonstrating the capabilities that led to the NIH grant to Livermore. Today, Turteltaub is the grant's principal investigator, assisted by fellow biochemist Karen Dingley and a team of CAMS scientists. As an NIH Research Resource for biological AMS, Livermore is charged with providing biological researchers throughout the country with access to carbon-14 AMS analysis in their research, developing new methods and instrumentation for the use of AMS in biomedical research, demonstrating new applications, and educating the biomedical research community on AMS. All of these functions have been under way at Livermore for many years, which makes award of the grant particularly gratifying as recognition of a job well done.

All NIH Research Resource grants focus on collaborative work in order to educate the biosciences community. For this grant, Turteltaub and his cohorts are working to expand support for the use of AMS and to export its use to as many researchers as possible.

In work that is just beginning, they are also developing new experimental methods so that AMS can be applied to as many types of biological research as possible. They are developing the sample preparation and analytical methods necessary to reduce handling, automate processes, and increase sample throughput. The team will explore new spectrometer components that allow the direct interface of bioanalytical instrumentation to the spectrometer for simplified, rapid, on-line analysis. Finally, further development of the small carbon-14 spectrometer will help to bring AMS machines to more bioanalytical laboratories.

Measuring Damage to Molecules

Turteltaub, Dingley, and other researchers at Livermore are already involved in several collaborations with researchers in the U.S. and England to examine the effects of substances produced by cooking meat. Both 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline



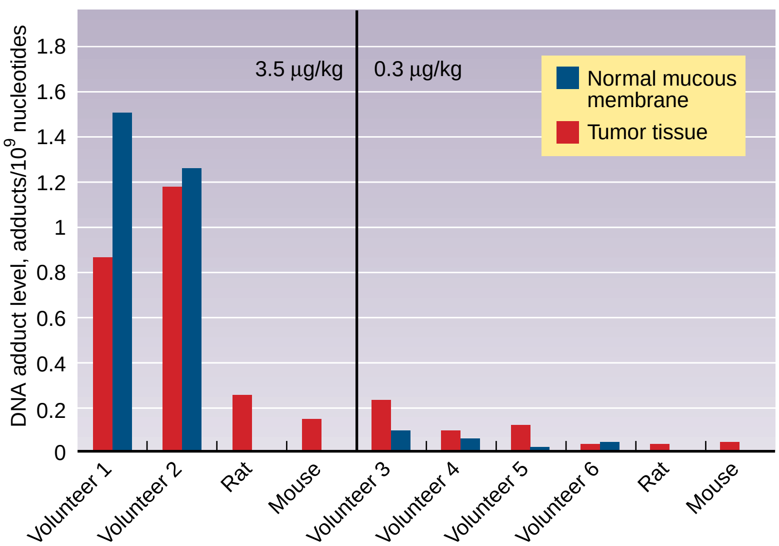
Taking a 1-hour plane trip exposes a person to 5,000 times more ionizing radiation than participating in an accelerator mass spectrometry study of a drug or toxin. Everyone on Earth takes in about 2.5 millisieverts of radiation every year. (A sievert is a Système International [SI] unit for radiation dose. It is equal to 8.38 roentgens. One microsievert is one millionth of a sievert and a nanosievert is one billionth of a sievert.)

(MeIQx) are heterocyclic aromatic amines that have been shown to cause cancer in laboratory animals when administered at high doses. The team has used AMS to establish whether DNA and protein adducts (damage) can be detected in laboratory animals and humans when they take in a smaller, more typical dietary amount of these substances.

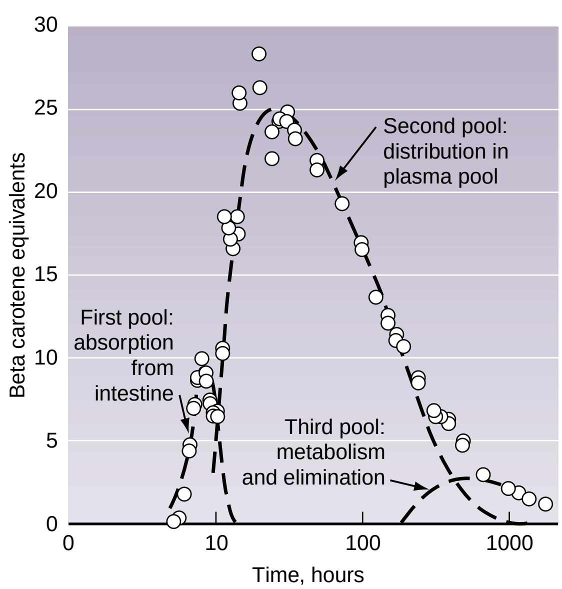
In numerous experiments using carbon-14-tagged PhIP and MeIQx molecules, the team has confirmed not only that adducts can be detected at low doses, but also that humans may be more sensitive to these substances than mice or rats. These results are important because researchers in the fields of toxicology, pharmacology, and nutrition currently make two basic assumptions: that data obtained from high-dose experiments can be accurately extrapolated to more typical environmental levels and that animal models are valid. While all the data are not yet in on the veracity of either assumption, AMS provides the sensitivity and precision needed to address each of them thoroughly.

Dingley performed the first AMS experiments using biomolecules labeled with tritium (hydrogen-3). With tritium-labeled PhIP and carbon-14-labeled MeIQx, she found comparable sensitivities at low doses. (The lowest doses were equivalent to the amount of PhIP you would take in when eating a single well-cooked hamburger.) These experiments demonstrated the feasibility of using tritium AMS for biomedical studies as well as the feasibility of using both tracers in experiments to study how two substances, given at once, may affect each other.

Tritium is widely used in biological tracing and has some advantages over carbon-14. It is relatively easy to label a molecule with tritium, whereas carbon-14-labeled molecules must be custom synthesized, which can be expensive. While the use of detection techniques other than AMS requires relatively high tritium dosages and large samples, AMS eliminates those disadvantages. In fact, Livermore's experiments have demonstrated that AMS could be used to detect tritium in biological samples with a 100- to 1,000-fold improvement over scintillation and other decay counting techniques.



Rodents and humans exhibit significantly different responses to high and low doses of MeIQx, a chemical that appears in meat after it is cooked. MeIQx causes damage to DNA known as adducts.



Three "pools" of carbon-14-tagged beta carotene can be identified from blood, urine, and feces collected for 3 months after ingestion. The first peak is due to carotene metabolizing to retinol in the intestine, from where it is quickly excreted. The largest pool in plasma reaches its highest point a day after dosing but is then drawn into a deeper pool, perhaps the muscular tissue, over the next 5 to 10 days. This pool is slowly metabolized back to a circulating form and finally, in the third pool, is eliminated.

From Ants to Elephants

AMS is ideal for measuring extremely small samples, certainly the case when ants are the subject of study.

Research Opportunities Grow with New and Better Equipment

A continuing thrust of the Center for Accelerator Mass Spectrometry (CAMS) at Livermore has been the development and application of new AMS technologies as well as the improvement of existing ones. Three entirely new systems for measuring carbon-14, tritium (hydrogen-3), and plutonium-239 have come on line in recent weeks and months. Tweaking of the 15-meter-long, multipurpose AMS unit, in place since the inception of CAMS in 1989, never stops. After 11 years of development, the unit's cesium ion source is the most powerful in the world. Sample preparation methods and experimental protocols evolve and are standardized as Livermore's capabilities grow and as the demands of the biological research community expand. The focus now is to robotize the sample preparation process as much as possible.

For several years, researchers at Livermore have been working to develop a smaller, less expensive spectrometer dedicated to carbon-14 biological research. Biological AMS experiments can be run more quickly than most other AMS applications such as radiocarbon dating. A dedicated bioresearch unit would free the larger machine for other AMS work.

Testing for the first such small unit began in June (see photo on p. 12). One-tenth the size of the larger machine, the small spectrometer will provide higher throughput for biological samples. It also will serve as a testbed for new sample preparation and delivery technologies.

John Knezovich, director of CAMS, says, "These smaller units are just beginning to appear. Livermore is one of four institutions in the world using one. Now, virtually every AMS unit, of whatever size, is custom-built. But when they have been developed to the point where the cost for one is below $1 million, then we can expect to see a proliferation of small AMS machines operating in universities and pharmaceutical companies, making significant scientific advances."

Livermore's new tritium system (below left) is the result of a collaboration with AccSys Technology of Pleasanton, California, and was funded by a National Institutes of Health/National Cancer Institute Small Business Innovation Research grant. The system was specifically designed to be small, simple, and inexpensive, making the technique of tritium AMS more broadly accessible.

Physicist Mark Roberts was primarily responsible for the system's design and for bringing it on line. Biochemist Karen Dingley, who performed the first AMS experiments using tritium as a biological tracer, has been developing experimental methods, including sample preparation and handling protocols. One of the system's first uses is in experiments to improve current methods to measure the rate at which cells divide. Cancer is a disease in which cells divide uncontrollably. Researchers want to know if exposure to cancer-causing chemicals results in an increased rate of cell division before a tumor appears. Normally, experiments such as this would require large doses of toxic chemicals or radioactive isotopes. With AMS, only a very small amount of tritium in drinking water does the trick. Then, the tritium in newly synthesized DNA can be measured. The level of tritium incorporation in DNA indicates the approximate rate at which new cells are synthesized and thus the rate of cell division.

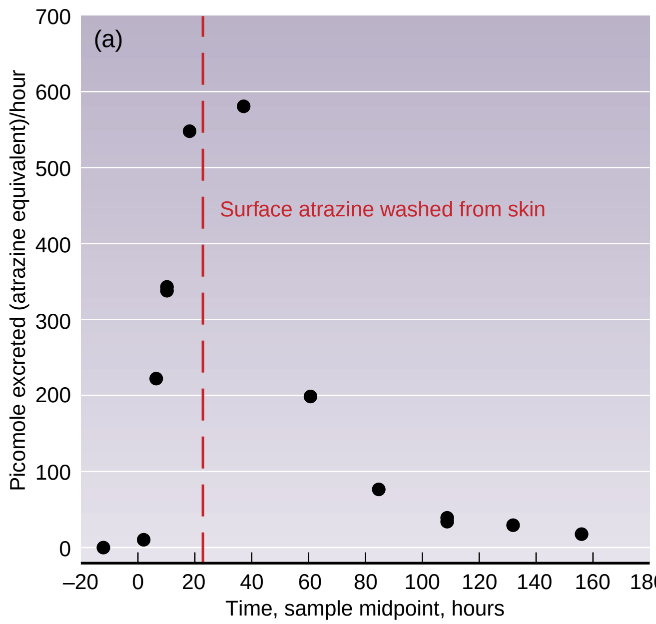
Physicist Jeff McAnich has led the development of a new heavy-isotope spectrometer (below right) that makes it easier to run plutonium samples on a routine basis. The initial focus of this work is the measurement of plutonium concentrations and isotope ratios in urine bioassays of Marshall Islanders who are being resettled to Bikini Atoll and other islands used in the 1950s for weapons testing. The unit will be able to measure plutonium-239/240 as well as plutonium-241. Other heavy isotopes such as neptunium-237 may also be studied in the future.

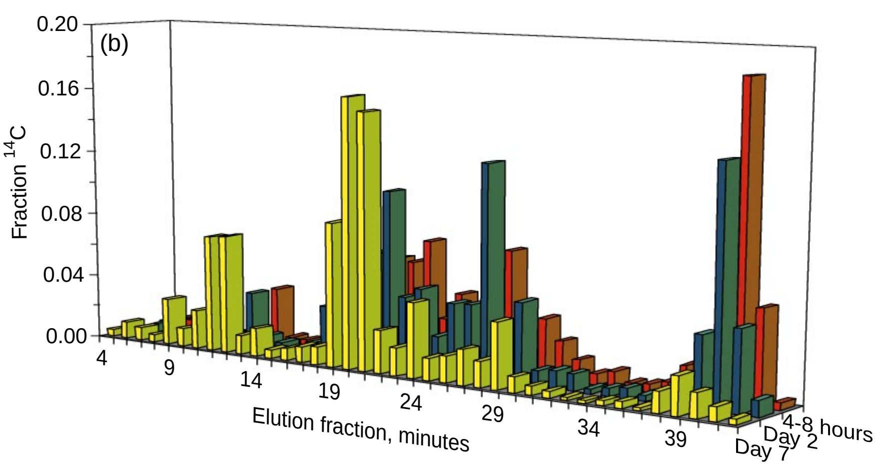
Livermore's Center for Accelerator Mass Spectrometry has developed specialized spectrometers for tritium (photo at left), heavy isotopes such as plutonium (photo at right), and carbon-14 (see p. 12), in addition to its large, multipurpose machine.

CAMS worked with UC Riverside to follow the path of carbon-14-tagged food and insecticides through colonies of Argentine ants housed at the university. Once native to Brazil and Argentina, the Argentine ant is now the most prevalent pest around homes in California, the Caribbean, the Mediterranean, and South Africa. No available control strategy works effectively because each colony is home to several queens, any of which can regenerate the colony if other queens are destroyed. Learning how nutrients make their way to the queens is essential to finding an effective control method.

Work on nutrient dynamics was part of the largest AMS collaboration in place before the new NIH Research Resource was established. It involves CAMS and four UC campuses. Several campus—lab collaborations are ongoing at Livermore and other laboratories managed by UC, and the UC Office of the President has described this one as the most successful of all. Livermore's principal investigator for the Consortium for Ultra-Low-Level Tracing is physicist John Vogel, assisted by Bruce Buchholz, who was trained as a nuclear engineer.

Vogel has been involved with CAMS from the start and has been responsible for many of the technological advances that have helped to establish AMS as an increasingly routine biomedical research tool. His work with UC Davis on nutritional studies has earned him the rank of adjunct full professor of nutrition. "One never knows where a career will go," he chuckles.





(a) The level of atrazine, an herbicide, peaks in the body about 20 hours after the wearer of an atrazine skin patch has it removed. (b) Atrazine metabolizes to products in the body that change markedly over time.

The CAMS–UC Davis experiment that followed a single dose of folic acid for 200 days was no fluke. Many over-the-counter products that we take with barely a thought stay in our bodies for a remarkably long time. A similar experiment followed a single dose of beta carotene in blood, urine, and feces samples for 3 months. The three primary "pools" of beta carotene or retinol, the vitamin A that beta carotene metabolizes to, are clearly visible, first in the intestine, then in blood plasma, and finally in a circulating form with a 38-day half-life. The existence and equivalence of the slowly changing pool that starts 9 days after dosing would be undetectable without AMS.

Metabolic studies of vitamins and other nutrients are of more than passing academic interest. At present, little is known about vitamin metabolism except that it seems to vary widely among individuals. Folic acid, one of the B vitamins, is required for the production of red blood cells, DNA, and RNA. A deficiency can cause anemia and is related to heart disease and certain birth defects. A deficiency in beta carotene can cause blindness, a serious problem in less developed parts of the world. In fact, a new variety of carroty-orange "golden" rice, rich in beta carotene, has recently been developed in Switzerland for use in undeveloped countries.

In 1998, a University of California at Riverside doctoral candidate won the annual award given by the *International Journal of Mass Spectrometry and Ion Processes* for best student paper. Her fundamental work in protein chemistry netted her a trip to Amsterdam as well as a cash prize. She could not have won without Livermore's biological AMS facilities because her subject, nematodes, supplied only the smallest of samples.

Dr. Andy Clifford, professor of nutrition at UC Davis, has headed all of this important nutrient metabolism work with CAMS. As a result of his research to date, he has been awarded follow-on grants from NIH to conduct definitive AMS studies in humans. His experiments are now under way using healthy human volunteers aged 18 to 60.

CAMS researchers are working with clinicians at UC San Diego to develop a diagnostic technique sensitive enough to detect the growth protein that cancerous tumors produce. Most of this protein stays at the site of the tumor, but a very small amount leaks out into the blood. Different kinds of tumors—ovarian, prostate, breast, liver, and so on—produce slightly different kinds of growth proteins. A blood test that could detect the protein would be an effective tool for an early diagnosis. The test could also be used to determine whether follow-up treatment was working; ideally, the protein level would drop to zero over time.

Yet another current study is of very large animals—elephants—but small samples. A hormone imbalance in bull elephants can produce raging behavior known as musth. The only way to obtain a sample from an enraged elephant is from its feces. But most of that is undigested fiber, leaving researchers with little usable material from which to measure the carbon-14-tagged hormone precursors that the elephant has ingested. AMS comes to the rescue again.

Science in Revolution

For all this work to date, biological AMS is still very new. Knezovich acknowledges this, saying, “We at Livermore are still doing lots of missionary work, educating the research community about this powerful technology. But the NIH grant makes clear that the biological research community is convinced of the worth of AMS.”

Livermore’s AMS experts are part of an apparent revolution in which biology may be replacing physics as the haute discipline. Research in biosciences now accounts for more than 40 percent of federal funding for basic research, fueled in part by an aging population and increased needs for health care.

As the use of AMS for biological research grows and matures, related applications will quickly be found. CAMS has already participated in a study of the effects of atrazine, a commonly used herbicide, on a group of volunteers in California who wore a skin patch of atrazine for 24 hours. Radioactivity in their urine was too low to be counted efficiently with liquid scintillation counting. But with AMS, uptake and elimination could be followed easily. Samples were even chromatographically separated to determine how the subjects metabolized the compound during exposure, a day after exposure, and several days after exposure. These biomarkers provide the data needed to develop an assay for occupational exposure to this chemical.

From agriculture to nutrition, from toxicology to chemotherapy—the potential uses of AMS for our better health are almost endless.

—Katie Walter

Key Words: biomedical research, carbon-14, Center for Accelerator Mass Spectrometry (CAMS), Marshall Islands, MeIQx, National Institutes of Health (NIH), nutrition, pesticides, PhIP, plutonium, radioisotope tracing, tritium.

For further information contact John Knezovich (925) 422-0925 (knezovich1@llnl.gov).

About the Scientist

JOHN KNEZOVICH has been the director of the Center for Accelerator Mass Spectrometry since May 1998. He received his B.A. in biology from the University of the Pacific in 1977 and his Ph.D. in chemical ecology from the University of California at Davis in 1983. He was previously a group leader for Environmental Chemistry and Toxicology. Knezovich is an environmental chemist with extensive experience in the design and application of experimental approaches for determining the fate, transport, and toxicity of contaminants in the environment. He originally came to Lawrence Livermore as a student guest in 1977 and returned as a postdoctoral scientist in 1983. Knezovich serves on University of California, state, and federal advisory panels that oversee research on toxic substances. He has written more than 60 scholarly articles and publications.