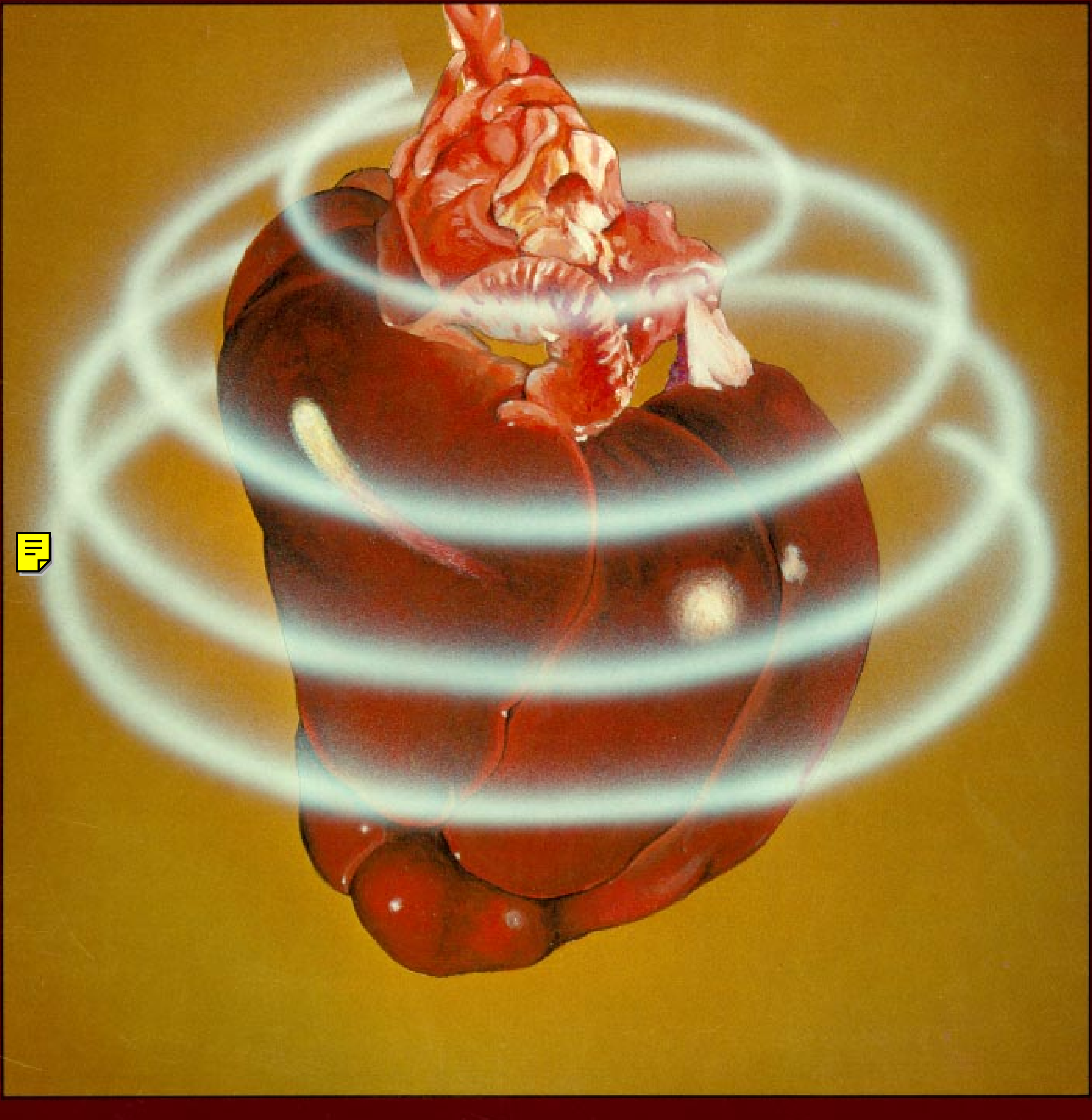
NUMBER 8 SUMMER 1983

NUCLEAR CHEMISTRY

Los Alamos Science

LOS ALAMOS NATIONAL LABORATORY



EDITOR'S NOTE

Nuclear chemistry is one of the lesser known and lesser publicized efforts at Los Alamos. At first glance the origin and fate of minute amounts of rare isotopes may seem much less exciting than big new laser projects or the novel abstractions of modern physics. But throw away your prejudice, gather some patience, and enter the fascinating world of isotopes and elements. Here meticulous care leads to determining the efficiency of nuclear explosions and discovering new elements in the debris. Tiny amounts of isotopes or elements provide unique signatures of events long past, such as the earth's collision with a large meteor that may have caused the dinosaurs' extinction, the existence of a natural nuclear reactor in the earth's crust, and the history of solar neutrino flux over billions of years. Here also the introduction of rare stable isotopes into complex systems provides a means for mapping the circulation patterns of the atmosphere and tracing the pathways of metabolism in living creatures.

The story of nuclear chemistry at Los Alamos began forty years ago when nuclear chemists fashioned highly radioactive, short-lived isotopes into gamma-ray sources to monitor the effectiveness of implosion weapons. Immediately after the Trinity test these scientists manned specially designed Sherman tanks to gather radioactive debris from the ground beneath the blast that would tell them the efficiency of the explosion. Jere Knight, nuclear chemist by profession and long-time ghost writer by natural talent and good will, has pooled his talent with Jim Sattizahn to present a fast-moving history of nuclear and radiochemistry at Los Alamos. It goes from weapons diagnostics for the Trinity and the Mike tests (the fabulously high neutron fluxes of the latter test produced two new elements) to the current panoply of research in biomedicine, geochemistry, nuclear waste storage, and basic nuclear science.

This history, rich in scientific detail, is followed by an article, written for the research scientists involved by Roger Eckhardt, on geochemical migration of radionuclides. Research in this area is providing a rational basis for decisions on underground storage of nuclear waste. For the past ten years Los Alamos scientists have done painstaking laboratory and field work to determine how water, the great mover of the earth's crust, may affect the migration of radionuclides in the tuffs at the Nevada Test Site. The question under study is: Should this site be chosen as a waste repository and, should a breach develop in the canisters containing the waste, will this site provide an adequate geologic barrier to migration?

There are at present over 10,000 tons of spent fuel at various places around the country waiting for a method and a site of storage to be chosen, and this number is expected to increase to 239,000 by the year 2000. The present plan is to characterize the geochemistry of three potential sites (tuff, basalt, and salt) by 1985 and have one operational by the mid 1990s. The responsibility involved in the final choice is awesome, so it is with interest and gratitude that we learn of the high-quality scientific effort being brought to bear on this decision.

The Los Alamos nuclear chemists are expert not only at tracing isotopes but also at separating both radioactive and stable isotopes and incorporating them into compounds for biomedical and environmental research. About ten years ago Nick Matwiyoff, leader of the stable isotopes program, realized that labeled compounds could be introduced into living systems and traced with nuclear magnetic resonance to monitor metabolic activity. In our cover story "Metabolism As It Happens" he and his coauthors describe the development of this emerging methodology and its potential as a tool for diagnosis or prevention of metabolic disorders. Great pains were taken to simplify this difficult subject, but the nonexpert may still need extra strength and patience to follow all the complexities of biochemistry. The reward is understanding the rich potential of a field still in its infancy.

Darleane Hoffman, highly respected as a scientist, is the first woman to become leader of a technical division at Los Alamos. She has for the last four years led and managed the diverse activities of the Isotope and Nuclear Chemistry Division. What is she like? How did she succeed in a man's world? In a short piece by Judy Lathrop we get a glimpse into the life of this remarkably clear-headed and attractive person. She is not only scientist, boss, wife, and mother, but also-as her colleagues quickly add—a trusted good friend. Her ability to make happen what she knows can happen without compromising the human gifts we most associate with the feminine side is an inspiration to those of us who sometimes wonder whether we can manage it all. ■

Necia Grant Cooper

Cover illustration by Rod Furan.
A nuclear magnetic resonance experiment with a perfused hamster liver—a marriage of physics and biology. The descending spiral around the liver describes the path of the induced magnetization vector. In these experiments the nuclear magnetic moments of stable isotopes make it possible to trace the biochemical pathways for resynthesis of glucose in the liver and to probe the regulation of the process. This new research technique, which promises to have widespread clinical uses, is described in the article *"Metabolism As It Happens."*

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EDITOR'S NOTE

NUCLEAR AND RADIOCHEMISTRY, PAST AND PRESENT

NUCLEAR MAGNETIC RESONANCE STUDIES

COMMENT

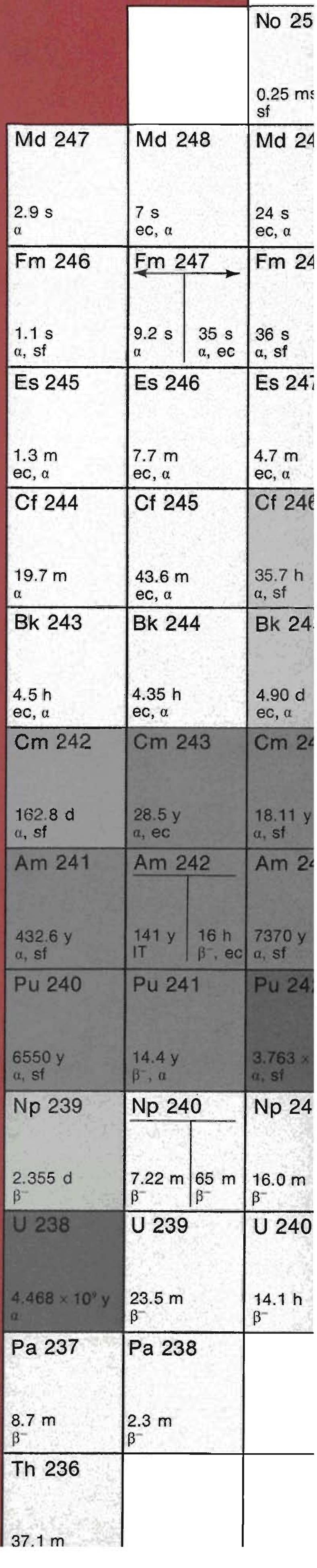
PEOPLE

Tracking the Isotopes

by Jere D. Knight and James E. Sattizahn

The story of nuclear and radiochemistry at Los Alamos is a story that reflects in many ways the growth and development of the Laboratory itself. It began in the early days before the test of the first nuclear explosive, when the Laboratory's overriding concern was to design and build such an object and to ascertain how well it would work, if at all. A group of radiochemists was set up under R. W. Dodson, and as soon as they could get their laboratories and equipment in running order, there was an urgent need for their services on tasks such as the preparation and calibration of radioactive sources and the measurement of fission products, as well as for their ideas on the application of radiochemistry to other problems. Later, as design ideas began to take shape in the form of full-scale models of the explosive system, large-scale radiochemical preparations were required, preparations far outside the scale usually dealt with in university laboratories. A well-known example was the chemical processing and mounting of lanthanum-140 sources emitting trillions of gamma rays per second. These sources were used for short-time-scale radiography of imploding systems, the so-called RaLa (for radioactive lanthanum), or Bayo Canyon, experiments.

Implosion was one of the ways chosen to achieve criticality in a nuclear bomb: a ball of fissionable fuel, say plutonium, was to be squeezed into a critical configuration by detonating a carefully tailored surrounding layer of conventional high explosive. The unexploded system resembled a very large apricot in which the plutonium and an intermediate layer of dense, neutron-reflecting metal are the pit. An important question was how rapidly, how uniformly, and how deeply the pit was squeezed when the high explosive was set off. A relatively simple way to study this process was to employ radiography on a similar configuration in which the plutonium was replaced by a nonfissionable but comparably dense metal. If one could somehow have a very strong gamma-ray source at the center of the pit and an array of gamma-ray detectors outside the complete bomb, one could measure the flux of gamma rays reaching the outside as a function of time during the course of the



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Data from the fifth edition of Karlsruher Nuklidkarte.

Since the earliest days the Laboratory's nuclear and radiochemists have been tracking isotopes to assess the performance of nuclear weapons. Today their interests also include geochemistry, biomedicine, and basic nuclear science.

explosion. These data would provide information on the change in amount of dense absorber between the source and the detectors and thus on the change in configuration of the pit caused by the implosion. The key to this diagnostic scheme was to find such a gamma-ray source. The fission products barium-140 and lanthanum-140 (the same isotopes that led to the discovery of fission) provided the solution. Barium-140 has a half-life of 12.8 days and decays to lanthanum-140, which has a 40-hour half-life and emits an energetic gamma ray with its decay. As the operation was finally worked out, the lanthanum-140 was extracted from a large batch of its barium-140 "parent" (obtained from Oak Ridge) and put into a compact receiver in the center of the pit to be tested. Chemical separation and further purification of the lanthanum-140 were carried out by a group headed by Gerhart Friedlander at a temporary building a mile or two from the firing site in Bayo Canyon. There were then no elegant "hot cells" nor sophisticated remote-handling apparatus. To minimize radiation exposure, the radiochemists rigged up the chemical processing equipment so that the essential operations could be performed and monitored from a distance with a system of cables, mirrors, and telescopes. The final lanthanum-140, concentrated into a volume of 0.1 milliliter, was then heavily shielded and trucked to the firing site, where another simple remote-control rig transferred it into the implosion test assembly. The source, implosion assembly, and gamma-ray detectors were destroyed in the test explosion, but the barium-140 supply back at the processing site was available to serve another day: because of its relatively long half-life it could "grow in" one or more new lanthanum-140 sources for use a few days later. The RaLa implosion tests became a regular diagnostic practice; with steady improvements in technique and with increasingly strong sources, they were continued until 1962, when they

What Are Radiochemistry and Nuclear Chemistry?

The definition of the terms "radiochemistry" and "nuclear chemistry," and the delineation of the presumed difference between them, is a problem that bedevils the toilers in this vineyard whenever they try to tell what they do. Historically, radiochemistry appears to have been the first in line. When Madame Curie was carrying through her laborious chemical procedures to isolate and identify the radioactive elements and to establish their transformations, she was doing radiochemistry. It was not until some time later that the nature of the nucleus and its reactions became clear. In most of the early researches, chemical manipulations were essential, and by the late 1930s a recognizable body of techniques and strategies had evolved to deal with the rapidly growing list of radioactive species. And it was by such chemical manipulations that Otto Hahn and Fritz Strassmann first demonstrated the occurrence of nuclear fission. Examining the products resulting from neutron irradiation of uranium, with an atomic weight around 238, they found unmistakable evidence for radioactive barium with an atomic weight around 140; they were forced to conclude that the barium came from the uranium by a process that could only have been some kind of a splitting of the uranium nucleus. Their employment of chemical techniques to study nuclear phenomena may be considered nuclear chemistry.

Unfortunately for those who would compartmentalize science into neat bins, our subject has broadened greatly in the span of time since the discovery of nuclear fission. Nowadays, its practitioners range from the "pure" radiochemists, whose primary concern is with the chemistry involved, to some "nuclear chemists" who are concerned only with nuclear physics problems and who rarely get their hands on any radioactivity—or vice versa. In our article we occupy something of a middle ground. Those pursuits in which the chemical operations are clearly of first importance we call radiochemistry, and those in which the problems of nuclear structure and reactions are of first importance we call nuclear chemistry. If in telling our story we turn out to have been inconsistent in employing this criterion, we plead guilty and ask our radiochemist/nuclear chemist critic to cast the first stone. ■

Wartime photo showing transfer of a heavily shielded gamma-ray source at the RaLa chemical processing site in Bayo Canyon. Motive power is being supplied by group leader Gerhart Friedlander and anchorperson Norma Gross, a WAC assigned to Los Alamos. Such sources were used to study the process of implosion, a method for achieving a critical configuration of the fissionable fuel in a nuclear weapon.

were supplanted by other diagnostic schemes.

The Trinity Test

The task that was to become the dominant occupation and responsibility of the nuclear and radiochemists up to the present day was one that began with the first nuclear explosion, the Trinity test of July 16, 1945 at the Jornada del Muerto site in central New Mexico. That task was the measurement of the efficiency of the explosion.

The efficiency of a nuclear explosion, like that of an industrial process such as the conversion of coal into electrical energy, is measured by the fraction of the energy content of the valuable starting material, the fuel, that appears as usable output energy. Thus, in the case of an explosion produced by nuclear fission of a fuel such as plutonium-239, the efficiency is given by the fraction of the plutonium-239 originally in the device that actually underwent fission, or "burned." The nuclear chemist proceeds to derive this fraction by gathering a small sample of the debris from the explosion and performing radiochemical separations to isolate, individually, the plutonium and at least one radioactive fission product. By measuring the plutonium sample in an alpha-particle counter, he obtains the number of remaining plutonium nuclei—those that did not fission—which we shall call p. By measuring the fission-product sample in a beta- or gamma-ray counter, he obtains the number of fission-product nuclei. He then employs an empirical constant to convert this number to the number of fissions that had occurred in the debris sample, a quantity we shall designate by f. (The empirical constant is derived by measuring the same fission product in a calibration standard of plutonium-239 in which a known number of fissions has been induced.) The efficiency of the explosion, ϵ, is then given by

$$\epsilon = \frac{\text{fissions}}{\text{original }^{239}\text{Pu nuclei}}$$

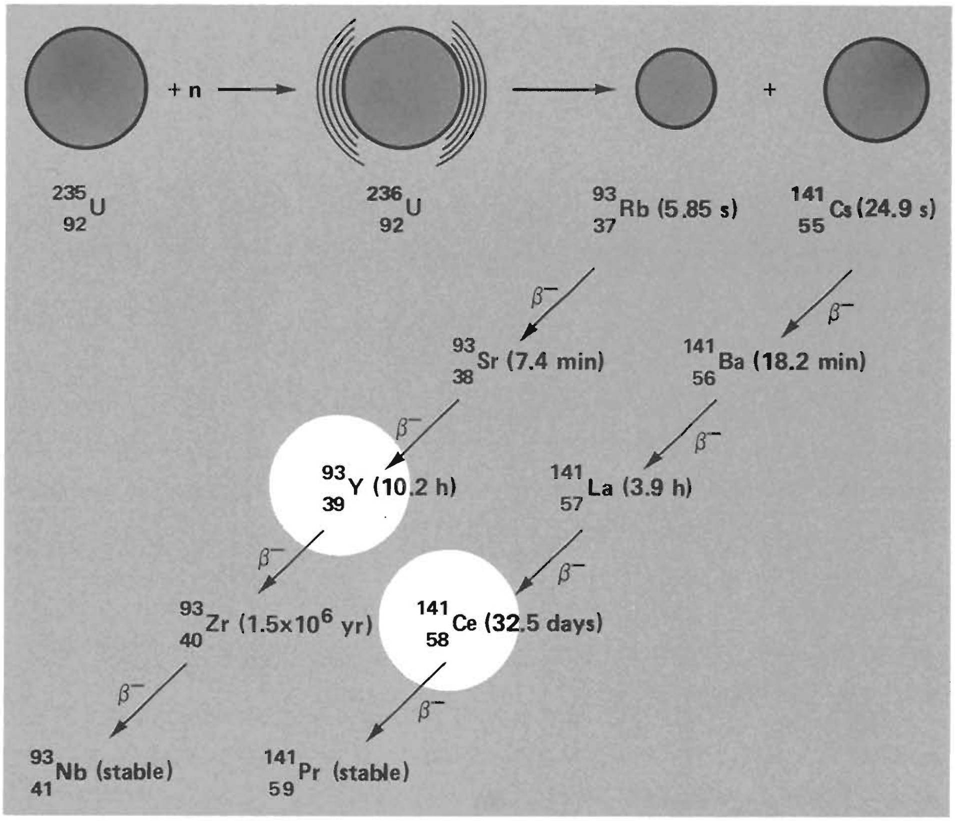
$$= \frac{\text{fissions}}{\text{fissions} + \text{remaining Pu nuclei}}$$

$$= \frac{f}{f+p} \; .$$

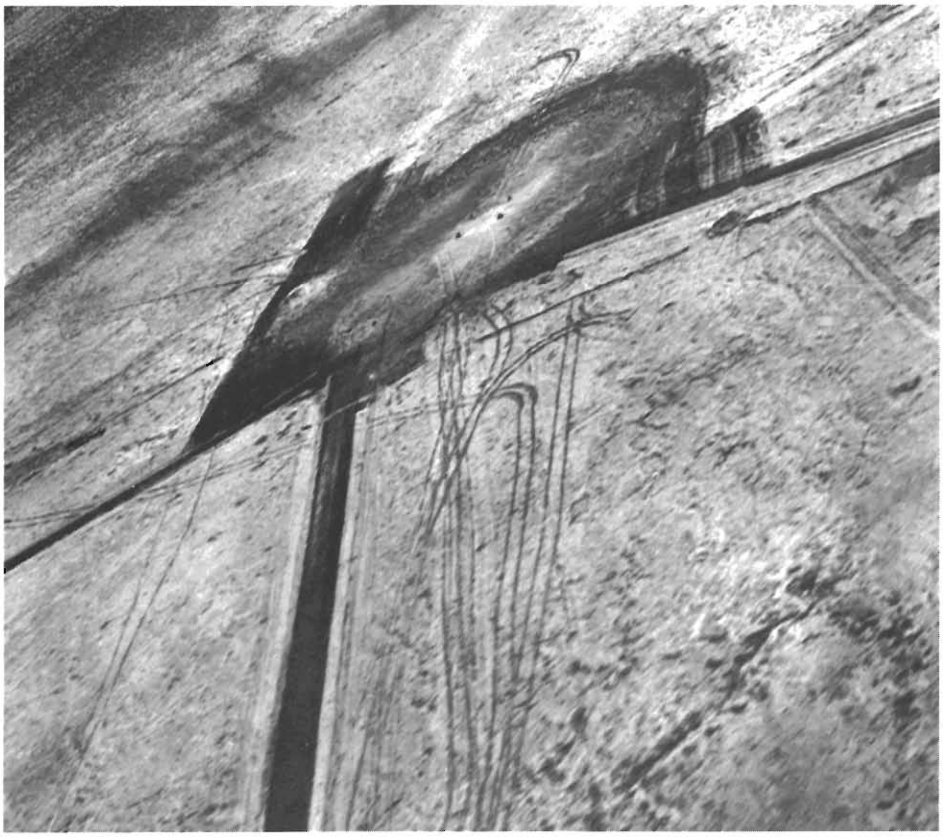
Thus, we see that we are able to determine the explosion efficiency by measuring the fission products and the unburned fuel in a small fraction of the explosion debris *without having to know explicitly the fraction of the total debris represented in that sample.* In actual practice we can perform this measurement on only ten trillionths of the total debris created by a 10-kiloton test, if the efficiency is the only performance parameter desired. Also, in actual practice there are a number of nontrivial complications that we have swept under the rug: some of the plutonium-239 is consumed by the nonfission reaction $^{239}\text{Pu}(n,\gamma)^{240}\text{Pu}$ (that is, plutonium-239 + neutron → gamma rays + plutonium-240); some additional plutonium-239 may have been created by neutron reactions with any uranium-238 present in the device followed by beta decay $[^{238}\mathrm{U}(n,\gamma)^{239}\mathrm{U} \xrightarrow{\beta^-} {}^{239}\mathrm{Np} \xrightarrow{\beta^-} {}^{239}\mathrm{Pu}]$; and chemical fractionation may have caused our sample to become enriched or depleted in the fission product chosen for analysis, so that f and p may not be found in the ratio in which they originally occurred. There are methods for dealing with these and other complications.

By multiplying the efficiency by the number of plutonium-239 nuclei known to have been put in the device, we can calculate the total number of fissions that occurred in the explosion. This is the number that the nuclear chemist actually determines, and it can easily be converted into a yield, or total energy released. For practical reasons nuclear explosion yields have traditionally been expressed in terms of tons or kilotons of TNT equivalent. Conversion of the chemist's number to kilotons involves multiplying total fissions by a nominal energy release per fission and inclusion of minor additional energy contributions from sources such as radiative neutron capture. There are other measures of the energy release in a test explosion (fireball expansion rate, atmospheric pressure wave, underground seismic signal, and so on) but the direct nuclear chemistry measurements provide the standard energy release to which all the other measurement techniques are calibrated.

The determination of the energy released in the Trinity explosion employed just these principles and measurements, albeit with equipment that would be considered primitive by modern standards. The job was performed by a team headed by Herbert Anderson and Nathan Sugarman, the latter on temporary assignment from the Manhattan Project at the University of Chicago. Their final report of the measurement, dated September 25, 1945 and still bearing a secret classification, contains some interesting notes on the planning and execution of this unprecedented operation. Since it was recognized that, if the explosion went as it was



The energy generated by both nuclear power reactors and so-called atomic bombs arises from the neutron-induced fission of certain isotopes such as uranium-235 and plutonium-239. Shown here is a typical fission event: absorption of a neutron by uranium-235 leads to an excited state of uranium-236, which splits into two primary fission products, in this event rubidium-93 and cesium-141, within about 10^{-21} second. (Not shown are the additional neutrons emitted as the excited nucleus splits; these neutrons make the fission reaction a self-sustaining, or chain, reaction.) Each of the neutron-rich primary fission products undergoes a series of beta decays leading eventually to a stable isotope. As the residues of a nuclear weapon test, the fission products contain information about the weapon's performance. For example, the number of fissions that occurred can be determined by assaying a sample of the explosion debris for one or more of the fission products. A radiochemist setting out to do this by assaying for one of the fission products in the decay chains shown here would most probably pick yttrium-93 or cerium-141. The preceding chain members decay too rapidly, and the subsequent members decay too slowly (or not at all). The excited uranium-236 nucleus can also split initially into any one of a large set of possible primary fission-product pairs. The resulting mixture of primary fission products and their decay products, which contains various isotopes of about three dozen elements, is the almost universal diet of Los Alamos radiochemists. Although a nuisance when a particular isotope must be isolated, the fission-product mixture is a rich lode of material for basic research in nuclear chemistry, including investigations of the fission process and studies of individual nuclides on the neutron-rich side of stability. Such studies at Los Alamos have resulted in the discovery of at least fifteen new nuclides with half-lives ranging from less than a second to millions of years.



The crew that collected samples of radioactive debris from the Trinity crater rode to its destination in a Sherman tank outfitted with lead shielding and bottles of breathing air. The samples were gathered through a trapdoor in the bottom of the tank. The lower photo is an aerial view of the crater and the tracks left by the tank.

supposed to, the best samples would probably be found in the ground beneath the blast, means had to be devised to get at this material. Two Sherman tanks were made available for the purpose. One of them was fitted with lead-shielded compartments for the driver and the observer/sampler, who gathered earth samples through a hole in the floor of his compartment with a vacuum cleaner or by driving a hollow pipe into the ground. The second tank was equipped to fire rockets into the center of the crater from a distance of 500 yards; the rockets were fitted with sampling noses and with cables for retrieval. Both methods worked, and a good set of samples was brought back to Los Alamos for processing and radiochemical analysis.

One's first reaction on reading these accounts today is astonishment that such measurements could be made with useful accuracy at all, given the primitive instruments available at the time. Apparatus now considered indispensable—high-resolution gamma-ray detectors, multichannel pulse-height analyzers, and stable transistorized electronics, to name just a few—did not exist. In point of fact, the efficiency and yield numbers generated by this work were probably accurate to ±10 percent, a quite respectable error even today. The nontrivial lesson to be learned is that ingenuity and scientific craftsmanship can achieve impressive results even with rudimentary equipment.

More Weapons Testing and a Radiochemistry Group

The experience gained in the Trinity test was soon put to use in the summer of 1946, when similar diagnostic measurements were made on the two test explosions of Operation Crossroads at Bikini Atoll in the Marshall Islands. There was still no specifically designated nuclear and radiochemistry group at Los Alamos, most of the experienced personnel having left in the great diaspora following

Samples of airborne debris from the Operation Crossroads tests of 1946 were gathered by drone aircraft. Shown here is a sampling unit being installed behind the cockpit of a drone B-17. The filter paper in the sampling unit was specially treated so that the debris would stick to its surface.

the end of World War II. Thus, when the call came for a working group to carry out the task at Crossroads, a team of experienced individuals was gathered from Los Alamos, Argonne, Oak Ridge, and various universities under the leadership of William "Buck" Rubinson.

This time, two kinds of postexplosion debris samples were gathered: from the atmosphere, by drone B-17 and F-6F aircraft, and from the sea, by drone boats. To provide early information in the field, a temporary radiochemical laboratory with appropriate radiation counting equipment was set up nearby at Kwajalein Atoll. The shorter lived products from the explosion (such as zirconium-97 and molybdenum-99 from fission and neptunium-239 from neutron capture on uranium-238) were analyzed at Kwajalein, and the longer lived products (strontium-89, zirconium-95, barium-140, and cerium-144 from fission) were analyzed in samples taken back to Los Alamos. The total plutonium content of the individual samples was measured at both laboratories, but measuring the ratio of plutonium-239 to total plutonium, which involved fission counting at a reactor, was of course done at Los Alamos. [This ratio provides information about the fraction of a weapon's original plutonium-239 content that is "wasted" in nonfission neutron reactions such as $(n,2n)$ and (n,γ).]

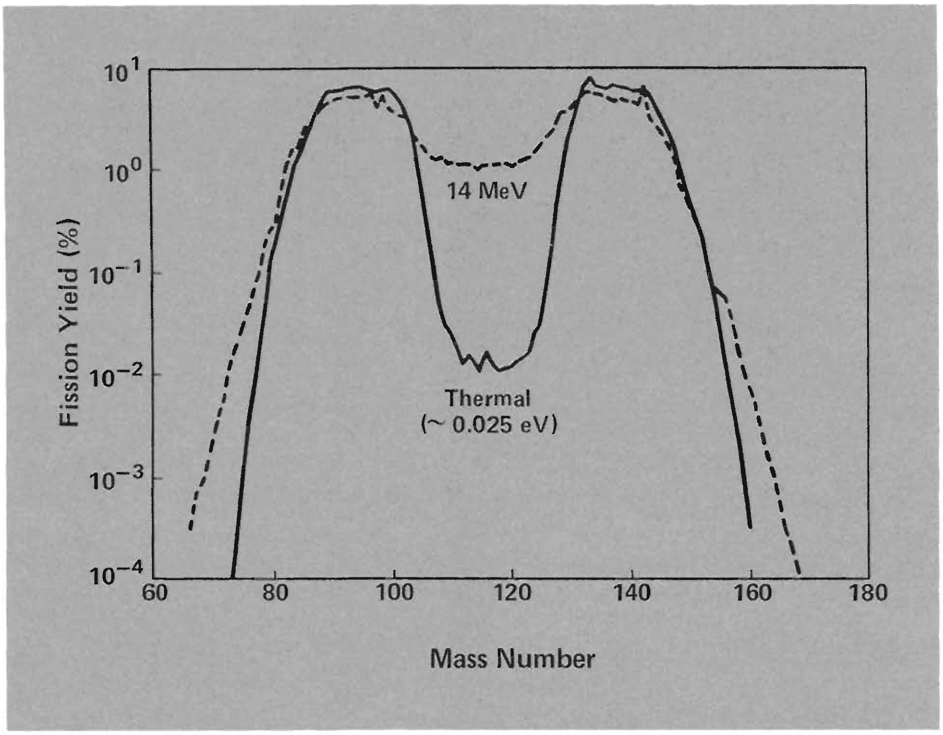
The fission-product measurements for Operation Crossroads showed for the first time in a practical way the consequences of the occurrence of noble-gas elements in fission-product decay chains. Fission products such as strontium-89 and barium-140, which derive in large part from the noble-gas ancestors 3.15-minute krypton-89 and 13.7-second xenon-140, did not "track" well with other fission products derived from chains with very short-lived or insignificant noble-gas ancestry. It was clear that even less than a minute spent as a gas during decay of a predecessor was enough to cause significant chemical fractionation of that fission product relative to others and to neptunium and plutonium. No way has ever been found to exploit this fact for study of bomb-cloud phenomenology; its only effect has been to restrict the choice of fission-product nuclides suitable for weapons test diagnostics.

The period beginning in late 1946 and early 1947 saw the formation at Los Alamos of a permanent nuclear and radiochemistry group led by R. W. Spence and soon to become a part of J Division, the test organization. This change had two important effects. First, it provided the organizational focal point for dealing in a more coherent way with the spectrum of contributions that nuclear and radiochemists could make to a weapons development laboratory. More important from a scientific standpoint, it set the stage for the transition of the nuclear chemists from their status as primarily consumers of basic science information to producers as well.

Operation Sandstone in 1948, in which three new experimental fission-weapon designs were tested at Eniwetak Atoll, was an important landmark for the new group and for the entire Laboratory. The successful conduct of these tests and the information gained from them confirmed to the defense establishment and to the fledgling Atomic Energy Commission the importance of continuing Los Alamos as an integral part of the national defense research and development effort. From a more technical standpoint these tests showed that radiochemical analyses of atmospheric samples alone could provide essential efficiency information and in addition detailed information on the performance of individual components of the test device. The demonstrated success of air sampling followed by radiochemical analysis was important not only to the nation's domestic nuclear weapons testing program but also to the development of its atmospheric surveillance system for detecting and evaluating possible foreign weapons tests. It was such a system that picked up evidence of a nuclear event in the Soviet Union in August 1949, and radiochemical analysis of the evidence was able to show that the event was indeed the test of a nuclear explosive device.

Continued Growth and the Mike Test

Following the Operation Sandstone tests, and with them the assurance of a continuing contribution to the Laboratory's future, members of the newly organized nuclear and



The neutron-induced fission of uranium-235 results in a wide range of products. The graph above shows that the yield of these fission products has a relative minimum in the mass region corresponding to symmetric and nearly symmetric fission and that the yields in this region increase with the energy of the neutrons inducing the fission. Yield curves for other fissile isotopes exhibit similar shapes and variations with neutron energy.

radiochemistry group (now a part of J Division) began a program of research and development along two complementary lines. First and perhaps the most immediately urgent was development of radiochemical procedures, instrumentation, and calibrations to support both programmatic functions and research in this field. The second was the research itself. Of particular interest was the study of fission-product yields and decay properties. Careful measurements were made of the mass distributions of fission-product yields from the fission of uranium-235, uranium-238, and plutonium-239 induced by various reactor neutron spectra. Similar measurements were made for fission induced by monoenergetic 14-MeV neutrons. [Neutrons with this energy are a product of the reaction $^3H(^2H,n)^4He$ induced by bombarding targets containing tritium (hydrogen-3) with deuterons (hydrogen-2 ions) from the Laboratory's Cockcroft-Walton accelerator. The same reaction between tritium and deuterium at thermonuclear temperatures is the dominant reaction and energy source for controlled thermonuclear power.] The latter results, subsequently reported at one of the Gordon Research Conferences on Nuclear Chemistry, are believed to be the first of their kind. Studies were also begun on the decay properties of individual fission products and a variety of other nuclides and on nuclear cross sections, in particular cross sections for reactions with 14-MeV neutrons. Some of this work led in 1949 to the group's first discovery of a new radionuclide—an isomeric state of niobium-97 denoted by ^{97m}Nb. The scheme for decay of zirconium-97 to ^{97m}Nb and its decay to the ground state was reported in *Physical Review* and became the first in a large number of research papers to be published by Los Alamos nuclear chemists and their collaborators from other groups and institutions.

An important part of the Laboratory's scientific history, and one that contributed significantly to the development of nuclear and radiochemistry, was the creation in 1949 of a special arrangement with the University of New Mexico to provide scientific instruction at Los Alamos. As part of this program, Professor Milton Kahn of the University's Chemistry Department began commuting from Albuquerque to give a two-semester night-school course entitled "Chem. 213. Radiochemistry." This program helped fill an urgent need: an opportunity for Laboratory employees to carry out graduate-level studies and eventually to earn advanced degrees while continuing their active commitment to ongoing Laboratory programs. Though the Ph.D. requirements included an academic year in residence at Albuquerque, in practice a candidate could and did return frequently to Los Alamos to take part in the activities of his group. After the year of residence, the candidate returned to Los Alamos to do his thesis research; the topic was chosen to be relevant to Laboratory interests, and the progress of the work was guided jointly by Professor Kahn and a Laboratory staff member. A total of thirteen University of New Mexico doctorates were awarded on radiochemistry and nuclear chemistry theses under this program. Doctoral thesis work of students from the Massachusetts Institute of Technology, the University of Wyoming, and the University of California, San Diego has also been performed here under similar arrangements.

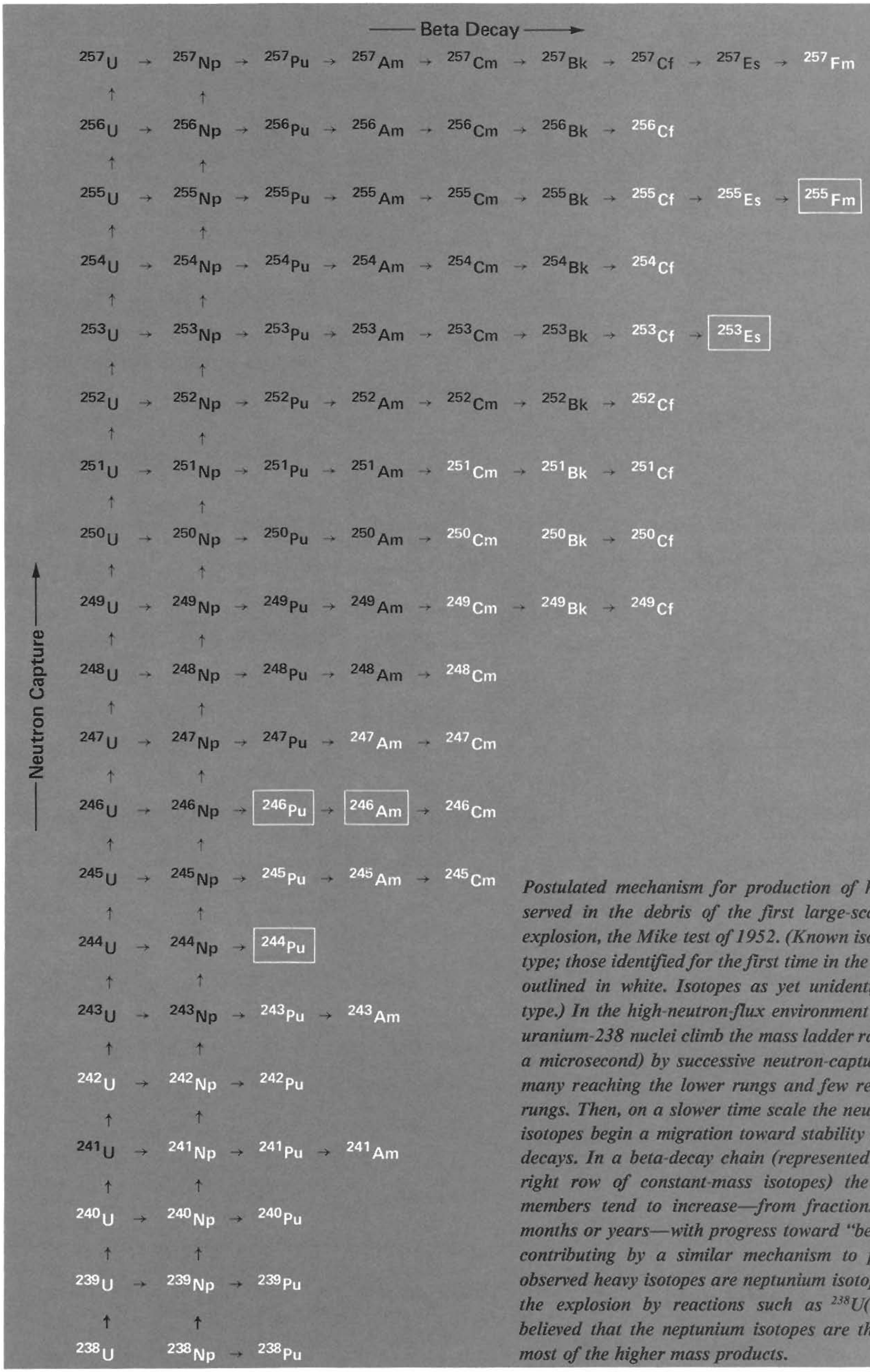
The major expansion in the Laboratory's weapons design and testing effort that began in 1950 had a corresponding impact on the size and scope of the group's activity. The first Nevada test series in early 1951, a test series at Eniwetak in the spring of 1951, and another Nevada test series in the fall of that same year presented the group with the sharp challenge not only of providing diagnostic information on a large number of fission explosions, but also of exploring and eventually developing means for obtaining quantitative diagnostic information on the phenomena associated with thermonuclear fusion components. The latter led to the first use of "radiochemical detectors": small amounts of suitable target elements incorporated into or near the device components to provide, by their level of activation, a measure of the neutron spectrum and fluence at these sites. The success of these early detector trials has led in succeeding years to a steady growth in the scale and sophistication of this diagnostic tool, to the point where nowadays the detector information is the most important radiochemical contribution to weapons testing.

The first large-scale thermonuclear explosion, the Mike test of 1952, handed the nuclear chemists an unexpected bonus: from its unprecedented neutron fluxes came a grand assortment of transuranium nuclides, including two new elements. Using samples of airborne debris collected by aircraft from the Mike cloud, a collaboration of nuclear chemists from the University of California Radiation Laboratory, Argonne National Laboratory, and Los Alamos undertook a thorough analysis of the transuranium elements by means of cation-exchange resin separations. These experiments showed a number of previously unknown radioactive species, among them an isotope of element 99 emitting 6.6-MeV alpha particles and a 7.1-MeV alpha emitter growing from an element-99 parent. Further work identified the first of these activities as $^{253}99$ produced by beta decay of californium-253 and the second as $^{255}100$ arising from beta decay of $^{255}99$. The new elements 99 and 100 were eventually named einsteinium and fermium, respectively. Also discovered in the Mike debris were the new nuclides plutonium-244 (the longest lived of the plutonium isotopes) and the beta emitters plutonium-246 and americium-246. Detailed examination of the abundances of the transuranium isotopes revealed what had happened. Some of the uranium in the device, together with some of the neptunium produced by various nuclear reactions during the explosion, was exposed to neutron fluxes high enough to produce strings of successive neutron capture reactions leading to uranium and neptunium isotopes with masses sixteen units or more heavier than those of the starting isotopes; these neutron-rich product isotopes then underwent successive beta decays culminating in the nuclides later observed by the radiochemists. Indeed, because of the new family of nuclear phenomena made possible by the great neutron densities achieved in the thermonuclear burn, the Mike debris turned

Ion exchange is a powerful technique for separating chemically similar elements. Chemist Deva Handel here loads an ion-exchange column with a solution of mixed rare earths. The columns (light vertical tubes at right center) contain a resin similar to the zeolite in water softeners. Flow of a complexing agent causes the rare earths to migrate through the resin, each at a different rate. The plastic tubing on the left carries the emerging solution to test tubes mounted on an automatic sample changer.

A Spin-off in Space

The success of radiochemical detectors for weapons diagnostics led directly to the development of a device performing a similar function in quite a different environment—space. Radiation exposure of spacecraft and their crews was of course a concern of the nation's manned space-flight program, but measuring the kind and amount proved an awkward task. The instruments that do the job well in the laboratory require power, room, and attention that are at a premium in a spacecraft. Through previous collaborations on NASA space experiments, a member of the nuclear and radiochemistry group became aware of the problem and organized the designing of a radiochemical dosimeter that offered a partial but economical solution. Designed to measure neutron spectra and weighing approximately one pound, the dosimeter contained target specimens of uranium-238, yttrium-89, scandium-45, and titanium-46, -47, -48, -49, and -50. After a nine-day journey in space aboard the Apollo spacecraft that rendezvoused with the Soviet spacecraft Soyuz in July 1975, the dosimeter was returned to Los Alamos for radiochemical analyses within twenty-four hours after splashdown. The analysis of the radioactivities induced in the target isotopes, in conjunction with the body of neutron cross-section data derived from the group's experience with radiochemical detectors for weapons diagnostics, gave information on total neutron fluxes and on fluxes within various energy bins up to about 40 MeV. The data, apart from their intrinsic interest, were of value in interpreting other experiments carried out during the space mission. One noteworthy result was that the overall neutron exposures in the spacecraft had been lower by a factor of about 2.5 than was expected on the basis of calculations from previous flights. ■



Postulated mechanism for production of heavy isotopes observed in the debris of the first large-scale thermonuclear explosion, the Mike test of 1952. (Known isotopes are in white type; those identified for the first time in the Mike test are also outlined in white. Isotopes as yet unidentified are in black type.) In the high-neutron-flux environment of that explosion, uranium-238 nuclei climb the mass ladder rapidly (in less than a microsecond) by successive neutron-capture reactions, with many reaching the lower rungs and few reaching the higher rungs. Then, on a slower time scale the neutron-rich uranium isotopes begin a migration toward stability by successive beta decays. In a beta-decay chain (represented here by a left-to-right row of constant-mass isotopes) the half-lives of the members tend to increase—from fractions of a second to months or years—with progress toward "beta stability." Also contributing by a similar mechanism to production of the observed heavy isotopes are neptunium isotopes formed during the explosion by reactions such as ^{238}U(^{2}H,n)^{239}Np. It is believed that the neptunium isotopes are the predecessors of most of the higher mass products.

out to be a proverbial gold mine for basic nuclear science as well as for the science and technology of thermonuclear explosions. We return to this exciting subject in a later section.

The remaining years of the '50s up to the moratorium on testing of November 1, 1958 were a period of hectic activity and vigorous scientific growth for the group. During this period there were four weapons test series at Nevada and three in the Pacific, for all of which the group provided essential diagnostic information. In addition, research on radiochemical procedures and on nuclear properties proceeded along several lines, some of them prompted by the needs of weapons diagnostics, others by ideas, special sources, and facilities deriving from the test activities. An especially important factor in this work was the availability of state-of-the-art radiation measuring equipment, including 4π beta counters, magnetic discrimination instruments for positron counting, multichannel pulse-height analyzers for alpha and gamma scintillation spectrum measurements, and a magnetic beta-ray spectrometer.

Diagnostics for Nuclear Rocketry

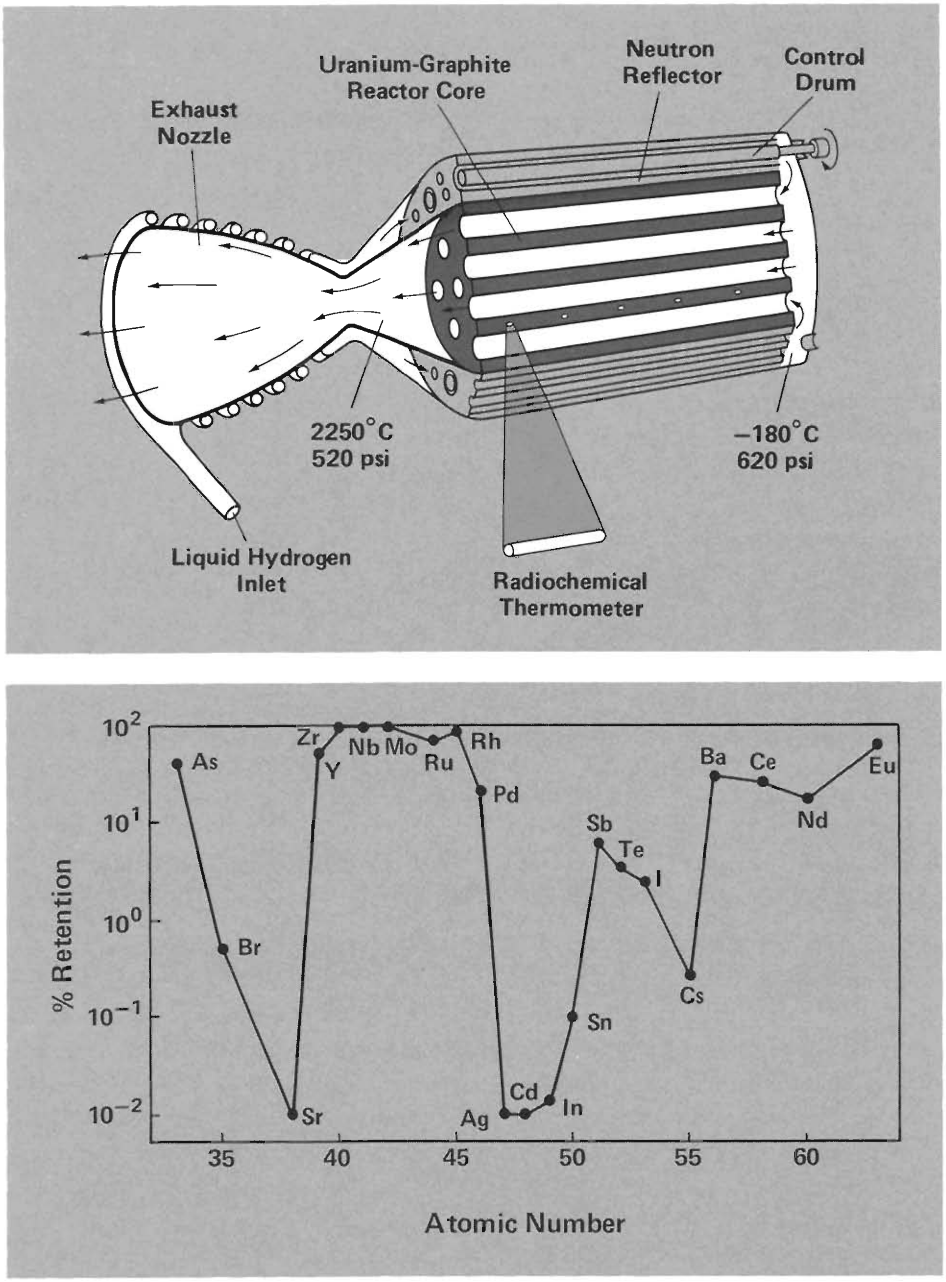
The middle '50s saw another significant change for the nuclear and radiochemistry group: R. W. Spence, who had created the group in late 1946 and led it through nine years of expansion, was appointed second in command of a new division set up to study nuclear rocket propulsion, and his place as group leader was taken by G. A. Cowan. An early member of the Manhattan Project at Princeton University and the University of Chicago and with Los Alamos for Operation Crossroads, Cowan had been with Spence's group since 1949 until he was brought to the J Division office in early 1955. Shortly after assuming the group leadership in November 1955 (the group was then known as J-11), he initiated what was to become a second major program for the group over the next fifteen years: the diagnosis of nuclear rocket performance by radiochemical techniques.

The program to investigate the use of nuclear reactors for rocket propulsion, the Rover program, represented a major new initiative for the Laboratory. The basic concept was to heat a low-molecular-weight gas—hydrogen was the obvious choice—by passing it through a compact reactor and to direct the now high-temperature gas stream through a nozzle as a high-specific-impulse propellant. Practical exploration and development of this concept involved diagnostic needs for which radiochemical techniques were well suited: measurement and calibration of fission-energy release, analysis of fission distribution in the reactor fuel elements, assays of the amount of fuel and fission products escaping from the reactor into the propellant, and a variety of problems associated with creation and migration of radioactivity under high-temperature conditions.

For a reactor with uranium-impregnated graphite fuel, the design type provisionally selected for Rover, there were three concerns of special importance to high-temperature operation: the loss of uranium fuel by diffusion out of the fuel elements, the loss of delayed-neutron-emitting fission products that provide the margin of reactor control, and the escape of fission products in general to the propellant stream. With these concerns foremost in mind, the group began immediately an experimental study, in the temperature range expected for the reactor, of the rates of diffusion and volatilization into a gas stream of a large number of elements from fission of uranium in a graphite matrix. Although some data were available from previous studies conducted elsewhere, it was necessary to have a wider and self-consistent data base suited to the Rover components. From the large amount of these data three important results emerged: none of the uranium diffused out; although substantial amounts of the delayed-neutron emitters bromine and iodine were lost at temperatures of 2000 degrees Celsius and above, more than half of the delayed-neutron species were retained; and some of the fission-product elements, such as silver and cadmium, were found to be very mobile. In one experiment the sharp variation in mobility was exploited to measure the half-life of palladium-115, a newly discovered fission-product isotope. Uranium-loaded graphite slugs were irradiated with neutrons, allowed to decay at room temperature for varying lengths of time, and then heated to expel the more volatile products, among them being the cadmium-115 granddaughter of palladium-115. (This procedure is somewhat analogous to a chemical "milking" experiment.) Analyses for the cadmium-115 showed the half-life of palladium-115 to be 47 ± 3 seconds.

The studies of the variations in diffusion rates with element and temperature led also to the development of a means for indirect determination of temperature distribution in the reactor. Small cartridges of graphite loaded with radioactive tracers selected to cover a range of diffusion rates were emplaced at various locations in the reactor during assembly. These "radiochemical thermometers" were recovered after a reactor test run and analyzed for tracer content. Comparison of the tracer losses with losses from identical units put through calibration runs in ovens back at the laboratory gave the approximate temperatures at the reactor locations. Our records show that in a typical full-scale reactor test we employed sixty such thermometers, each one loaded with the tracers barium-133, americium-241, and plutonium-239, listed in order of decreasing diffusivity.

By far the largest component of the nuclear and radiochemistry group's participation in the Rover program was the detailed post-mortem of the fuel component of the various reactors after full-scale tests. The reactors were disassembled at the Nevada Rocket Development Site, and the fuel elements were returned to Los Alamos for determination of fission distribution, total

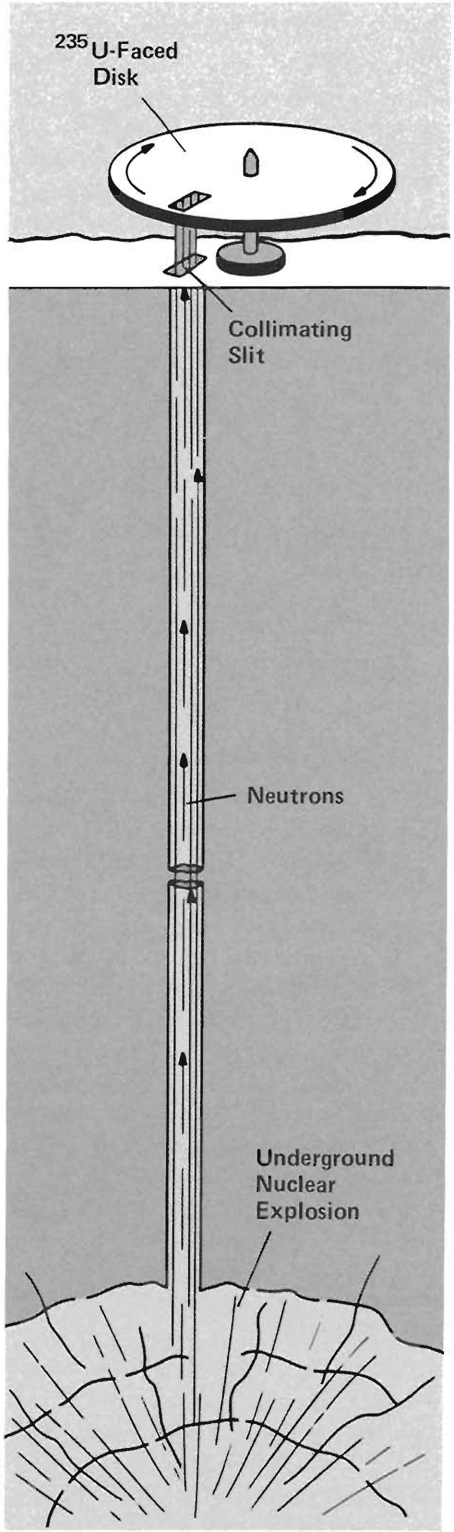


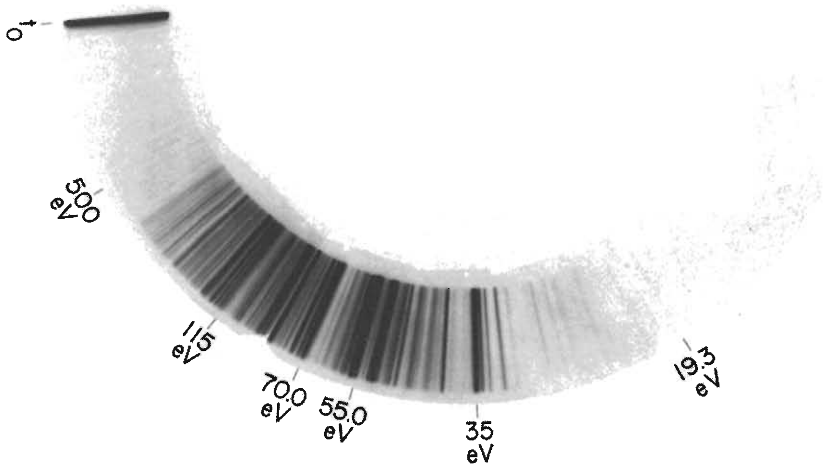
From 1955 to 1970 Los Alamos was investigating the principles of nuclear rockets, rockets propelled by a low-molecular-weight gas heated to high temperature by passage through a nuclear reactor. The upper figure shows schematically the design of one of the rocket engines built and tested by the Laboratory. [From R. W. Spence, International Science and Technology, 58-65 (July 1965).] The hydrogen not only provided the rocket's thrust as it exited through the exhaust nozzle but also cooled the nozzle as it entered the system in liquid form through the coiled pipe. The Laboratory's nuclear and radiochemistry group was called upon to study various aspects of rocket reactor behavior, including the diffusion of fission products from the graphite fuel matrix at the expected operating temperatures. The lower figure shows data from one such study in which members of the group measured the amounts of various fission-product elements remaining in a graphite sample after it was held at 2400 degrees Celsius for 4 minutes. The variation of diffusivity with element and with temperature led to the development of "radiochemical thermometers" for determining temperatures within the reactor core during a test run. The thermometers contained radioactive isotopes of elements covering a range of diffusivities. The losses of the isotopes measured after the test run gave the temperatures at various reactor locations.

fission, and fission-product loss to the graphite moderator and to the atmosphere. This large-scale effort, extending from 1958 to 1967, had important consequences for future undertakings of the group. It brought to the radiochemistry site a versatile chemical hot-cell facility and a complement of up-to-date radiation-measuring apparatus with computerized data-processing equipment (the latter included a PDP-8 computer acquired in 1966). Even more important than the facilities, it brought the group valuable experience and a broadened outlook in state-of-the-art radiochemical operations on the 100-curie scale.

Scientific Applications of Nuclear Explosions

In the middle and late 1950s the nuclear chemistry group undertook another activity that was to make a significant contribution to basic nuclear science: the specific application of nuclear explosions to the measurement of quantities and the production of materials that lay outside the reach of conventional laboratory facilities. This research direction, initiated and actively promoted by Cowan, had its first noteworthy success with the "wheel experiment" carried out during a nuclear weapon test in 1961. This experiment was aimed at measuring the variation in mass symmetry of uranium-235's fission at its neutron resonances. [A little explanation may be in order here: for the neutron-induced fission of, say, uranium-235, the term "mass symmetry" describes the extent to which the compound nucleus uranium-236 (the intermediate product formed by absorption of a neutron) splits nearly in half, and the term "neutron resonance" describes the enhanced absorption of neutrons with energies corresponding to the differences between that of uranium-235 and those of semistable states of the compound nucleus.] A disk faced with uranium-235 was set spinning in front of a slit at the upper end of a vacuum pipe leading to an underground

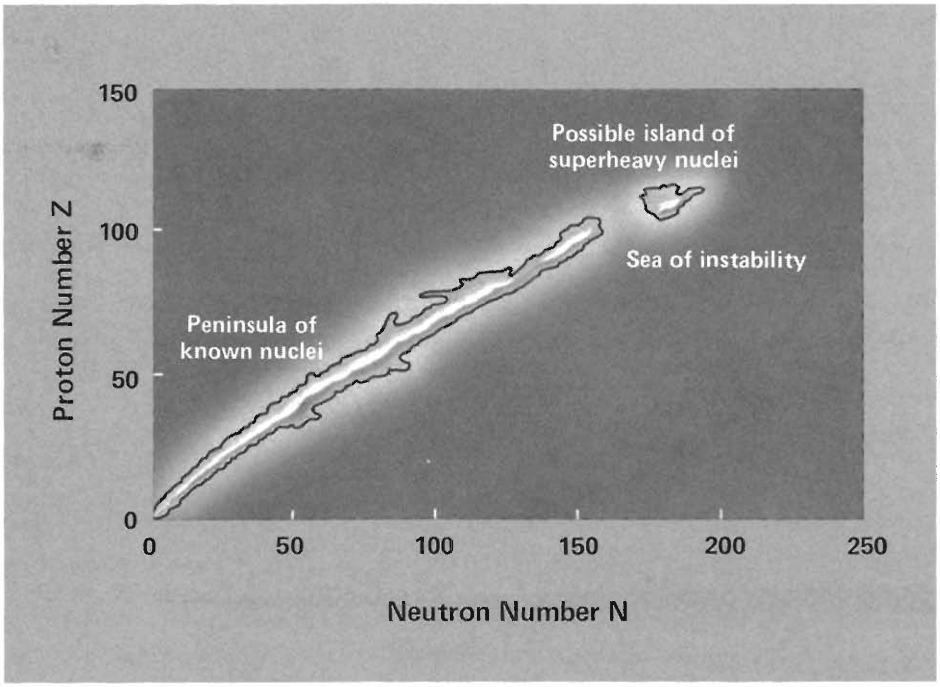




Shown schematically at left is the setup for the "wheel experiment" to determine the variation in mass symmetry of the fission induced in uranium-235 by neutrons with energies corresponding to its neutron resonances. The neutron source was an underground nuclear explosion, which produces, essentially in a single burst, neutrons with speeds ranging from one-fifth to ten millionths the speed of light. By the time the neutrons reach the target disk, they have been separated in energy by time of flight; that is, neutrons with different energies reach the target at different times—the fast first, the laggards later. Because the disk is rotating, the neutrons arriving at different times strike the target at different locations. Those neutrons with resonant energies induce many fissions and create bands of fission products in the uranium-235. Shown above is an autoradiograph of the fission products on the disk's surface. The energy scale was derived by identifying the time of the explosion t_0 ***(revealed on the disk by a large number of fast-neutron-induced fissions) and a few prominent resonances.***

nuclear explosion. The pulse of neutrons from the explosion, separated in energy by time of flight, created in the uranium-235 a series of fission-product bands corresponding to the resonances. Radiochemical analysis of the individual bands for total fissions and for silver-111 and cadmium-115, products of nearly symmetric fission, gave the symmetric-fission yields at the resonances. This and subsequent experiments with improved techniques showed that the symmetric-fission yields fall into two groups averaging 0.59 and 1.11 times the symmetric-fission yield for fission induced by thermal neutrons. The resonances with the lower and the higher symmetric-fission yields, respectively, could be identified with states in uranium-236 with total angular momentum J of 4 and 3. Similar results were obtained for the resonances of plutonium-239, but in this case the higher and lower symmetries are associated with the $J = 1$ and the $J = 0$ states of plutonium-240.

Another scientific application of nuclear explosions was one that had been brought to light in startling fashion with the Mike test: the production of very heavy nuclides. The results of that test had given rise to a wave of hope amongst some of the heavy-element research fraternity. They reasoned that since



Attempts to reach a region of predicted semistable nuclides in the vicinity of Z = 114 and N = 184, the "island of stability," have occupied nuclear scientists since the '50s. According to current thinking, the best prospect of reaching it lies in single-step collisions, with minimum excitation, between neutron-rich projectiles and neutron-rich targets—in effect a quick, shallow underwater trip to the island. (Adapted from computergraphics by Ray Nix.)

the Mike device was designed to achieve other technical goals—the production of heavy elements was, after all, only incidental—it did not seem unreasonable that other devices of this kind could be modified to achieve even higher instantaneous neutron fluxes and thus provide a path to synthesis of still heavier elements and possibly even the superheavies, the predicted semistable nuclides in the vicinity of the doubly closed-shell nuclide $^{298}114$. (The stability of nuclides with approximately 114 protons and 184 neutrons is predicted by the shell model of the nucleus. This model explains the observed stability of nuclides with "closed" shells containing certain "magic" numbers of protons or neutrons. Other examples of doubly closed-shell nuclides are calcium-40 and lead-208.) Moreover, by the '60s there was reason to believe that the high neutron fluxes could be achieved in explosions producing much less radioactivity and releasing much less energy, say of the order of a few kilotons. Between 1962 and 1969 several sophisticated devices modified for production of heavy elements were actually fired in underground tests in Nevada, some by Los Alamos and some by Livermore. Although radiochemical examination of the products of these tests turned up valuable nuclear information, it also clearly brought to light a fundamental limitation on heavy-element synthesis—spontaneous fission. The fluxes achieved in these later tests were high, but the rapidly increasing probability of spontaneous fission with increasing isotopic mass set an effective limit on the masses of the products attainable. The heaviest product recovered was fermium-257, with a half-life of 100 days. [The heavier isotope fermium-259, discovered in 1976 by a Los Alamos/Livermore team employing the $(^3\text{H},p)$ reaction on a fermium-257 target, decays by spontaneous fission with a half-life of only 1.5 seconds.] There has been some speculation on the possibility of reaching the predicted "island of stability" near $^{298}114$ by irradiating suitable heavy-element targets in a very high-flux nuclear explosion (or perhaps one bootstrapped on another) to make an "end run" around the zone of nuclides prone to spontaneous fission, but the prospects do not at present appear sufficiently promising to warrant a test. Meanwhile, this heavy-element work has indicated that a large area on the neutron-rich side of the isotope chart represents nuclei known to have been produced (or to be producible) in nuclear explosions but remaining undescribed and currently unavailable by any other known means. Here the limitation lies not in the technology of thermonuclear explosives but in the technology of sample recovery. Development of the means to retrieve and partially process samples from an underground explosion within five or ten minutes of the event could open up a large new field of nuclear studies.

Tools Old and New

In science it is axiomatic that new advances follow the introduction of new tools and new techniques. Pressed by insistent demands for accurate and timely diagnostic data, the group and its instrumentation czar J. P. Balagna had early on recognized the necessity for first-rate equipment. New tools of the trade were tested and incorporated into the group's instrumentation complex as soon as they appeared on the scene. A particularly useful addition was an isotope separator purchased in 1964. With it we could separate interfering isotopes of various elements and thus perform for the first time accurate analyses for second- and third-order (and in some cases even higher order) products of neutron reactions. This capability significantly broadened the range and utility of the radiochemical detector technique for weapons diagnostics. The isotope separator has been used as well for experiments in basic nuclear science, including a tour de force involving tightly scheduled operations at Oak Ridge, Los Alamos, and the Nevada Test Site in which the fission cross section of 6.75-day uranium-237 was measured at neutron energies from 43 eV to 1.83 MeV.

Semiconductor radiation detectors, silicon units for charged particles and soft x rays and lithium-drifted germanium units for gamma rays, also became available to the group in the '60s. With their fifty-fold increase in energy resolution over the then standard sodium iodide scintillation detectors, semiconductor detectors made it possible to identify and precisely assay individual radionuclides in mixtures and thereby permitted us to redirect a great deal of radiochemical processing and counting time to new measurements. At Los Alamos as elsewhere, the new detectors also brought about a jump in the detail and range of information to be obtained from basic nuclear research.

There has been another component of the group's facilities and capabilities that has expanded its interests to areas not commonly associated with nuclear and radiochemistry. Ever since the first atmospheric tests in Nevada in 1951, explosion-cloud sampling had been performed by the U.S. Air Force with manned aircraft; the actual cloud sampling was directed at close range by a scientist from the group serving as a crew member in the sampling squadron. With the cessation of atmospheric testing in 1963, the sampling director, some of the aircraft, and, most important, the accumulated experience became available for other studies. One of the first studies involved flights through the discharge plumes of the Rover reactor tests to provide information on the radioactivity carried out by the propellant gas. Then, in the middle to the late '60s, the rapidly increasing national concern over environmental pollution steered the sampling efforts toward investigation of airborne pollutants. The aircraft sampling facilities were used to survey and measure forest-fire clouds, industrial smokestack discharges, and regional air quality. By microscopic examination, neutron-activation analysis, and other specialized techniques group members were able in many cases to establish telltale "fingerprints" of individual sources by which their contributions to atmospheric pollution could be tracked far from the point of origin.

The mid '70s brought to the nuclear and radiochemistry group a state-of-the-art mass spectrometry facility that made possible quantitative measurement of isotopes by direct counting of atoms. Its development represented the convergence of two trends, one in supply and one in demand. The demand lay in the pressure to extract more detailed and sophisticated diagnostic information from the debris of nuclear weapons tests. On the supply side, steady improvement in the apparatus and techniques available to mass spectrometry was bringing it within reach as a cost-effective adjunct to the diagnostics program. The most common diagnostic task requiring mass spectrometry is determining the isotopic compositions of the uranium and plutonium fuels remaining in the debris. From such data is obtained the fraction of the fissile fuel consumed by nonfission reactions. But, as in other pursuits, with every boon comes a burden. Mass spectrometers are notoriously finicky, and every sample must be meticulously prepared to free it of all other elements, real or suspected, that come with it in the raw debris. Still, the effort spent in setting up the facility and getting it to work as it should has begun to pay off. The mass spectrometrists now not only supply essential isotope data on fissile elements and on radiochemical detector elements but also have made possible some new initiatives in basic research.

One quite recent initiative that demands the capabilities of the mass spectrometry facility is the use of "heavy" methane molecules as tracers to study atmospheric circulation and mixing patterns. ("Normal" methane contains the common isotopes carbon-12 and hydrogen-1; in the heavy methanes these isotopes are replaced by the rare but stable isotopes carbon-13 and/or hydrogen-2.) In a 1980 trial experiment carried out in collaboration with the National Oceanic and Atmospheric Administration, heavy methanes were released at Norman, Oklahoma, and their concentrations were measured in samples collected at a network of stations and by the U.S. Air Force. Evidence of the heavy methanes was found even at East Coast locations. Since the samples contained much larger concentrations of normal methane, state-of-the-art mass spectrometry was essential to the experiment. The mass spectrometers currently in use at Los Alamos can measure about one part of methane-20 in a billion parts of normal methane and one part of methane-21 in 200 billion parts of normal methane. (These discrimination capabilities may be increased by a factor of a thousand with new techniques involving sophisticated particle-identification apparatus at the Laboratory's vertical electrostatic accelerator.) The success of the trial experiments has led to preparations for further experiments on a continental scale.

Members of the group also, of course, have access to other Laboratory facilities of great value for their programmatic and research interests. Among these facilities, until it was shut down in 1974, was the old cyclotron brought here during World War II, which provided beams of low-energy charged particles. This cyclotron was the world's first practical source of "lightweight alpha particles," or helium-3 ions, and nuclear chemists had measured excitation functions (cross section dependence on energy) of the reactions between helium-3 and target nuclei ranging from carbon to uranium.

Another valuable facility, the vertical electrostatic accelerator/FN Tandem Van de Graaff complex, came on line in 1964; it was the first to offer accelerated beams of tritons with energies up to more than 20 MeV. With these tritons nuclear researchers could study nuclei by reactions such as $(^3H,p)$, $(^3H,\alpha)$, and $(^3H,\alpha p)$. Also available were high-energy neutrons [from the $^2H(^3H,n)^4He$ reaction] for measuring excitation functions of (n,xn) reactions at neutron energies well above 14 MeV, a regime of considerable interest to the weapons program. This accelerator complex has served as the focus for a nuclear chemistry/nuclear physics (the boundary line is sometimes indistinct) collaboration on researches of frontline interest: the fission process in a wide range of nuclei and excitation, the properties of the heaviest nuclei, and the processes occurring when complex nuclei are bombarded with other complex nuclei.

In addition, the charged particles available from the Van de Graaff provide a way—often the only way—to study neutron reactions of isotopes important to radiochemical weapons diagnostics. As an example, consider the isotope yttrium-86, which is among the neutron-deficient yttrium isotopes formed by (n,xn) reactions when yttrium-89, the common stable isotope of yttrium, is employed as a radiochemical detector in a nuclear device. Analyses of debris samples for the various yttrium isotopes, together with the known (n,xn) cross sections, are expected to provide values for the flux of neutrons from the thermonuclear fusion of deuterium and tritium. But the flux determination is complicated by the fact that some of the yttrium-86 is consumed by further neutron reactions, such as (n,γ), (n,p), and (n,np). To correct for this complication, information about the cross sections of these parasitic reactions is needed but is difficult or impossible to obtain directly. The cross section for, say, the (n,p) reaction can be determined indirectly, however, by creating excited states of yttrium-87 [the intermediate product of the (n,p) reaction] and measuring the fraction of these excited nuclei that decay by proton emission. The excited yttrium-87 nuclei are produced through the reaction $^{86}Sr(^3He,^2H)^{87}Y$ by employing helium-3 ions from the Van de Graaff and a target of strontium-86 (an uncommon but stable isotope available from Oak Ridge). This example illustrates that by using well-known nuclear physics strategies we can circumvent the difficulties sometimes arrayed against us when we try to study a nuclear reaction as it actually occurs.

The Laboratory's reactor complex, in particular the series of high-neutron-flux installations (of which the Omega West reactor and the Pajarito Site critical assemblies are the current representatives), has also been an essential and versatile resource for the nuclear and radiochemistry group. Nuclear explosive devices are essentially one-shot neutron reactors specially designed to achieve very rapid power buildup and energy release, and the great variety of radiochemical procedures needed to diagnose their performance has to be developed and calibrated with more conventional neutron sources.

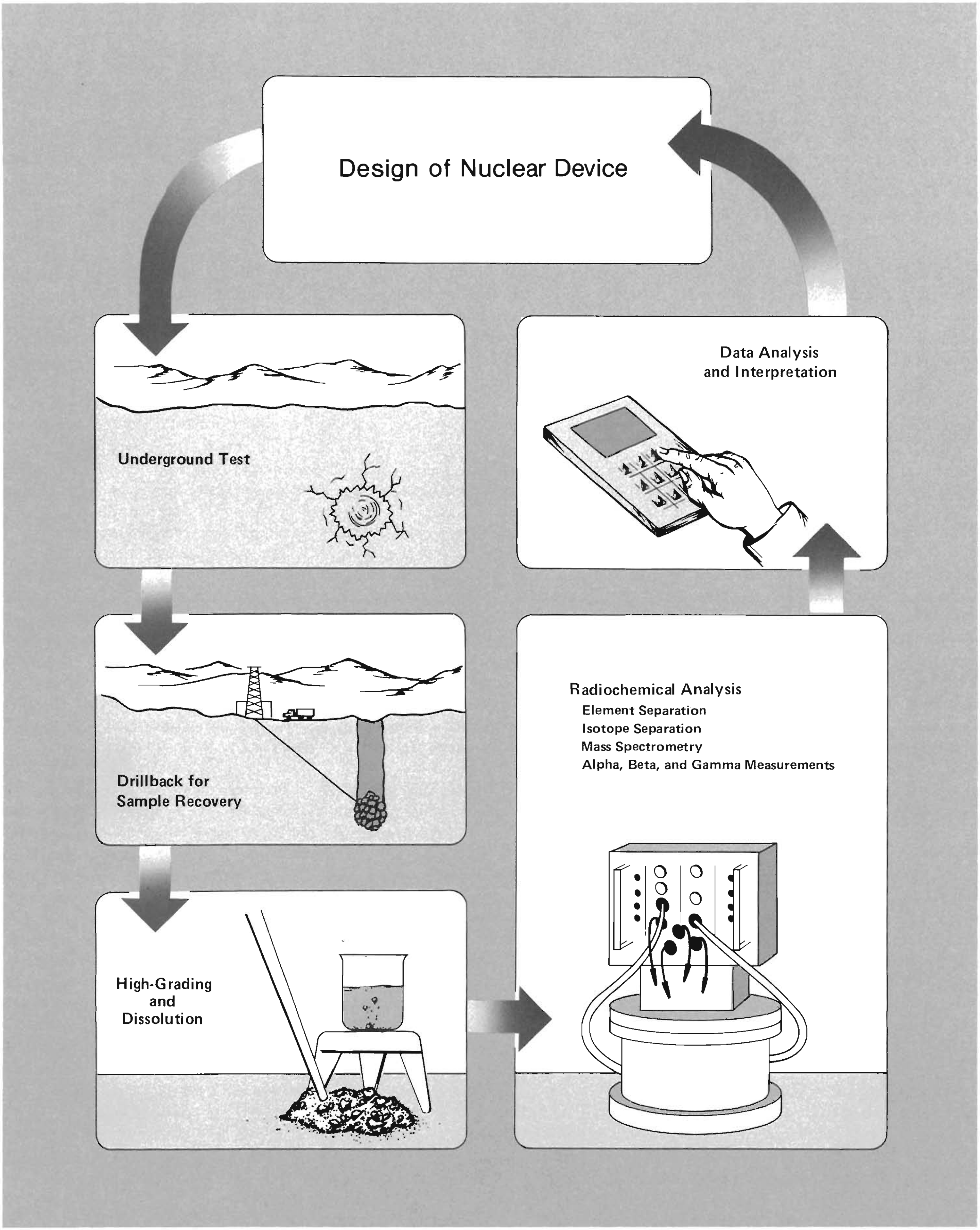
Mentioned last only because of its more recent vintage, the Laboratory's meson physics facility (LAMPF) has considerably broadened the scope of the group's nuclear chemistry research. We elaborate on this topic in a later section.

From Underground Testing to Isotope Geochemistry

The Limited Test Ban Treaty of 1963, which restricted all nuclear test explosions to underground, had a far-reaching effect on the course of nuclear and radiochemistry at Los Alamos. Compared with atmospheric testing, underground testing had both drawbacks and advantages for radiochemical diagnostics. Access to the debris was slower—days compared to hours—and the material of interest in the debris had to be extracted from large amounts of fused earth, usually containing unwanted products from neutron activation of trace elements in the earth. But for most diagnostic purposes the benefits more than compensated for the drawbacks. The debris stayed where it was made, available for recovery in large amounts at any time and with simpler control of personnel radiation exposure.

The experience and expertise gained in dealing with the products associated with underground nuclear explosions contributed to some noteworthy accomplishments and new initiatives. For example, starting with 85 kilograms of bastnäsite ore from a rare-earth mine near Mountain Pass, California, D. C. Hoffman and her colleagues succeeded in isolating a plutonium fraction that, when subjected to mass-spectrometric analysis at the General Electric Research Laboratory, showed the presence of plutonium-244. (Bastnäsite is a rare-earth mineral whose formation should have concentrated also any primordial plutonium.) Reported in 1971, this discovery of plutonium-244 in nature had significant consequences for our ideas about nucleosynthesis and the distribution of elements in the solar system. In other high-sensitivity measurements another staff member and his postdoctoral associate, in collaboration with associates at Livermore and Hanford, obtained evidence for niobium-92 and -94 in natural niobium. These investigations were the precursors of what is today a large and far-ranging research effort in isotope geochemistry.

The group's capabilities in applying radiochemical techniques to measuring very minute quantities of elements in earth samples, coupled with the interests of group member C. J. Orth, led recently to the discovery of evidence in support of a controversial explanation for the extinction of many plant and animal species, including the dinosaurs, that



Radiochemical diagnostics provide essential feedback to nuclear weapon design. A variety of analytical techniques are called upon to produce the necessary range of information about a weapon's performance during a test explosion.

marks the boundary between the Cretaceous and Tertiary geologic periods. A Berkeley team headed by Luis and Walter Alvarez proposed that an interruption of the food chain was responsible, an interruption caused by a blotting out of the sun by debris from the impact of an extraterrestrial object. They based this hypothesis on unusually high abundances of iridium found in marine sediments from Italy at a stratum coincident with the Cretaceous-Tertiary boundary. (Iridium is a platinum-family element exceedingly scarce in the earth's crust and somewhat less scarce in extraterrestrial matter.) Similar iridium "anomalies" were subsequently discovered at other locations, but all were found in marine sediments and could possibly be attributed to concentration by ocean processes. Orth and his radiochemistry team members, in collaboration with experts in Cretaceous stratigraphy and plant life from the U.S. Geological Survey, selected a likely drill site in northern New Mexico and succeeded in discovering, in *freshwater* sediment, a strong iridium concentration spike that correlated exactly with the Cretaceous-Tertiary boundary as defined by changes in abundances of pollen species. Since the peak concentration of iridium in normal earth samples is only parts per trillion, very sensitive analytical techniques involving neutron activation and radiochemical separation were required to detect the anomaly. This research area is fast moving, and we expect future developments to broaden the scope of our contributions still further.

The Winds of Change

The period from the mid '60s to the early '70s was a time of transition for the Laboratory, a transition from concentration of effort on nuclear weapons to participation also in a broad spectrum of other high-technology topics of national concern. Nuclear and radiochemistry entered a corresponding transition. Significant new research was initiated on nuclear structure and reactions (at the electrostatic accelerator complex and at LAMPF), on the application of new radioisotopes to medicine and of stable isotopes to atmospheric studies, in isotope geochemistry and cosmochemistry, and on the migration and containment of nuclear wastes. Discussion of research in the latter area has been reserved for another article in this issue of *Los Alamos Science.*

Linked with the broadening range of research was a sequence of leadership changes following the appointment in 1970 of Harold M. Agnew as the third Director of the Laboratory. Group Leader Cowan was made Leader of the new Chemistry-Nuclear Chemistry Division, which was created to incorporate research in inorganic, physical, and nuclear chemistry. The nuclear and radiochemists under newly appointed Group Leader James E. Sattizahn became CNC-11.

Nuclear Chemistry at LAMPF

With the start-up of the Clinton P. Anderson Meson Physics Facility in the early 1970s, the chemists found new horizons opened to them in both applied radiochemistry and basic nuclear science. This 800-MeV linear proton accelerator and its array of auxiliary equipment, built to produce beams of protons and pi mesons (pions) at intensities several hundredfold greater than had been available anywhere else before, brought within reach a wide range of phenomena previously almost inaccessible to practical measurement. One of these phenomena is the interaction of pions with nuclei, and small teams of nuclear chemists began to study pion-induced nuclear reactions on targets ranging from carbon to uranium by measuring the radioactive products. (We note here a commonly used distinction made a little less inaccurate by use of the qualifier "many": many nuclear *chemists* study nuclear reactions by measuring radioactive products remaining in a target *after* bombardment; many nuclear *physicists* study nuclear reactions by measuring particles emitted from a target *during* bombardment.)

An interesting example is the triplet of pion-induced reactions that produce carbon-11 in a natural carbon (predominantly carbon-12) target:

$$\pi^- + {}^{12}\mathrm{C} \rightarrow \pi^- + {}^{11}\mathrm{C} + n \ , \qquad (1)$$

$$\pi^+ + {}^{12}\mathrm{C} \rightarrow \pi^+ + {}^{11}\mathrm{C} + n \ , \qquad (2)$$

and

$$\pi^+ + {}^{12}\mathrm{C} \rightarrow \pi^0 + {}^{11}\mathrm{C} + p \ . \qquad (3)$$

Once the cross sections for these reactions are known at various incident pion energies, they become good intensity monitors for negative and positive pion beams. One places a carbon-containing target in the pion beam for a few minutes, withdraws it, and measures the carbon-11 produced; a simple calculation gives the number of pions that struck the target. These reactions contain a basic physics interest as well. Examination of reactions 1 and 2 suggests that the incoming pion, whether negative or positive, simply hits a single neutron in the carbon-12 nucleus and knocks it out; reaction 3 is the same except that the positive pion passes its positive charge to a neutron during the collision and a proton emerges. Now it is considered as firmly established in nuclear physics that, at pion kinetic energies around 180 MeV (the so-called 3/2,3/2 resonance), the probabilities for the elementary reactions $\pi^- + n \rightarrow \pi^- + n$, $\pi^+ + n \rightarrow \pi^+ + n$, and $\pi^+ + n \rightarrow \pi^0 + p$ should be as 9 to 1 to 2. Thus, if a carbon-12 nucleus is merely a collection of neutrons and protons, the ratio of the cross section for reaction 1 to that for reactions 2 and 3 together should be 3. (When carbon-11 is measured, reactions 2 and 3 are indistinguishable.) When carefully measured by the nuclear chemists, however, the ratio came out to be not 3, but 1.6. This seemingly simple discrepancy presented the theoreticians with an interesting problem, and they have devised a model that solves it. The key to their solution lies in taking into account that, although reactions 1, 2, and 3 as we have written them may represent what happens *at the site of a collision inside a nucleus,* the emerging neutron (proton) is likely to hit and trade places with a proton (neutron) while on its way out of the nucleus and produce another mass-11 nuclide, boron-11 (nitrogen-11). Thus, the carbon-11 nuclei that the chemist sees may be fewer than the total that were initially produced in the collision. They represent the cases where "nothing went wrong."

The Discovery of Thorium-236

About a year before LAMPF's experimental program was officially launched with the production of the first mesons in 1973, the nuclear chemists conducted an experiment, Experiment Zero, that led to the first scientific discovery and publication from this new installation. By bombarding a target of uranium-238 with the high-intensity 100-MeV proton test beam that had just become available, they produced a new heavy isotope of thorium, thorium-236. With short bombardments followed by fast radiochemical isolation and electromagnetic isotope separations, they were able to identify and characterize this previously unknown isotope. It decays by beta emission with a half-life of 37.5 minutes. ■

Another research interest of the nuclear chemists at LAMPF concerns the "exotic" nuclei produced when 800-MeV protons strike nuclei, especially heavy ones. This interaction yields a wide assortment of particles and nuclear fragments ranging from pions and neutrons to nuclei only a little lighter than the target. Among these fragments are nuclei, many of them still unidentified, that represent the extremes of what can exist at all. Some of them have a great excess of neutrons, so great that their last few neutrons are barely held; these are described as almost neutron-unbound or near the "neutron drip line." There exist other nuclei at the opposite extreme: severely deficient in neutrons and rich in protons, that is, almost proton-unbound, or near the "proton drip line." These exotic nuclei are hard to find. Not only are they produced very rarely in nuclear reactions, but they have half-lives so short (seconds or less) that they cannot be isolated and identified by conventional radiochemical techniques. A team of nuclear chemists devised a method to identify some of these exotic products in flight, that is, as they emerge from the proton/heavy nucleus reaction. Measuring a product's time of flight from the production site to a detector gives its velocity; measuring its kinetic energy as it is stopped in the detector then gives its mass from the expression $E = \frac{1}{2}mv^2$ (the correction for relativistic effects is small); and measuring its rate of energy loss in the detector assembly gives its nuclear charge. Thus, by electronic means one establishes the identity of the isotope without chemically separating it or measuring its decay. In a series of experiments using thin targets of uranium and nickel, the nuclear chemists have identified five new examples of neutron-rich exotic isotopes: neon-27, magnesium-31, magnesium-32, aluminum-34, and phosphorus-39. (The heaviest stable isotopes of these elements are neon-22, magnesium-26, aluminum-27, and phosphorus-31.) Others are currently under study.

Present nuclear theory cannot predict accurately the boundaries of nuclear existence, and there is now evidence that even the exotic nuclei named above may be short of the neutron drip line. To illustrate this point, we pick an example from low-atomic-number nuclei measured elsewhere. The element beryllium, with an atomic number of 4, has a single stable isotope, beryllium-9. The question has been asked, "What is the heaviest possible isotope of beryllium?" A team at the University of California Bevatron made a search, employing an experiment similar to the one described above. At the upper end of the mass scale they found evidence for beryllium-11, -12 (both already known), and -14, but not for beryllium-13, -15, or -16. Thus, it appears that the neutron drip line occurs beyond beryllium-14 and that beryllium-16 is probably neutron-unbound. On the other hand, beryllium-11 is probably the heaviest odd-mass beryllium isotope that is neutron-bound. The difference between the odd- and even-mass isotopes is not surprising to nuclear scientists; it reflects the well-known preference of nuclear particles to occur in pairs.

So far we have talked in terms of merely establishing the existence of exotic nuclei. The data would be much more valuable if they told in addition *how stable* such nuclei are. The Los Alamos team is now at work on a new apparatus that should provide this kind of information. It will still measure time of flight, but with sufficient precision to give a spectroscopically useful isotope mass, which is a direct measure of nuclear stability. When operational, the instrument should yield masses on at least thirty exotic nuclei with accuracies of 200 keV or better, a figure that should lead to a new kind of thinking about these nuclei.

After the proton beam at LAMPF has passed through the last of the particle-generating targets, it is considered to have done its work. Attenuated to between 50 and 75 percent of its starting intensity and too diffuse for normal use, it is refocused slightly and sent to the "beam dump." But even in its death throes it can produce large amounts of radioactivity in the materials in its path. Inserting targets of their own into the beam-dump zone by means of a remotely controlled facility specially engineered for the purpose, radiochemists obtain a variety of radioisotopes that they isolate and prepare for experiments in nuclear medicine. A separate radiochemistry group has been built up to explore and develop this family of applications.

Another offering on the LAMPF menu, negative muons, has provided the chemists with a new probe of matter whose utility and applications they are still exploring. (A negative muon, loosely speaking, is a semistable, heavy version of an electron. These particles are available at LAMPF from the decay of negative pions.) Experimentally, one places a specimen of interest in a beam of negative muons and measures the intensities of the muonic x rays emitted as the muons captured by individual atoms de-excite through their levels in the atoms. Each element is identifiable by its set of muonic x rays, just as it is by its set of ordinary electronic x rays. The aspect of this phenomenology that attracts the attention of the chemists is one first observed over two decades ago: the muonic x-ray intensity patterns of the elements in a compound and the relative number of muons captured by the individual elements in a compound *vary with the compound's structure and state.* Thus, if one could learn something about the nature of this variation, one might use it as a tool to investigate chemical structure.

Beginning with an experimental program that got under way in the mid '70s, nuclear chemists have studied the intensity effects on a variety of target materials chosen to elicit chemical structure factors. Along the way they have turned up a number of curious results, some of which are beginning to make sense in terms of the chemical bond. For example, the relative probability of capture of a negative muon by the two elements in a binary compound had been expected to vary approximately as the atomic numbers of the two elements weighted by their stoichiometric abundances; thus, in boron nitride (BN) the ratio of the capture probabilities $c(\mathrm{B})$ and $c(\mathrm{N})$ would be 5/7, or 0.714. Actual measurement, however, gave a ratio of 0.258 ± 0.020; the nitrogen appears to have captured about 2.8 times its "fair share." Although no realistic theoretical model has yet been developed to calculate this ratio, some European workers have come up with an assumption and a recipe that do reasonably well for BN and other molecules composed of low-atomic-weight elements. The heart of the assumption is that muons are captured by an atom in proportion to the number of loosely bound electrons around the atom, a quantity that can be computed in a simple way from the ionicity of the B-N bond. We conclude with a case in which this concept has provided the first experimental evidence for the direction of the dipole in the diatomic molecule nitric oxide (NO). Our muon team measured the capture ratio $c(\mathrm{N})/c(\mathrm{O})$ for two gas targets, one consisting of 5 atmospheres of nitrogen (N_2) and 5 atmospheres of oxygen (O_2) and the other consisting of 10 atmospheres of NO. The capture ratios came out to be 0.834 and 0.959, respectively, and the ratio of the ratios was 0.870; that is, the nitrogen in the NO captured a small excess of muons. By our assumption, then, the loose electrons are slightly displaced toward the nitrogen end of the molecule. A detailed theoretical calculation of the molecular structure has led to the same charge orientation.

Fission Comes to the Group

The reader who has followed our account this far is likely to have acquired the impression that the nuclear and radiochemistry group was becoming rather large and diverse, with a range of programs and interests beyond that usually associated with a single discipline. As seen through the eyes of the participants this transformation has been undramatic but noticeable in many ways. It has reflected changes both within the community of members—in interests and capabilities—and outside—in the spectrum of national programmatic needs and the Laboratory's response to them. The transformation, like most transformations on the science and technology scene, had an important organizational facet: the question of how people and resources were to be organized and allocated for the strongest contribution to the Laboratory's goals. Consideration of the overall nuclear and radiochemistry effort from this standpoint received a great deal of attention and resulted, in late 1980, in the first major structural change since the group was formed in 1947. The nuclear and radiochemistry group, which had reached a membership of 100, was split into three groups, the first under Group Leader H. A. O'Brien and centered on research in medical radioisotopes, the second under Group Leader E. A. Bryant and centered on isotope geochemistry, and the third under Group Leader J. E. Sattizahn and centered on weapons diagnostics and other topics in nuclear and radiochemistry. In practice and as intended, the boundary lines are fuzzy. The groups continue to share the same building complex and facilities. Most important, they continue to enjoy the contacts and cross-fertilization of ideas that have contributed so significantly to past developments. ■

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AUTHORS

Jere D. Knight joined the Manhattan Project at the University of Chicago in 1942 after receiving a Bachelor of Science in chemistry from St. John's University (at Collegeville, Minnesota) and completing two years of graduate work at the University of Minnesota. In 1943 he transferred with the nuclear chemistry group under C. D. Coryell to Oak Ridge, where he worked on fission products, reactor neutron activations, and the first macro production of tritium. Following a return to the University of Minnesota and a Ph.D. in physical chemistry in 1948, he spent the next year and a half as an instructor in chemistry and research associate in physics at the University of Illinois. In 1949 he joined the nuclear and radiochemistry group at Los Alamos, where he has been involved in many of the developments described in this article. He was appointed a Laboratory Fellow in 1981. Since his retirement in 1982 he has served as a consultant to his former group.

James E. Sattizahn received his B.S. in chemistry from Lawrence College in Appleton, Wisconsin in 1942 and joined the Manhattan Project at Oak Ridge in March 1944. (He and the co-author of this article worked only 100 yards apart at Oak Ridge, but they never met until both had moved to Los Alamos.) The Bikini tests brought Sattizahn to Los Alamos on June 5, 1946 as part of the team assembled to carry out radiochemical diagnostics. He was an original member of the Laboratory's permanent nuclear and radiochemistry group formed in early 1947 and has served as its Leader since January 1, 1971. He received a Ph.D. in radiochemistry from the University of New Mexico in 1957 under the Laboratory's graduate study program.

Migration of Radioisotopes in the Earth's Crust

written by Roger Eckhardt for the
Isotope and Nuclear Chemistry Division

Radioisotopes trace out mechanisms important both to underground nuclear waste storage and to our planet's geology.

Water moves steadily in the earth beneath our feet, flowing in aquifers through the pores, fractures, and grain boundaries of rocks and minerals. This ubiquitous "universal solvent" interacts with the rock, helping gradually to reshape the geology of our planet's crust; it is the fluid most commonly associated with an important process called geochemical migration, that is, the selective movement of chemical elements in rock. A better understanding of the nature of this process has implications for a variety of human activities.

For example, burial of hazardous materials has often been chosen in the past as the safest method of disposal. But this choice sometimes ignored the pervasive nature of geochemical migration, leading to a number of serious cases in which hazardous material resurfaced in drinking water. A particularly important disposal problem is what to do with nuclear reactor waste. Storage in deep geologic sites is being considered, but it is imperative to evaluate the potential for migration of long-lived, highly radioactive waste away from the site and into the accessible environment.

There are also implications for locating ore deposits. A wide variety of ore bodies are thought to be formed as the result of selective dissociation of elements from a large body of rock followed by transport in water and eventual concentration and precipitation at another site. What conditions favor the dissolved forms of the elements, what favor the precipitated forms? A full understanding of such processes would serve as a rational basis for predicting the location of ore bodies in different geologic settings.

The complex phenomena that constitute geochemical migration have long intrigued and challenged geochemists. Much relevant information has come from studies of volcanism, of the processes that alter a rock's mineral composition, and of age determination techniques. Much remains to be learned if we are to understand the complexities of geochemical migration and its relationship to all the other geologic processes that shape our planet.

Radioisotopes

At Los Alamos we draw upon the Laboratory's background in nuclear physics and radiochemistry to study geochemical migration using radioisotopes. These methods have much to recommend them, starting with the fact that even trace amounts of radioisotopes are easily detectable. This is important because elements are frequently present in water only to the extent of parts per billion. The ease of detection is due, of course, to the radiation emitted by these isotopes. This radiation, characterized by type and energy, also helps establish a unique signature for any source of radioisotopes, especially when coupled with mass spectrometric measurements that give the relative isotopic abundances of both the radioactive and nonradioactive isotopes in a sample. The signature can then be used not only to identify the source of an element but also to follow its migration through the environment.

For example, the atmospheric nuclear weapons tests that took place over the ocean in the fifties injected large quantities of chlorine-36, a long-lived beta emitter, into the environment. This isotope is normally produced by cosmic rays in the atmosphere and had already been of use as a tracer in hydrology. The much larger quantities, however, could be uniquely associated with tests detonated in proximity to seawater: chlorine-36 is not a direct fission or fusion product but is generated by thermal neutron activation of the chlorine-35 isotope abundant in seawater. A recent study by researchers at the University of Arizona, New Mexico Institute of Mining and Technology, and the University of Rochester has shown that these tests caused a global "pulse" of chlorine-36 to be injected into the environment. This pulse lasted from 1953 to 1976 and, at its peak, rose to 1000 times the natural background level of chlorine-36 in the mid latitudes. In contrast to carbon-14 and tritium, which are still being generated by China's atmospheric testing, fallout rates for chlorine-36 have returned to natural background levels, making this isotope ideal for use as an environmental tracer. Because its long half-life eliminates ambiguities associated with loss of the isotope through radioactive decay, chlorine-36 should be especially valuable for studies of hydraulic flow and dispersive mixing as it incorporates itself into the water and geology of the earth's crust. Although these processes continuously reduce the concentrations of the tracer, recent advances in analytical chemistry, namely accelerator-based mass spectrometry, make possible its detection in amounts of 10^7 atoms per liter or less.

There are further advantages in the use of radioisotopes. As is well known from radioactive dating, individual radioisotopes decay at specific rates characterized by their half-lives; ages are determined using the ratio of the amount of radioisotope, or parent, to that of the decay product, or daughter. But because the daughter is usually a different element that is chemically distinct from the parent, fractionation processes can occur that physically separate the two. For example, conditions may have been such that the two elements had considerably different solubilities in the groundwater moving through the rock and one was transported away while the other remained in place. Deficiencies of parent or daughter elements in geologic samples compared to amounts predicted from nuclear physics may thus indicate geochemical migration. Samples from other geologic locations may reveal the destination of the transported element. Even more important, the chemical nature of the elements sheds light on the chemical nature of the fractionation process that took place in the sample. And comparison of parent to daughter ratios helps determine the geologic time at which the fractionation occurred (Fig. 1).

A particular advantage of analytical techniques based on mass spectrometry stems from the fact that amounts of both unstable and stable isotopes are measured. Even billions of years after a particular geological or nuclear event, stable isotopes and long-lived radioisotopes remain. Depending on the history of a sample, the relative abundances of these isotopes differ, providing the researcher a unique signature of the source. For example, the products of a uranium fission chain reaction can be distinguished easily from chemically identical products found naturally.

Initial motivations for much of the radioisotope migration research at the Laboratory have been related to nuclear weapons testing and nuclear energy. For example, how much, if any, of the radioactive material generated in the underground testing at the Nevada Test Site will be carried away by the groundwater? Or if radioactive nuclear wastes are stored underground, what mechanisms may cause migration of the radioisotopes and how fast would this happen? Yet, because geochemical migration is actually a complex group of interacting phenomena, answers to these particular questions also shed light on more general questions important to geology. (See "Mechanisms of Migration" for highlights of this broad picture.)

Migration from Underground Tests

From 1957 to the present there have been over five hundred underground nuclear tests at the Nevada Test Site, of which at least ninety-five were detonated at or below the

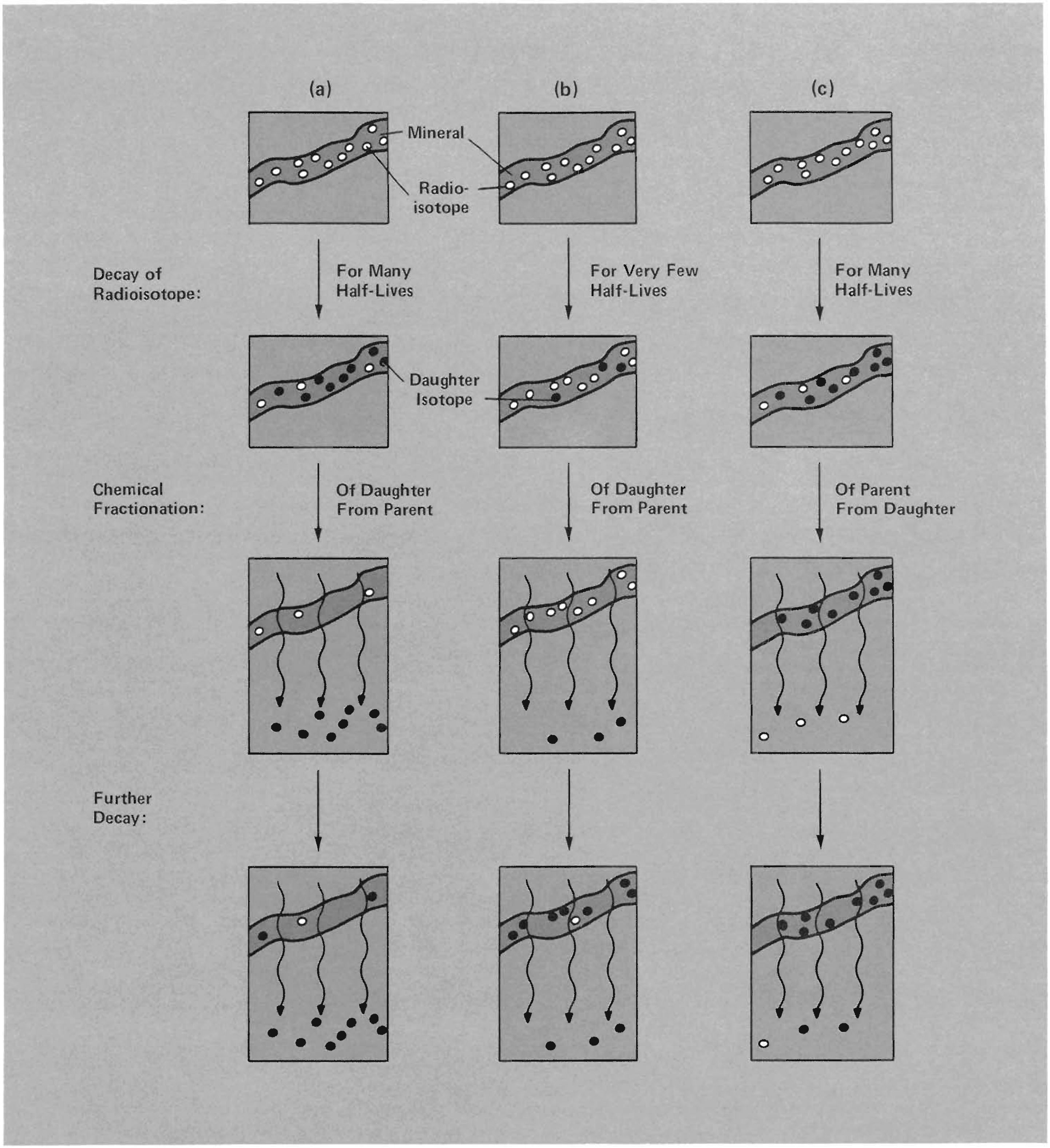


Fig. 1. Simple examples of fractionation of radioisotopes. Because the daughter isotope (solid circles) of a radioactive decay process is a different element than the original radioisotope (open circles), chemical and physical processes may eventually separate the two. The figure depicts a radioisotope-containing mineral in the same state at the top of each column. In (a) considerable decay occurs before fractionation, whereas in (b) only a small amount of decay occurs first. The fractionation process for these two columns is identical, say dissolution and transport of the daughter isotope by groundwater. The result of the difference in the time when fractionation occurs is seen at the bottom of each column. Column (a) has a high depletion of daughter in the mineral, whereas in (b) the depletion is much less. In (c) there is again considerable decay, but the fractionation process transports the original radioisotope, leaving an excess of daughter in the mineral.

Mechanisms of Migration

Most people are aware that rainfall, as it seeps into the earth, becomes laden with dissolved material or solutes. What they may not realize as fully is that the composition of the solutes can change drastically as the water passes from one geochemical environment to another, causing significant relocation of particular elements in relatively short geologic times.

Consider granite, a rock with a very low permeability to water. If a cube of such rock, 1 kilometer on a side and without fractures, has a typical hydraulic pressure gradient along one axis of 10 meters per kilometer, then 30 billion liters of water will flow through the rock in only 10 million years. If the water picks up uranium from the rock to the extent of 5 parts per billion, which is only 5 milligrams per 1000 liters, then a total of 150 kilograms of uranium will be transported out of the block. Although the degree of permeability and the strength of the driving forces determine how fast water flows, we see that large volumes can move through even very impermeable rocks, transporting significant quantities of trace elements.

The forces that act to produce migration are gravitational, chemical, and thermal. Gravity is the force responsible for hydraulic gradients. The rate of flow of groundwater is determined by this gradient, which, in turn, is generally a function of the topographic relief. A totally flat plain at sea level has a zero hydraulic gradient and zero flow.

For a given hydraulic gradient, flow is a function of permeability, which reflects texture and structure. Fractures may provide relatively low-resistance pathways through rock while a fine-grained or plastic rock may be relatively impermeable. In sedimentary and volcanic ash sequences, the permeability of individual strata may differ widely.

Another factor affecting flow is porosity, a measure of the space available in the rock for water. Porosity is generally related to permeability so that highly permeable rocks tend to be quite porous and vice versa. But for geochemical migration it is *connected* porosity that is important. This parameter determines the degree to which the entire bulk of the rock, as opposed to the surfaces of fractures, is exposed to flowing groundwater. Highly porous, permeable rock, such as some tuffs, are totally exposed, allowing elements to be leached from all minerals in the rock.

Above the water table, rock is unsaturated, and flow is limited although not stopped. As air replaces water, flow is gradually restricted to surface films that must be connected for liquid flow to occur. When the film becomes discontinuous, water movement is restricted to vapor transport in the air-filled pores, and transport in water becomes negligible.

A thermodynamic measure of the chemical force is the chemical potential, which, in aqueous systems, is directly related to solubility. For a given element, the chemical potential varies with the element's concentration and the environmental conditions such as temperature and pressure, acidity, the tendency for reduction or oxidation reactions, and the presence or absence of complexing ions or molecules in the water. In general, elements move from higher to lower potentials. For example, if the amount of dissolved mineral is less than the equilibrium concentration, then dissolution will take place, albeit often very slowly; if the amount exceeds the equilibrium solubility, then precipitation occurs.

The chemical processes contributing to

water table (Fig. 2). Considerable thought went into the engineering of the tests to insure that no radioactivity escaped into the environment, not even by way of underground water. However, it was realized that aspects of this latter path, such as the complex chemical partitioning of a radioisotope between the liquid, gaseous, and solid phases, were poorly understood. Hard data were needed.

Starting in 1972, an AEC (now DOE) program evolved that included the Los Alamos and Lawrence Livermore National Laboratories, the Desert Research Institute of the University of Nevada, the U.S. Geological Survey, and the Reynolds Electrical and Engineering Company. The program's mission was to gather and interpret data necessary for an understanding of radioisotope migration at the Test Site. Studies were eventually conducted at eight areas, including seven nuclear test sites and one area in which tritium was detected several hundred meters from the nearest underground test cavity. One of the most informative of these projects, and the one we will discuss here, measured migration by imposing an artificial hydraulic gradient on the normal underground water flow from a nuclear test cavity.

The cavity, the result of a test shot called Cambric, was located 73 meters below the water table within a Test Site water-supply aquifer. Since the detonation had occurred in 1965, nine years before the start of the study, it was predicted that the cavity, and the chimney resulting from later collapse above the cavity, had long since filled with water to the preshot level of the water table. Any radioisotopes present would constitute a potential source for transport, and it was felt that the postshot debris would contain sufficient amounts of radioisotopes to compare concentrations between rubble and groundwater. Table I gives the Cambric source geochemical migration can be very complex. A specific element can, in fact, move upstream against its potential treated as an isolated system if there is a net zero or negative potential change for the total system of interacting elements. It is quite possible in complex systems for one or more minerals in a rock to be dissolving while others are forming.

An illustration of this complexity is the variety of possible associations between metal cations in the minerals and the anions that may be available in groundwater, such as chloride, fluoride, carbonate, sulfate, or phosphate. For some conditions anions form stable complexes with cations, such as $PbCl_4^{-2}$, $Zn(HS)_3^-$, and HgS_2^{-2}, that increase the metal's solubility. For other conditions the anions do the opposite and form insoluble compounds, such as $CaCO_3$, $MgSO_4$, HgCl, or $Th_3(PO_4)_4$.

Temperature has a major effect on chemical potential. A mineral stable at 200 degrees Celsius may be unstable at 300 degrees. Solubilities typically increase with temperature but may be retrograde and decrease instead. Geothermal systems are particularly active zones for geochemical migration because thermal gradients combine with gravitational forces to produce convective groundwater circulation that exposes the minerals to a wide range of chemical environments.

Important chemical processes take place even in solid or crystalline materials. Trace elements such as the rare earths are frequently found as dissolved components in solid solution. These impurities can either be incorporated into the solid when it forms or diffuse into the lattice later. Conversely, diffusion out of the solid can release trace element impurities into the groundwater.

One of the most important aspects of geochemistry is the role of surfaces. For example, silicates tend to have a negative surface charge, due to exposed oxygen atoms, that attracts cations. Depending on the mineral, the cation, and the chemical environment, this attraction can cause a particular cation to spend most of its time sorbed on a surface, retarding its transport by moving water. Van der Waals forces, due to dipole interaction, and chemisorption, in which one or more chemical bonds are formed between the solute species and the surface atoms, also play an important role.

Because sorption is a surface effect, the crystal structure of a mineral is very important. With densely packed structures, only the external surface of the crystals can sorb species from solution. However, other minerals, such as zeolites, have a very open lattice structure that permits relatively easy access to interior passages. These show preferential sorption for species whose size matches the openings most closely.

Because the surfaces of the mineral in the pores are predominantly negatively charged, anions are typically repelled and cannot enter a small pore as readily as a neutral molecule or a cation. (Only a few minerals sorb anions, and then not very strongly.) This repulsion leads to the surprising result that anions can migrate through the rock more rapidly even than the water. The water molecules exchange with the stationary fluid in small water-filled pores, while the anions continue moving in the flowing stream.

The task, then, of the geochemist trying to understand migration in a given geologic setting is complicated. He must sort from a variety of factors those particular ones that apparently caused the equilibria to shift in one direction or another. ■

term, that is, the expected activity of selected radioisotopes if radioactive decay but no migration had occurred during the ten years before re-entry into the cavity. One important feature of this source term was the amount of tritium—enough to act as an easily measurable tracer for water from the cavity.

The geologic medium surrounding the Cambric cavity is tuffaceous alluvium, a deposit of gravel-like or sand-like debris that was originally volcanic material. This material was believed to constitute a good medium for hydrologic studies because it is quite permeable and does not have large fissures or cracks through which the water might flow selectively. Because the yield of Cambric was relatively low (about 0.75 kilotons), the test was expected to have had little effect on the local hydrology.

NATURAL MIGRATION. Two wells were drilled: the first, the satellite well, 91 meters from the cavity in the direction of normal water flow for the aquifer; the second, the re-entry well, at an angle through the lower chimney and cavity of the Cambric test (Fig. 3). The first question addressed was the extent of natural radioisotope migration since the detonation. To answer it, core samples were taken from the walls of the re-entry well starting just below the ground surface and continuing to 50 meters below the detonation point. Then, by using inflatable packers to isolate a zone in the well and perforating the casing in that zone, water samples were taken from five levels that ranged from just above the chimney to below the cavity.

Several interesting facts were determined about the distribution of the radioactive material. Ten years after the test, most of the radioactivity was still in the cavity region. No activity above background was found 50 meters below the point of detonation. Al-

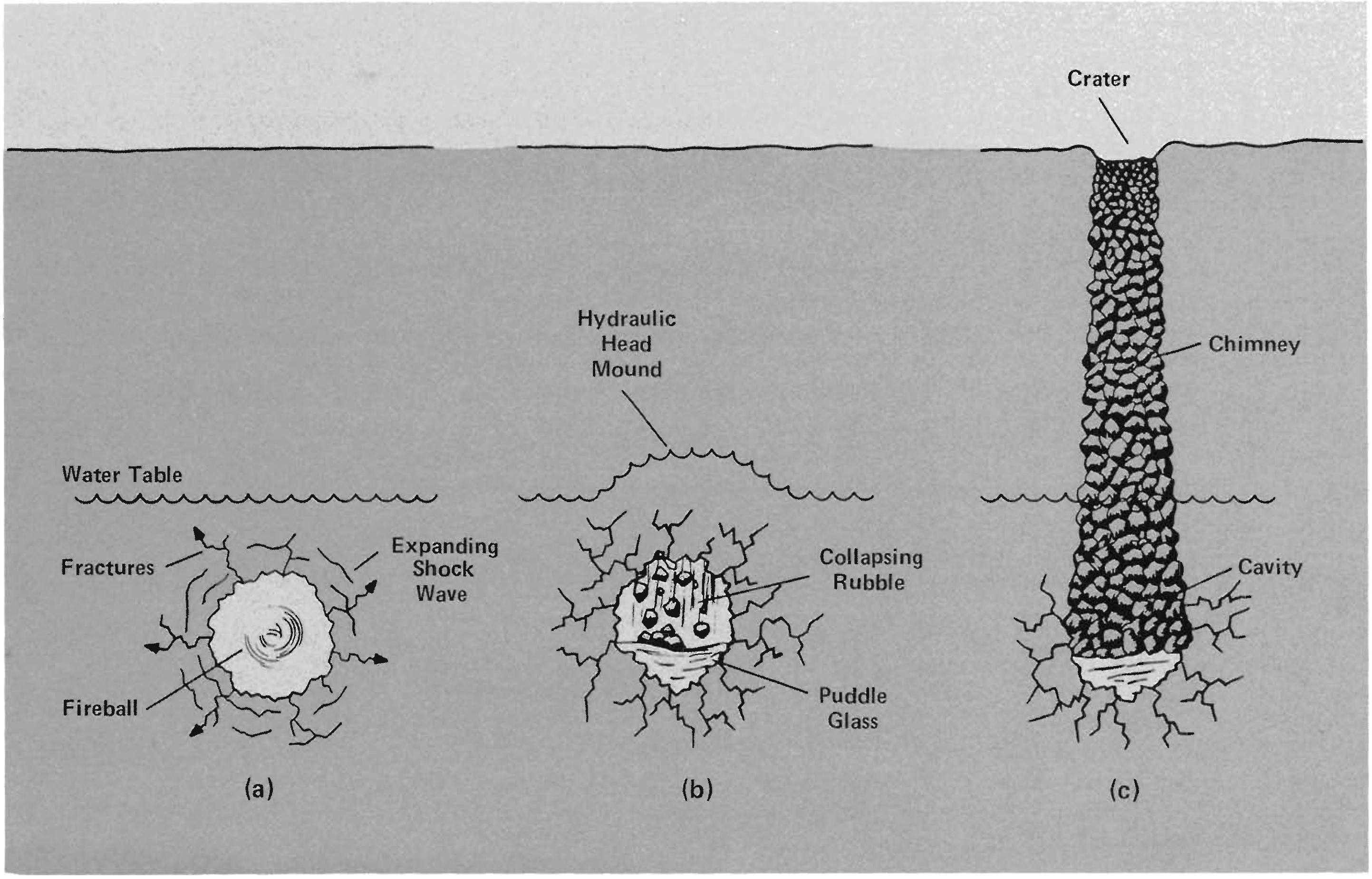


Fig. 2. Sequence of events in an underground nuclear explosion. (a) The explosion vaporizes the rock immediately surrounding the weapon, melts and spalls rock somewhat further away, and generates a compressional shock wave that creates a cavity and may fracture rock for several hundred meters. (b) After the explosion the gases cool and condense, forming puddle glass, or resolidified rock, at the bottom of the cavity that incorporates a significant fraction of the refractory radioisotopes, such as the rare earths, plutonium, zirconium, and the alkaline earths. At this point the cavity begins to collapse, and volatile radioactive species, such as the inert gases, move upward. Partly volatile species, such as the alkali metals, uranium, antimony, and iodine, may condense on rubble surfaces in the lower chimney. Also note that the shock wave has perturbed the water table, generating a mound in the hydraulic head. (c) A chimney of rubble forms above the collapsed cavity and, if it reaches ground level, forms the crater typical of underground tests. Eventually, the cavity and part of the chimney refill with water, dissipating the hydraulic head mound. The time for this last process ranges from days to years.

though some krypton-85 and tritium were found in the collapsed zone above the cavity, these two isotopes were concentrated in the cavity region. More than 99.9 per cent of the tritium was in the form of tritiated water (HTO), and the krypton-85 was dissolved in the water. In the lower cavity region, where the puddle glass was concentrated, strontium-90, cesium-137, and plutonium-239 were almost entirely incorporated into the solid material. As a result, only strontium-90 and tritium were present in the water from this region of highest radioactivity at concentrations above the federal guidelines for drinking water in uncontrolled areas.

TABLE I

SELECTED CAMBRIC RADIOISOTOPES TEN YEARS AFTER DETONATION

Calculated Source Term			Measured Distribution		
Isotope	Half-life (years)	Activity (curies)	Locations Detected	Form	Retention Factor on Solid in Cavity
^{3}H(T)	12.3	3.4×10^{4}	cavity, chimney	HTO	—
^{85}Kr	10.7	4.4	cavity, chimney	dissolved in water	—
^{90}Sr	29	34	cavity, chimney	retained with solids	2000
^{106}Ru	1.0	2.8	cavity	retained with solids	200
^{125}Sb	2.8	3.2	cavity	retained with solids	300
^{129}I	1.6×10^{7}	6.7×10^{-5}	cavity	dissolved in water	—
^{137}Cs	30	99	cavity, chimney	retained with solids	20,000
^{144}Ce	0.78	0.4	—	—	—
^{147}Pm	2.6	33	cavity	retained with solids	$>10^{6}$
^{155}Eu	5.0	6.4	—	—	—
^{239}Pu	2.4×10^{4}	classified	cavity	retained with solids	$>10^{7}$

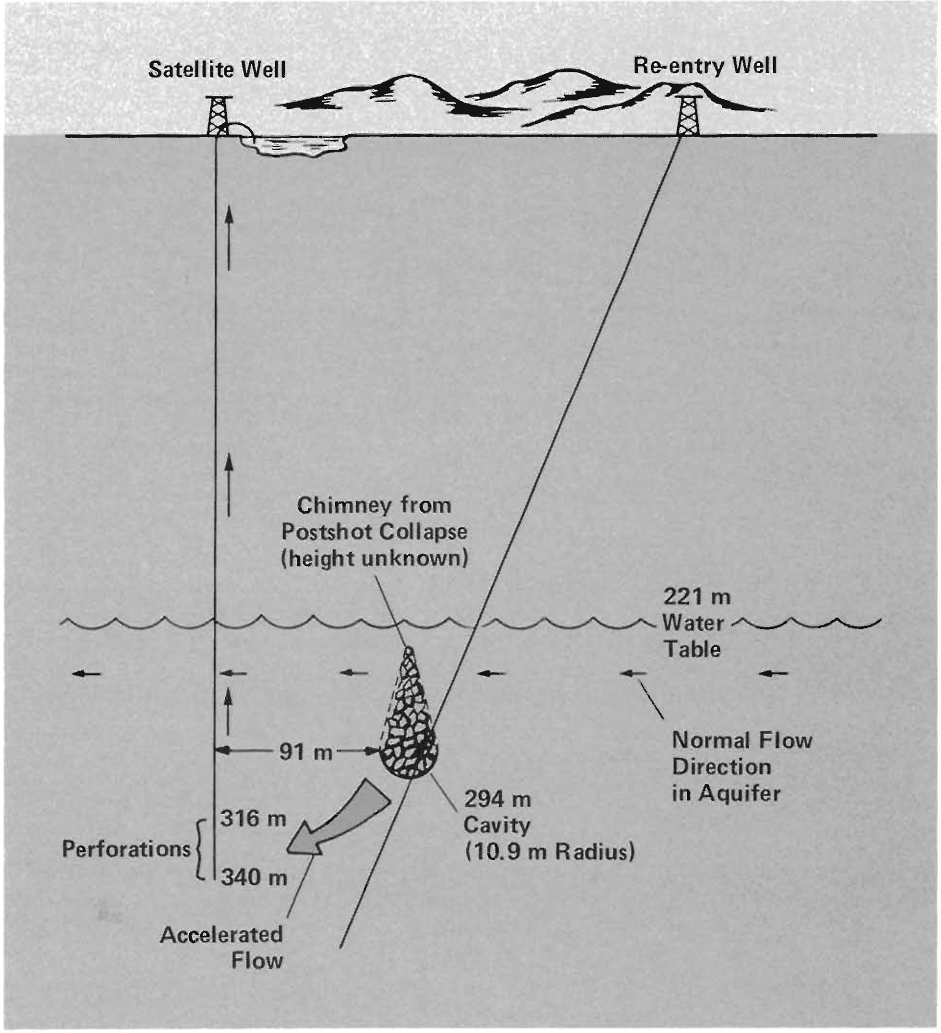


Fig. 3. The Cambric radioisotope migration study. The Cambric underground test was detonated about 9 years earlier at a depth of 73 meters below the water table. Accelerated flow of radioisotopes from the cavity (large arrow) was induced by pumping water from a satellite well 91 meters away in the direction of the normal flow of groundwater.

Retention factors were calculated to measure how effectively strontium-90, for example, had been retained in solid material. The ratio of strontium-90 activity in a solid sample to tritium activity in a water sample from the same location was compared to the same ratio for the Cambric source term. The high retention factors for strontium-90, ruthenium-106, antimony-125, cesium-137, promethium-147, and plutonium-239 (Table I) indicate that these isotopes were either fused into or highly sorbed on the solid material, or both.

ACCELERATED MIGRATION. The next step was to generate an artificial hydraulic gradient by pumping water from the satellite well. It was planned that pumping would draw water from the cavity and permit the study of radioisotope migration under field conditions. Pumping began in October 1975 at a rate of 1 cubic meter per minute; two years later the rate was increased to 2.3 cubic meters per minute. Early in 1978, after a total of 1.4 million cubic meters had been pumped, significant amounts of tritium were detected, signaling the arrival of water from the cavity region.

The tritium concentration grew, peaked at just below the maximum permissible concentration for drinking water in controlled areas (3 nanocuries per milliliter), and is now decreasing. By October 1982, 42 per cent of the tritium available from Cambric had been removed by this pumping. The concentration data are shown in Fig. 4 along with a theoretical fit to the data of a typical model for fluid flow through a dispersive medium.

Neutron-activated chlorine-36 produced

by the Cambric explosion was also detected by mass spectrometry in water pumped from the satellite well. The same flow model also fit these data. The major difference between the migrations of tritiated water and chlorine was that the chlorine arrived and peaked 20 per cent sooner. This difference was felt to be due to the anion exclusion effect in which anions are repelled by the negative surface charge in the small pores of the aluminosilicates. As a result, anions cannot enter these pores as readily as cations or neutral water molecules. The net result is that the anions migrate on a more direct path than the water.

Another radioisotope of great interest was iodine-129. Because of its biological activity and lack of sorption on most minerals, this radioisotope is considered a particular hazard. A method was devised, using neutron activation followed by counting of the iodine-130 product, that measured the aqueous concentration of iodine-129. Significantly, water from the cavity region had an iodine-129 concentration that was at least a factor of five lower than that recommended for drinking water in uncontrolled areas.

Iodine-129 was also detected in water drawn to the satellite well—at concentrations 5000 times lower than in the cavity water. These data are also plotted in Fig. 4. Fluctuations in the low concentrations make it difficult to determine a maximum; however, iodine-129 appeared to arrive at the satellite well at about the same time as the tritium. If iodine was present as an anionic species, its arrival and peak, like those of chlorine-36, should have preceded tritium's. Examination of the complex behavior of iodine in dilute solutions offers a possible explanation: for the pH and oxygen content of Cambric water, iodine may exist predominantly as the neutral molecule HIO and thus experience little or no anion exclusion effect.

The only other radioisotopes detected at the satellite well have been krypton-85 and, possibly, minute amounts of ruthenium-106.

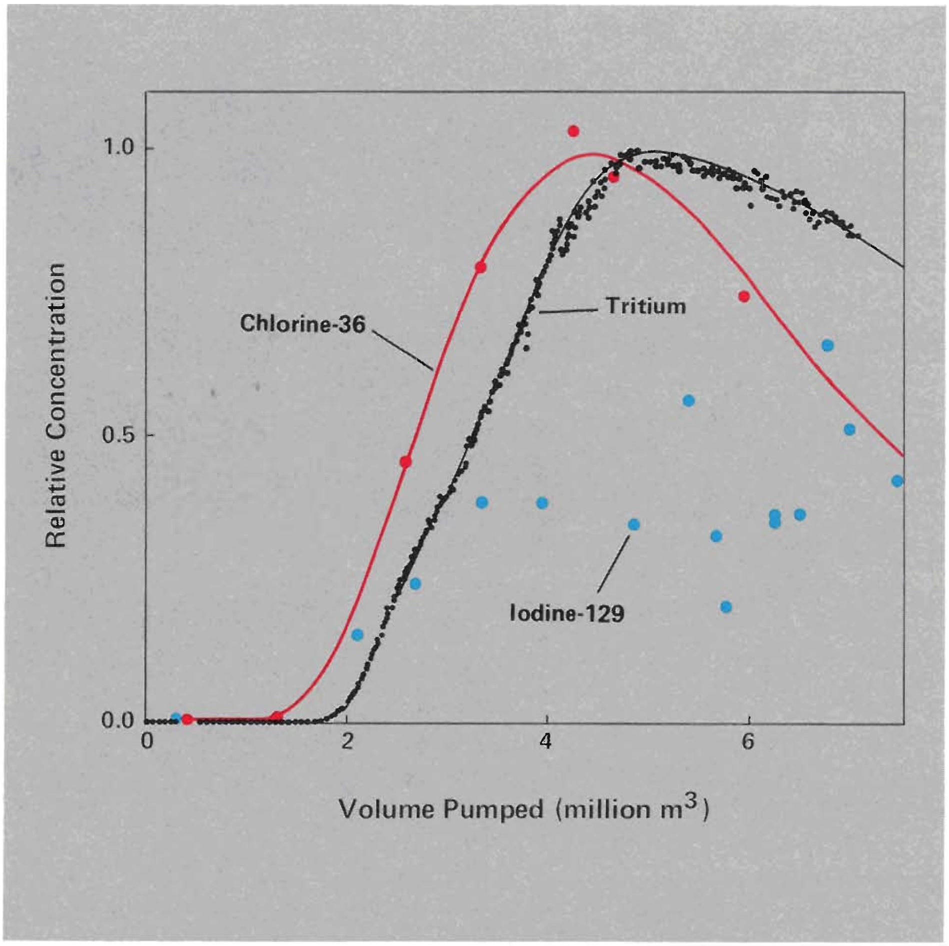


Fig. 4. Migration of radioisotopes from the Cambric cavity. The detection of tritium (black) signaled the arrival of water from the cavity. Concentrations of chlorine-36 (red) arrived earlier and peaked sooner than the tritium, most likely because of repulsion between the negative chloride ions and the negatively charged pore surfaces of the rock. On the other hand, iodine-129 (blue) appeared not to be accelerated by this effect, probably because its dominant species in the groundwater was the neutral molecule HIO. The solid curves are calculated from a model of dispersive fluid flow.

The krypton, dissolved in water, has been arriving with concentrations about one-third as great as expected based on tritium. Because ruthenium-106 has the lowest retention factor (Table I) and a very complex chemistry, it might be expected to migrate in minute amounts.

The main conclusion from the accelerated migration study is that, in general, the sorption of radioisotopes is sufficiently high to preclude the accelerated migration of most radioactive material, particularly cationic species, in the near future. However, pumping and analysis of the water will be continued to investigate the possible arrival of other poorly retarded species.

Migration from a Natural Fission Reactor

A unique opportunity for the study of radioisotope migration presented itself in the mid '70s with the discovery of the remnants of natural fission reactors in the Oklo uranium mines in equatorial Africa. About two billion years ago—nearly half the age of the earth—thick lenses of uraninite were formed that were compact enough and had the right mix of neutron-moderating elements so that fission chain reactions started. The reactors operated for a few hundred thousand years, leaving behind a set of nuclear reaction products different from those normally present in uranium ore.

Several features made the study of these fossil reactors important. The migration or containment occurred over geologic time periods. Radioisotopes present at Oklo were identical to those of modern reactor waste products. The average thermal loading within the reactor zones during operation was estimated to be about 50 watts per square meter, several times greater than the local thermal loadings proposed for reactor waste repositories. Most important, this was an opportunity to quantitatively characterize the migration or retention of more than thirty elements, the products of nuclear fission, neutron capture, and radioactive decay.

Starting in 1974, samples from Oklo were sent to the Laboratory for geochemical and migration studies. It was hoped that by examining various decay-product signatures a fair amount could be learned about which elements migrated, when the migration occurred, and what mechanisms operated to cause the migration.

REACTOR CHARACTERISTICS. Natural uranium consists essentially of two isotopes: the abundant uranium-238 and the fissionable but less abundant uranium-235. The ratio of uranium-235 to uranium-238 has been found to be 0.0072 with only slight variations throughout the world. However, within the thirteen reactor zones so far discovered at Oklo, there were significant deficiencies of uranium-235. Based on the normal ratio of uranium isotopes, it was estimated that about 12,000 kilograms of uranium-235 had fissioned. In fact, the nuclear product inventory at Oklo corresponds to 1 to 2 per cent of the plutonium and fission products and 10 to 20 per cent of the uranium in the United States spent fuel inventory as of 1980.

The rock surrounding the reactor zones is generally pelitic sandstone, a fine-grained sedimentary rock. The uranium is uraninite, a crystalline uranium dioxide. Examination of this mineral revealed crystallites characteristic of those produced by radiation. A correlation between the abundance of these crystallites and the degree of uranium-235 depletion suggested that the grains of uraninite had been thermally and mechanically stable since the reactors started. Also, the discovery of uranium samples with quite different degrees of depletion located within a few tens of centimeters of each other clearly showed that the uranium had not been chemically homogenized in the 2 billion years since the reactor operated.

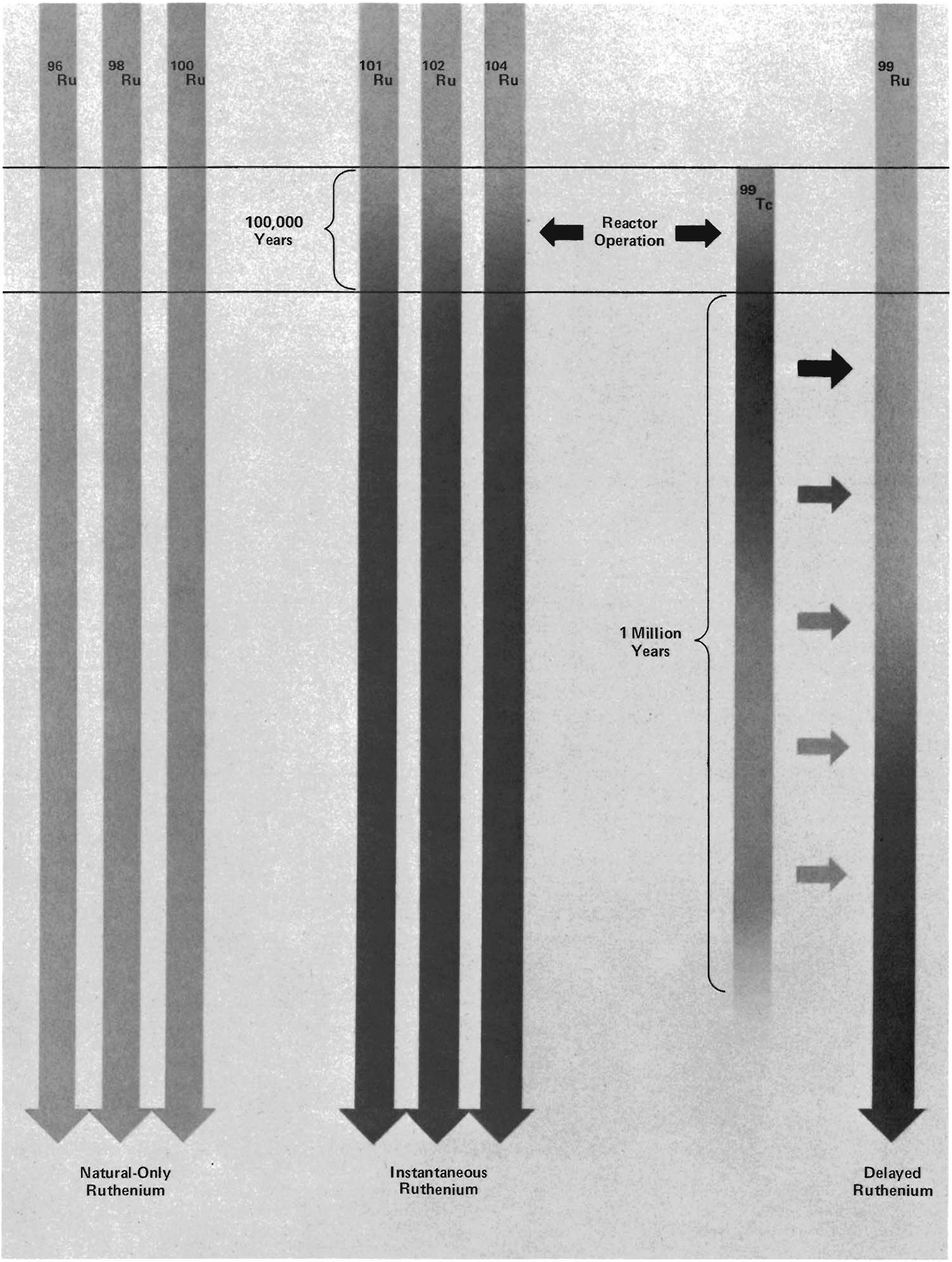
The two uranium isotopes decay ultimately to different stable lead isotopes at different rates. This fact is frequently used to date rocks, and in this case analysis of uranium and lead isotopes showed that uranium mineralization occurred 2 billion years ago. Likewise, analysis of the uranium fuel and the fission product neodymium indicated that the reactors became critical very soon after mineralization.

What were these zones like during the time of reactor criticality? Neutron fluences were as high as 10^{21} neutrons per square centimeter, a remarkably high fluence. Water in the rock was the most likely neutron moderator for the reactors, and this water, heated by the fission energy, generated hot aqueous solutions that circulated convectively through the rock. In fact, fluid inclusions in mineral grains formed at temperatures between 450 and 600 degrees Celsius have been found up to 30 meters from the reactor zones. Much of the unique physical character of the rock surrounding the reactors can be attributed to the dissolution and modification of minerals from heat and radiation and the chemical redistribution associated with circulating hydrothermal fluids.

FRACTIONATION. The isotopic composition in the reactor zones can be visualized as two components: a natural component found in materials throughout the earth and a nuclear component generated by the various nuclear processes. Total isotopic abundances were measured in the Oklo samples by mass spectrometric techniques and the natural component was then subtracted. The nuclear component remaining was compared to the predicted abundance resulting from nuclear reactions. Any difference, that is, any loss or gain for a given isotope, was indicative of migration.

Of particular interest was the evidence for fractionation between technetium and ruthenium. Technetium does not occur naturally on the earth in significant quantities, but the Oklo reactors generated approximately 730 kilograms of technetium-99. This isotope is of special concern in the storage of radioactive wastes because its 0.2 million year half-life implies storage times on the order of millions of years.

Natural ruthenium is composed of seven isotopes. These can be split into three groups depending on the relationship of each isotope to the fission process (Figs. 5 and 6). Three isotopes, the "natural-only" isotopes (96, 98, and 100), are not produced by fission. Knowing the amounts of these allows the natural component of all seven to be easily accounted for. Another three, the "instantaneous" isotopes of ruthenium (101, 102, and 104), are produced by the decay of short-lived technetium fission products. Amounts of these isotopes were generated while the reactors were operating. The last is



96 Ru
98 Ru
100 Ru
101 Ru
102 Ru
104 Ru
99 Ru
99 Tc
100,000 Years
Reactor Operation
1 Million Years
Natural-Only Ruthenium
Instantaneous Ruthenium
Delayed Ruthenium

Fig. 5. Ruthenium isotopes and their relationship to the Oklo reactor. The three "natural-only" isotopes on the left were not produced by any of the fission reactions and thus remained at their original concentrations except as modified by neutron-capture reactions. Fission reactions increased the concentrations of the three "instantaneous" isotopes during the 100,000 years of reactor operation. Because these isotopes are stable, their concentrations remained constant after reactor shutdown. Ruthenium-99, the "delayed" isotope, was produced by the gradual decay of reactor-generated technetium-99. The half-life of technetium-99 is 0.2 million years so that about 96 per cent changed to ruthenium-99 during the first million years after shutdown.

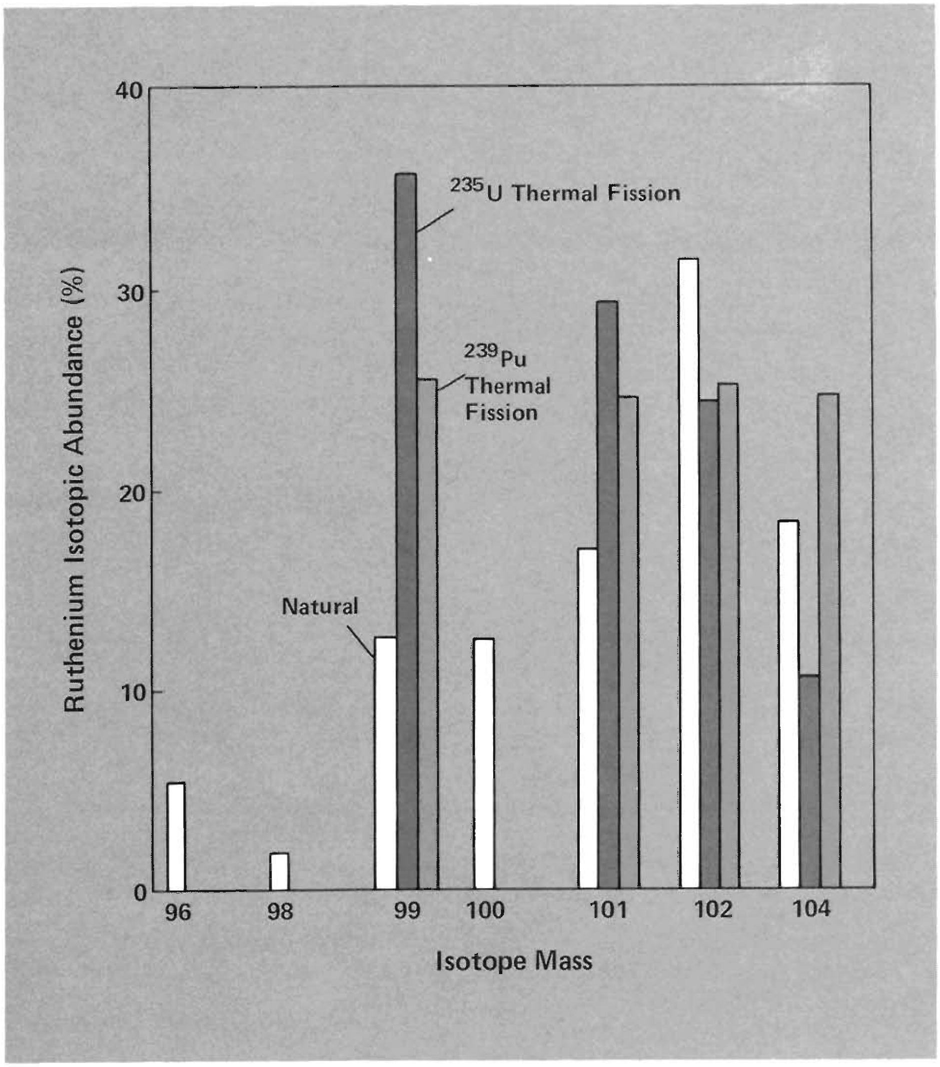


Fig. 6. Relative abundances of ruthenium isotopes in normal rock (white) and, likewise, as generated by the thermal-neutron-induced fission of uranium-235 (black) and of plutonium-239 (grey). Samples from the Oklo reactor zones usually contained ruthenium with the isotopic composition of the uranium-235 fission process; however, a few per cent of the fissions took place in plutonium-239 that had been produced from uranium-238 by neutron capture and subsequent beta decay. Samples from the periphery contained various proportions of natural and fission-generated ruthenium. Natural ruthenium was easily corrected for in a given sample using ratios based on abundances of the natural-only isotopes (96, 98, or 100).

the "delayed" isotope ruthenium-99, which, because it is the decay product of technetium-99, serves as a marker for fission-generated technetium. Any fractionation that separates ruthenium and technetium before technetium-99 decays would be observed today as a depletion or enrichment of delayed ruthenium relative to instantaneous ruthenium.

A detailed analysis based on the ideas only briefly outlined here showed that technetium-ruthenium fractionation had indeed occurred at the Oklo reactors. In fact, the half-life of technetium-99 places an upper limit on the time between reactor shutdown and fractionation. The basic conclusion was that chemical or physical processes caused technetium to be lost in greater abundance than ruthenium from the reactor zones at some time less than a million years after reactor shutdown. Analysis of samples from outside the reactor zones showed that the lost technetium (as well as other lost fission products) were contained within a few tens of meters of their source.

TRANSPORT MECHANISM. How were technetium and other elements carried out of the reactor zones? One clue was the physical distribution of technetium (now ruthenium-99). Generally, the direction of movement of this isotope was upward, consistent with convecting hydrothermal fluids as the carrier.

To examine this feature in more detail, it was postulated that the insoluble oxides of ruthenium and technetium were further oxidized to form soluble oxyion complexes (Table II). Because virtually nothing is known about the chemical conditions at Oklo during the thermal period, only this simple case of oxyions was considered. A pH and oxygen content typical of the reactor geology were assumed, and thermodynamic data were used to calculate the solubilities of the species as a function of temperature. As can be seen in Fig. 7, the solubilities vary

TABLE II

OXIDATION TO SOLUBLE OXYIONS

Insoluble		Soluble
RuO_2	→	RuO_4^{-2}
RuO_2	→	$Ru(OH)_2^{+2}$
TcO_2	→	TcO_4^-

widely (encompassing 50 orders of magnitude). The band in Fig. 7 around 10^{-10} moles per liter represents estimates of the concentrations of technetium, ruthenium, and neodymium in the aqueous fluid at Oklo. The width of the band is the uncertainty in the estimates. Also included is the solubility curve for the neodymium cation, which is retrograde.

Examination of Fig. 7 shows that at some temperature the thermodynamically controlled solubilities of neodymium, technetium, and ruthenium fall within the concentration band. Above the band, the solubility would cause the transport away of too much material, and below the band, of too little. However, this conjunction occurs at *different* temperatures for each species. RuO_4^{-2} will achieve the necessary solubility in the vicinity of 350 degrees Celsius, TcO_4^- around 150 degrees. Because of its retrograde solubility, neodymium achieves the necessary solubility *below* 25 degrees Celsius. The solubility temperature ranges for ruthenium and technetium agree with temperatures estimated for the reactor zone during its thermal period. Thus, the transport of ions by convecting hydrothermal fluids can account for the deficiency of these two elements but not for that of neodymium.

But there is a further complication. If the fluid temperature was 150 degrees Celsius, just hot enough to remove the appropriate quantity of technetium, then neither

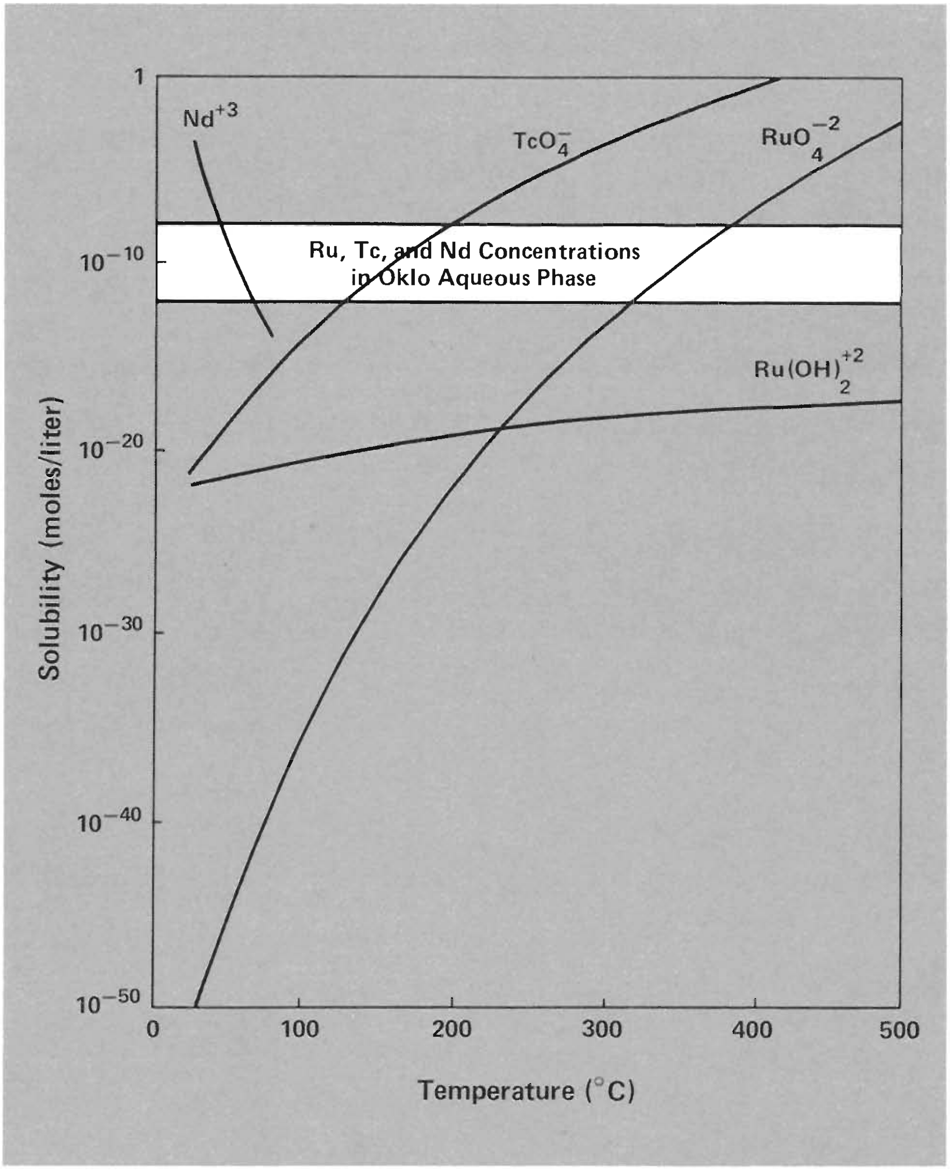


Fig. 7. The temperature dependence of the equilibrium solubilities for various ionic species of ruthenium, technetium, and neodymium. The range of estimated concentrations of these three elements in the groundwater at Oklo is superimposed on the diagram as the horizontal band centered at 10^{-10} moles per liter. Solubilities within this band indicate chemical processes and conditions that could have removed elements from the reactor zones at the appropriate rate. Solubilities above the band may have also occurred if other processes limited the rate of dissolution. The fact that $Ru(OH)_2^{+2}$ never intersects the estimated concentration band suggests that the dissolution of tetravalent RuO_2 was not a factor in the transport of ruthenium. (Uncertainty ranges for the solubility curves have been eliminated for clarity.)

ruthenium nor neodymium would have been transported away. On the other hand, if the fluid temperature was 350 degrees, hot enough to remove the necessary quantities of ruthenium, the solubility for TcO_4^- would have been so high that *all* the technetium would be gone. Since roughly equal quantities of each were removed from the reactor zone, it is likely that the rate of migration was controlled by diffusion of each element from the host mineral rather than by subsequent transport away from it as determined by the solubilities.

The retrograde solubility of neodymium suggests that it was redistributed after the thermal period. The fact that it appears to have been redistributed on a smaller scale than ruthenium or technetium agrees with this suggestion.

Although ruthenium and technetium migrated during the thermal period of the reactor, the overall transport was relatively insignificant. These elements were transported only a few tens of meters within a million years after reactor operation. Rates of transport were thus on the order of 10^{-5} meters per year whereas fluid movement could have been up to 5 meters per year. In other words, the pelitic sandstones surrounding the reactors had a remarkable ability to contain these nuclear products even after they were released from the uraninite. The extreme temperature dependence of the solubilities suggests a suitable mechanism for this retention capacity. As the fluids left the hot reactor zones and began to cool, they became supersaturated with respect to the ruthenium and technetium oxyions, and their reduced form could have been deposited in the rocks adjacent to the hot regions. If this mechanism is correct, aureoles reflecting temperature profiles around the hot zones should be found. That is, supersaturation for RuO_4^{-2} occurs around 200 degrees higher than for TcO_4^-, and ruthenium should be found closer to the reactors than technetium. This temperature dependence may also help explain the apparent fractionation of ruthenium and technetium during the thermal period.

In general, our studies have revealed the broad outline of a possible mechanism for the geochemistry of the Oklo reactors. Much work remains before the details are understood. However, the possibility of long-term storage of radioactive waste in underground sites has certainly been demonstrated by the existence of these natural reactors.

Nuclear Waste Storage

The National Waste Terminal program, initiated in 1976 and presently directed by DOE, is investigating several sites as potential repositories for high-level nuclear waste. One of these, Yucca Mountain, is located near the southwest corner of the Nevada Test Site and is being studied jointly by the Los Alamos, Sandia, and Lawrence Livermore National Laboratories and the U.S. Geological Survey. Our role in the research at this site is to develop part of the scientific base to be used in deciding whether Yucca Mountain will be appropriate as a repository.

Such a determination necessarily involves a wide range of studies. Rock samples at various depths to 6000 feet need to be analyzed for thermal and mechanical properties, porosity, permeability, extent of fractures, mineral composition, sorption properties for different elements, and so forth. The water there is very mild, containing mostly low concentrations of sodium and bicarbonate ions. Nevertheless, its physical and chemical properties, such as the amounts of dissolved oxygen, organic material, microorganisms, and other constituents that might effect migration, must be evaluated. One of the most important tasks is the realistic modeling of the geochemistry so that predictions can be made with confidence. Here we will discuss only a couple of examples to illustrate the link between laboratory or field experiments and model development.

RETARDATION. The rock underlying Yucca Mountain is primarily welded and nonwelded tuff, which are volcanic materials. Welded tuff is hard, dense, and has excellent thermal properties. Nonwelded tuff below the water table there contains large amounts of clay and zeolitic minerals, and the rock is highly porous but impermeable. In fact, this latter rock can hold an average of 25 per cent water by volume. Radioisotopes in the flowing water in fractures can diffuse into the rock matrix and be trapped in the static water or be sorbed on pore walls. Retardation describes the group of processes that, through interactions with rock, remove elements from solution in moving water and is an important parameter to measure and model.

A variety of experimental techniques have been used on Yucca Mountain tuff to measure sorption, an important process in retardation. They include the batch process on either crushed rock or tablets of whole rock, flow through columns of crushed material, continuous circulation of groundwater through crushed material, and radioisotope transport through cylinders or blocks of rock that contain real or artificial features. Quite similar sorption parameters are obtained for all these techniques if care is taken in both experimental procedure and in proper mathematical interpretation of the measurements.

For example, Fig. 8 shows batch sorption data for cesium as a function of depth in a series of layered volcanic materials. Although the sorption ratio can easily be experimentally reproduced within a range of 20 per cent, it varies with depth by more than two orders of magnitude, depending on the mineral composition of each sample.

The principal phases that aid in sorption and cause the dramatic changes in Fig. 8 include hydrated volcanic glasses, smectite clays, and zeolites. The glasses are the least important for sorption, but they are very reactive and can alter to other minerals with changes in hydration if heated in the presence of water. Smectite clays are reversibly expandable with highly sorptive properties, although the properties can be modified in a detrimental way by prolonged exposure to heat. Zeolites, with their open lattice structure, are the most highly sorptive minerals.

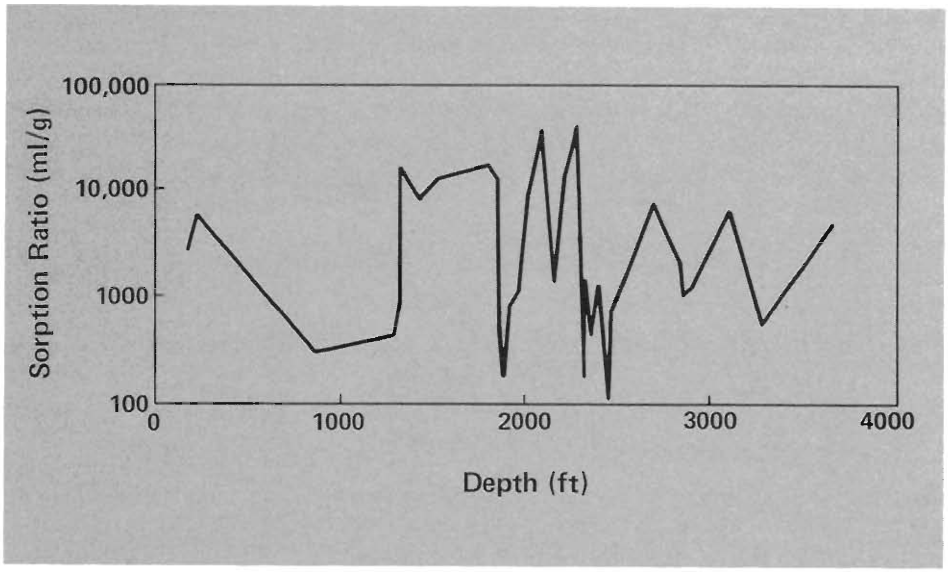


Fig. 8. The sorption ratio for cesium as a function of depth into earth. Because the rock at the Yucca Mountain site is made up of series of layers of volcanic material, each with its own mix of minerals, the sorption of cesium varies dramatically. Experimental reproducibility of the data shown here is about 20 per cent.

Because tuff samples may be composed of several sorbing minerals, we are currently developing ways to predict sorption ratios by combining the effects of several minerals. One approach uses the sorptive mineral content, or *SMC*, a weighted sum defined by

$$SMC = \sum W_i X_i \;,$$

where W_i is the weighting factor for each mineral phase and X_i is the per cent abundance of each phase.

Figure 9 demonstrates the results of this approach for the cesium sorption data. Weighting factors were determined relative to a zeolite, clinoptilolite, by directly measuring the sorption of cesium on pure minerals or by inferring it from measurements with mixtures. The resulting values are plotted in Fig. 9 along with the theoretical relationship (solid line) based on thermodynamic data. Note that almost all the data lie within the envelope (dashed lines) for an uncertainty factor of 3. Procedures similar to this should eventually allow us to predict sorptive properties for the tuffs along pathways to the environment. However, sorption of cesium is probably the simplest test of this concept. Any such procedure is critically dependent upon accurate determination of the mineral phases available to the groundwater.

KINETICS OF SORPTION. Another aspect that must be understood before an adequate transport model can be developed is the kinetics of sorption. This is especially true of tuff because its high porosity makes diffusion of waste elements into the rock matrix a very significant retardation process for radioisotopes.

Figure 10 shows the sorption of strontium onto a sample of tuff as a function of time (circles). Three types of fits were made to the data. The first and least successful, labeled simple diffusion, is based on diffusion into a plane sheet. In this case the equilibrium concentration of strontium between the grain surfaces and the pore water, defined by the sorption parameters, is reached instantaneously and sorption is treated as being linear with concentration. However, the number of available sorption sites may be limited so that at higher concentrations sorption eventually drops below the linear curve. This nonlinearity complicates the diffusion equations by giving the diffusion coefficient a concentration dependence. A finite-difference solution of the nonlinear diffusion problem was made and is also plotted in Fig. 10. The agreement with experimental data is better in this case but still does not reproduce the early time points. Attempts to adjust the

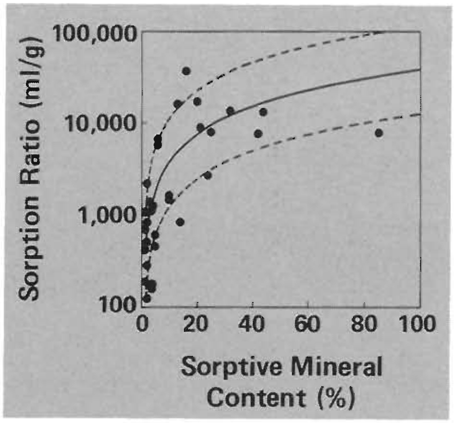
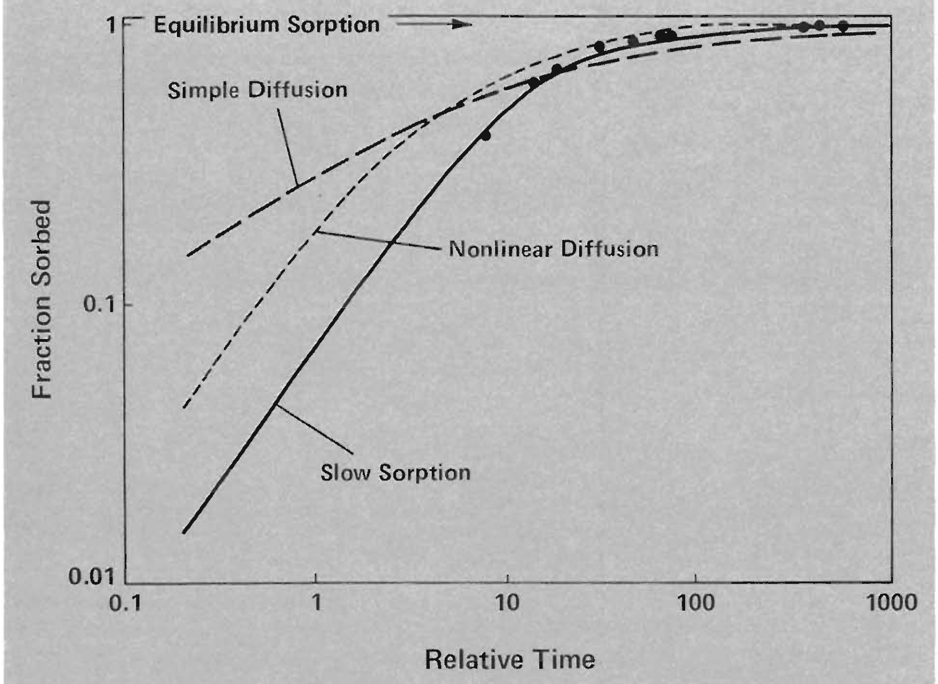


*Fig. 9. Sorption ratios for cesium are here plotted as a function of sorptive mineral content (*SMC*), a parameter that weights the sorptive qualities of each mineral in a sample. The solid line is calculated from thermodynamic parameters; the dashed lines indicate an uncertainty factor of ±3.*



MODEL EQUATIONS

Simple Diffusion

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \qquad D = \text{constant}$$

Nonlinear Diffusion

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \qquad D = f(C)$$

Slow Sorption

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \frac{\partial S}{\partial t}, \quad \frac{\partial S}{\partial t} = \lambda C - \mu S$$

C = concentration in solution
D = apparent diffusion coefficient
S = concentration of immobilized solute
λ, μ = rate constants

Fig. 10. Modeling the kinetics of sorption of strontium on tuff. The fraction of strontium sorbed on the rock approaches equilibrium amounts as relative time increases (relative time is Dt/L^2 *where* D *is the apparent diffusion coefficient for the material and* L *equals half the sample thickness). Simple diffusion involves a constant diffusion coefficient, whereas nonlinear diffusion involves a concentration-dependent coefficient. The best fit is achieved with a slow sorption model that includes a reversible immobilization of part of the material in the rock matrix.*

fit led to values for the adjustable parameters that were not consistent with other data.

The third type of fit, labeled slow sorption in Fig. 10, is like the first model except that the equilibrium concentration between grain surfaces and pore water is not reached instantaneously. The process was considered to be a reversible reaction with the solid tuff in which part of the dissolved species is free to diffuse within the material and part is immobilized in the tuff. Mathematically, a term representing the rate of removal of the immobolized component was subtracted from the simple diffusion equation. The agreement between the data and the shape of the curve based on this approach was quite good. Moreover, the parameters determined from the best fit agreed with other data.

The consequences of these kinetic studies may be profound for migration of waste in which the radioisotopes are carried by rapidly moving water in fractures. The sorption rate constants determined by us will be used in calculations with the nonequilibrium version of our three-dimensional transport code (TRACR3D) to model the results of flow in fractured rock. Our models are becoming detailed enough so that we can extrapolate from the short time scales of laboratory experiments to the long time scales required for storage of nuclear waste.

In general, our studies of the geochemistry of the Yucca Mountain site, although far from complete, so far indicate that the geochemical properties and setting of the site will strongly inhibit the movement of radioisotopes by flowing groundwater to the accessible environment.

We have discussed examples from three areas in the study of radioisotope migration. There are many more published examples of such Los Alamos studies, and there are considerable possibilities for future investigations. The field remains vital and exciting because these radioisotopes act as tracers, illuminating processes generally hidden from us by complexity and by time in the earth's crust. ■

Further Reading

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AUTHORS

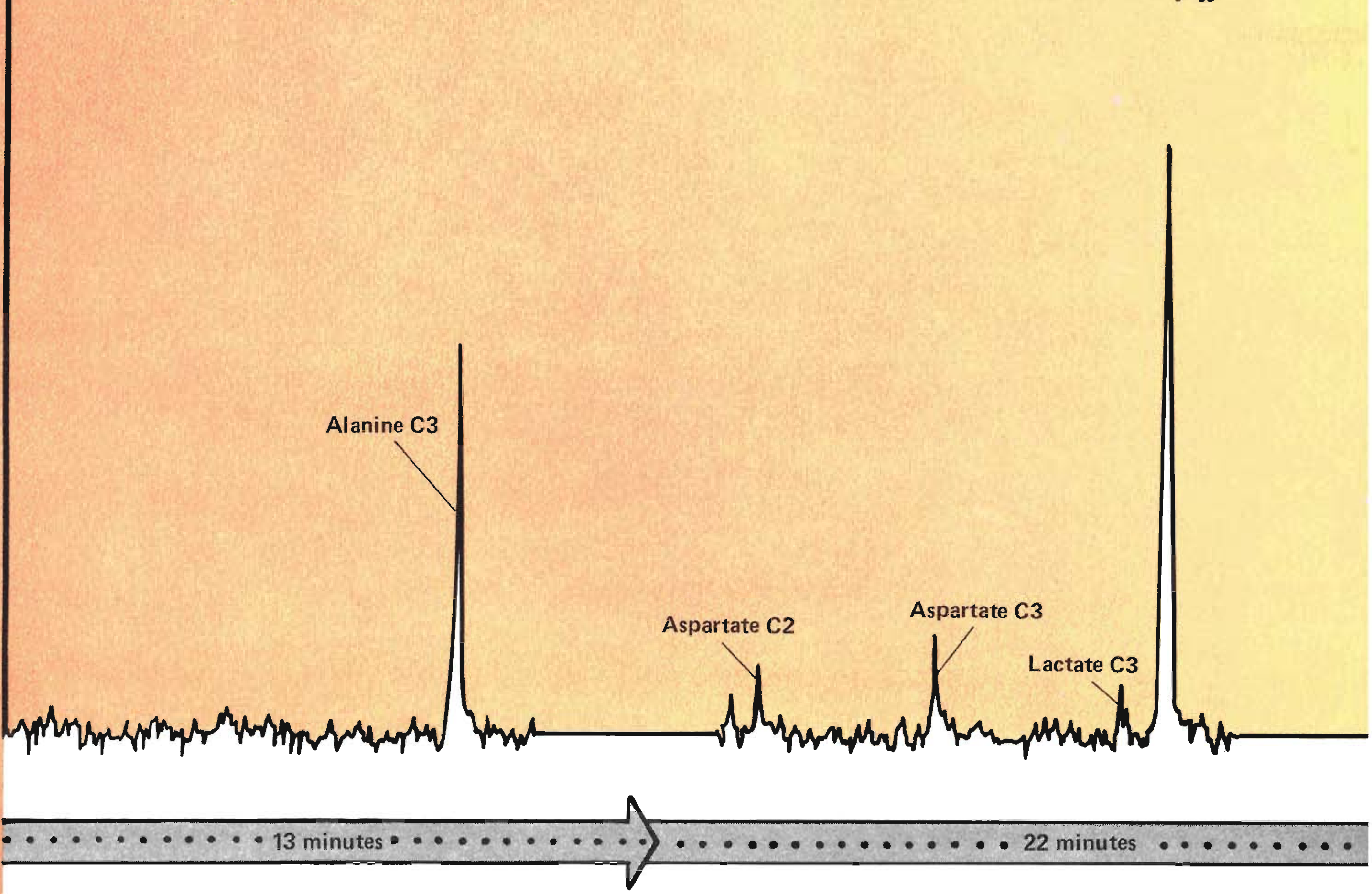
Many people from the Isotope and Nuclear Chemistry Division have contributed to the work described in this article. Those pictured above are: Tim Benjamin, Ernie Bryant, John Cappis, Dave Curtis, Clarence Duffy, Alex Gancarz, Darleane Hoffman, Ed Norris, Al Ogard, Jim Sattizahn, and Kurt Wolfsberg. Others, who weren't available for the photograph, are Bill Daniels, Bruce Erdal, Bob Rundberg, and Rosemary Vidale.

What happens to your body when you drink alcohol? How does your metabolism change when your oxygen supply is reduced? How does one know that an organ is healthy enough to be used as a transplant? Why do some people persistently have low blood sugar? The only way to find out in detail is to watch

Metabolism

as it happens

by James R. Brainard, Judith Y. Hutson, Robert E. London, and Nicholas A. Matwiyoff

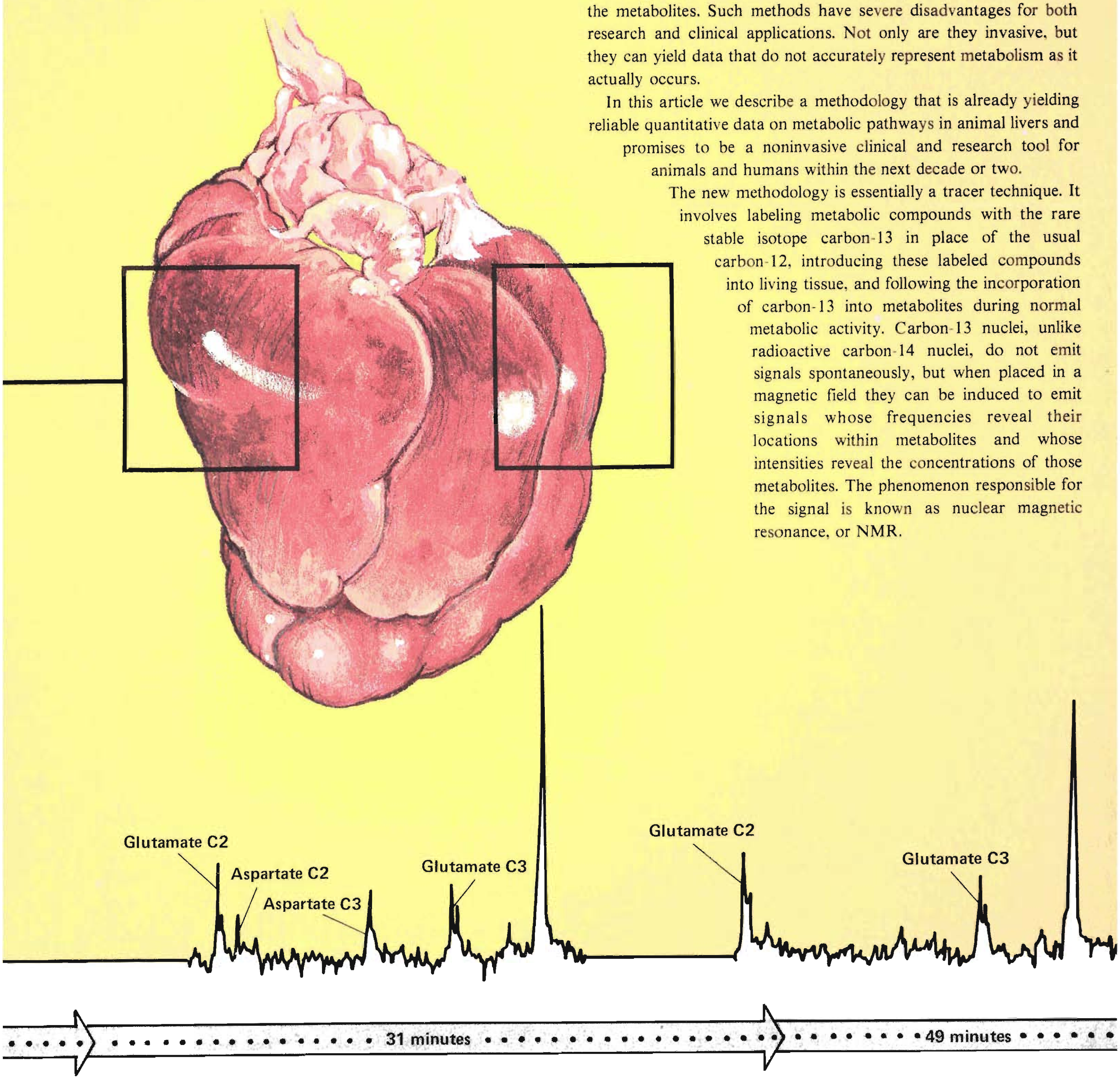


The liver is primary among the organs responsible for regulating metabolism. It is the first organ to process nutrients coming from the intestine, and since these nutrients have a highly variable composition, the liver must continuously adjust its metabolic activities to maintain relatively constant concentrations of nutrients in the blood stream. This regulation is achieved by a very complex network of chemical reactions with numerous checks and balances. Sometimes the network goes awry, and when it does, chronic disorders are the result. Abnormal metabolism of glucose, for example, accompanies diabetes, hypoglycemia, obesity, and glycogen storage diseases.

These diseases can best be diagnosed and treated—or prevented—if their molecular basis is understood. But until recently the only methods available to examine metabolic activity required removing body fluids or tissues, working up samples, and isolating the metabolites. Such methods have severe disadvantages for both research and clinical applications. Not only are they invasive, but they can yield data that do not accurately represent metabolism as it actually occurs.

In this article we describe a methodology that is already yielding reliable quantitative data on metabolic pathways in animal livers and promises to be a noninvasive clinical and research tool for animals and humans within the next decade or two.

The new methodology is essentially a tracer technique. It involves labeling metabolic compounds with the rare stable isotope carbon-13 in place of the usual carbon-12, introducing these labeled compounds into living tissue, and following the incorporation of carbon-13 into metabolites during normal metabolic activity. Carbon-13 nuclei, unlike radioactive carbon-14 nuclei, do not emit signals spontaneously, but when placed in a magnetic field they can be induced to emit signals whose frequencies reveal their locations within metabolites and whose intensities reveal the concentrations of those metabolites. The phenomenon responsible for the signal is known as nuclear magnetic resonance, or NMR.



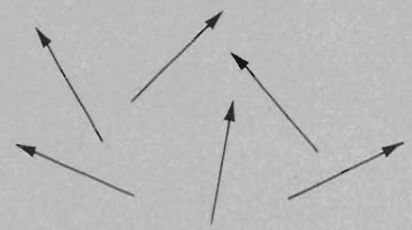
Principles of NMR Spectroscopy

Nuclear magnetic resonance (Fig. 1) has been used by physicists, chemists, and biochemists for almost four decades to examine the properties and the environment of certain nuclei, namely those with nonzero spins and hence nonzero nuclear magnetic moments. All nuclei with odd mass number (and some nuclei with odd proton number and odd neutron number) have this property, and the nuclei most commonly used in NMR studies of biological systems are hydrogen-1, carbon-13, and phosphorus-31.

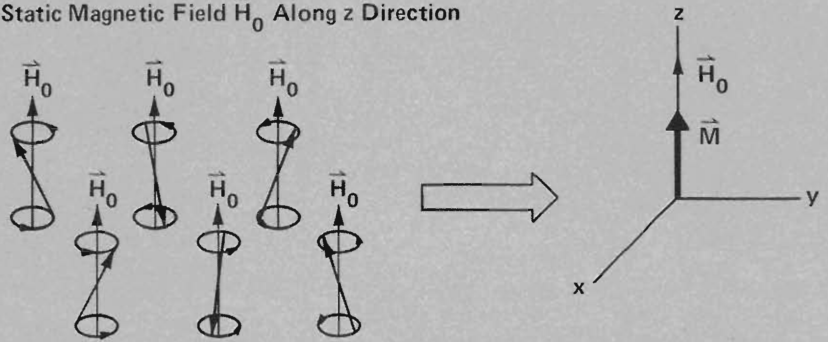
When a collection of (for example) carbon-13 nuclei are placed in a static magnetic field $\mathbf{H}_0$, the individual magnetic moments, which usually point in random directions, tend to line up either almost parallel or antiparallel to $\mathbf{H}_0$. (The two possible alignments reflect the fact that carbon-13 nuclei have a spin of $\frac{1}{2}$.) In addition, the magnetic moments precess around the magnetic field lines at a frequency, called the Larmor frequency, that is proportional to the product of carbon-13's nuclear magnetic moment and the magnetic field strength.

How do we detect these precessing nuclei? We use the fact that the alignment of the nuclear magnetic moments produces a net macroscopic magnetization **M** along the direction of $\mathbf{H}_0$ (the *z*-axis). This net magnetization is static, however, and does not give any information about the precession. Therefore we apply a second magnetic field $\mathbf{H}_1$, rotating at the Larmor frequency and perpendicular to the *z*-axis, to cause **M** to bend away from the *z*-axis toward the *x*-*y* plane. As **M** rotates around the *z*-axis at the Larmor frequency, it produces a fluctuating magnetization in the *x*-*y* plane that can be detected by sensitive radiofrequency pickup coils. A Fourier transform of the time-varying signal then yields the resonance signal at the Larmor frequency. Strong magnetic fields induce Larmor frequencies in the range of radio frequencies. For example, in a field of 1 tesla, the Larmor

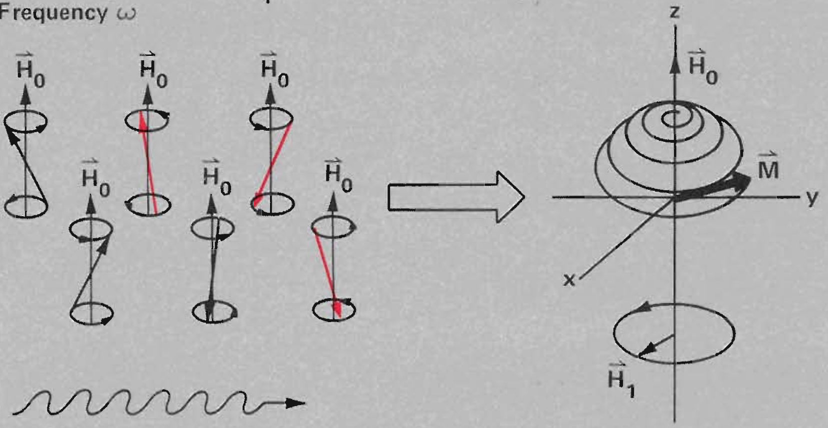
(a) No Magnetic Field



(b) Static Magnetic Field $\vec{H}_0$ Along z Direction



(c) Second Magnetic Field $\vec{H}_1$ in x-y Plane Oscillating at Frequency ω



(d) Signal Detected as $\vec{M}$ Rotates in x-y Plane at Frequency ω

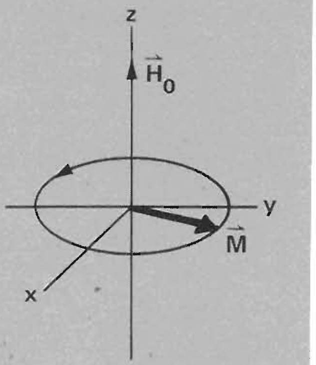


Fig. 1. Carbon-13 nuclear magnetic resonance.

(a) A carbon-13 nucleus has a spin of $\frac{1}{2}$ and a nonzero nuclear magnetic moment μ. The moments of a collection of carbon-13 nuclei are randomly oriented in the absence of an external magnetic field.

(b) In a static external magnetic field H_0 the moments become aligned almost parallel or antiparallel to H_0 and precess around the field lines at a frequency $\omega \propto \mu \cdot H_0$. More moments point "up" (in the direction of H_0) than "down" because the "up" state is lower in energy than the "down" state by an amount $\hbar\omega$. Consequently, the sum of the moments yields a net macroscopic magnetization M in the direction of H_0. Since the moments do not precess in phase, they do not produce a net magnetization in the x-y plane.

(c) To measure ω, one applies an oscillating magnetic field perpendicular to H_0. If the frequency of this field matches (is in resonance with) the Larmor frequency ω, it induces transitions between the two states. The resulting net absorption of energy could serve as the resonance signal, but most modern spectrometers measure the effect of the oscillating magnetic field on M. The oscillating field may be decomposed into two rotating fields, one of which, H_1, rotates in the same direction as the precessing nuclei. On a classical level we may say that H_1 exerts a torque on M such that M spirals down toward the x-y plane. The result is a net magnetization in the x-y plane that fluctuates at the frequency ω.

(d) H_1 is often applied as a radio-frequency pulse whose intensity and duration insure that at the end of the pulse M is rotating in the x-y plane.

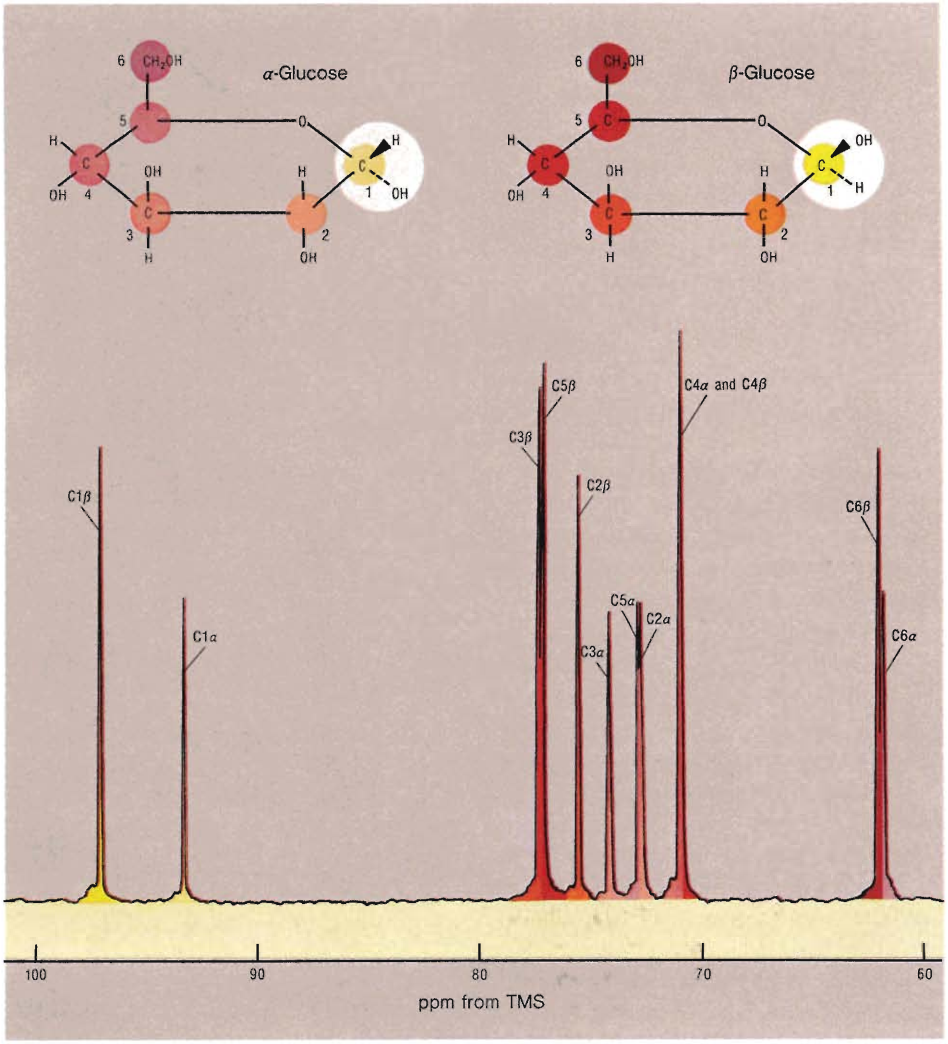


Fig. 2. NMR spectrum of the naturally occurring carbon-13 nuclei in glucose. All but two of the twelve different frequencies are clearly resolved in the spectrum shown here, which was obtained with a 2.3-tesla static magnetic field and a 25.2-megahertz rotating magnetic field. The resonance peaks are labeled and color-coded according to carbon position and anomeric form. The resonance frequencies are expressed as relative deviations (in ppm) from the resonance frequency of carbon-13 in the reference molecule tetramethylsilane, or TMS. This spectrum would have contained many more carbon-13 resonances had the protons of glucose been allowed to line up with the static magnetic field and produce magnetic fields that interact with the carbon-13 nuclei. These interactions are averaged—and a single resonance is obtained for each nonequivalent carbon location—by introducing a magnetic field oscillating at the Larmor frequency of the protons. The relative intensities of the resonances for the α and β anomers are related to their relative abundances.

frequency of carbon-13 nuclei is 10.7 megahertz and that of protons is 42.6 megahertz. Note that since the magnetic moment varies from one isotope to another, different isotopes in the same magnetic field have different Larmor frequencies.

Chemically bound nuclei, as opposed to free nuclei, also exhibit nuclear magnetic resonance but resonate at slightly different frequencies depending upon their chemical environment. Consequently, identical nuclei at nonequivalent locations within a molecule will give rise to separate resonance peaks. The ratio of the separation between the peaks to the frequency of any one peak is typically on the order of parts per million. These so-called chemical shifts in resonance frequency come about because the surrounding electrons tend to shield the nuclei from the applied magnetic field. Thus nuclei in electron-rich environments see a lower field and resonate at lower frequencies than do nuclei in electron-poor environments.

An illustration of chemical shifts is given in Fig. 2, the NMR spectrum of the naturally occurring carbon-13 nuclei in D-glucose ($C_6H_{12}O_6$). Since glucose contains six nonequivalent carbon atoms, one might predict that the spectrum would contain six distinct carbon-13 resonances. However, glucose exists in two anomeric forms, α and β, characterized by different configurations of the hydroxyl group (–OH) on carbon 1.

Consequently, its spectrum contains not six but twelve resonances corresponding to six carbons in two anomeric forms. The separation between the α and β peaks of carbon 6, a location relatively distant from the anomeric carbon atom, is testimony to the ability of NMR spectroscopy to detect rather subtle structural features. In addition, proper selection of instrumental parameters makes it possible to determine the relative amounts of different substances directly from the relative intensities of the resonances. For example, from the relative intensities of the resonances of the α and β anomers in Fig. 2, we can calculate that the β anomer predominates by a factor of 2.

For our experiments with ^{13}C-labeled molecules, we use a pulsed NMR technique in which the applied radio-frequency pulse has a bandwidth that covers the spread in carbon-13 resonance frequencies. A Fourier transform of the time-varying signal induced by the pulse then yields the frequencies of all the carbon-13 resonances.

Early NMR Studies of Metabolism *in Vivo*

About ten years ago one of us (Matwiyoff) began to explore the utility of applying carbon-13 labeling and NMR spectroscopy to monitor metabolic processes in living systems. The Laboratory was already involved in separating carbon-13 from carbon-12 and in finding methods to incorporate this isotope into organic molecules. Since carbon-13 is chemically identical to carbon-12, ^{13}C-labeled molecules could be introduced into living systems without compromising their biochemical activity. Moreover, since carbon-13 is normally present in organic molecules at very low levels (1.1 percent), its introduction through labeling would greatly enhance the sensitivity of NMR tracer studies. Such studies require subjecting living systems to magnetic and radio-frequency fields, but these fields are essentially nondestructive.

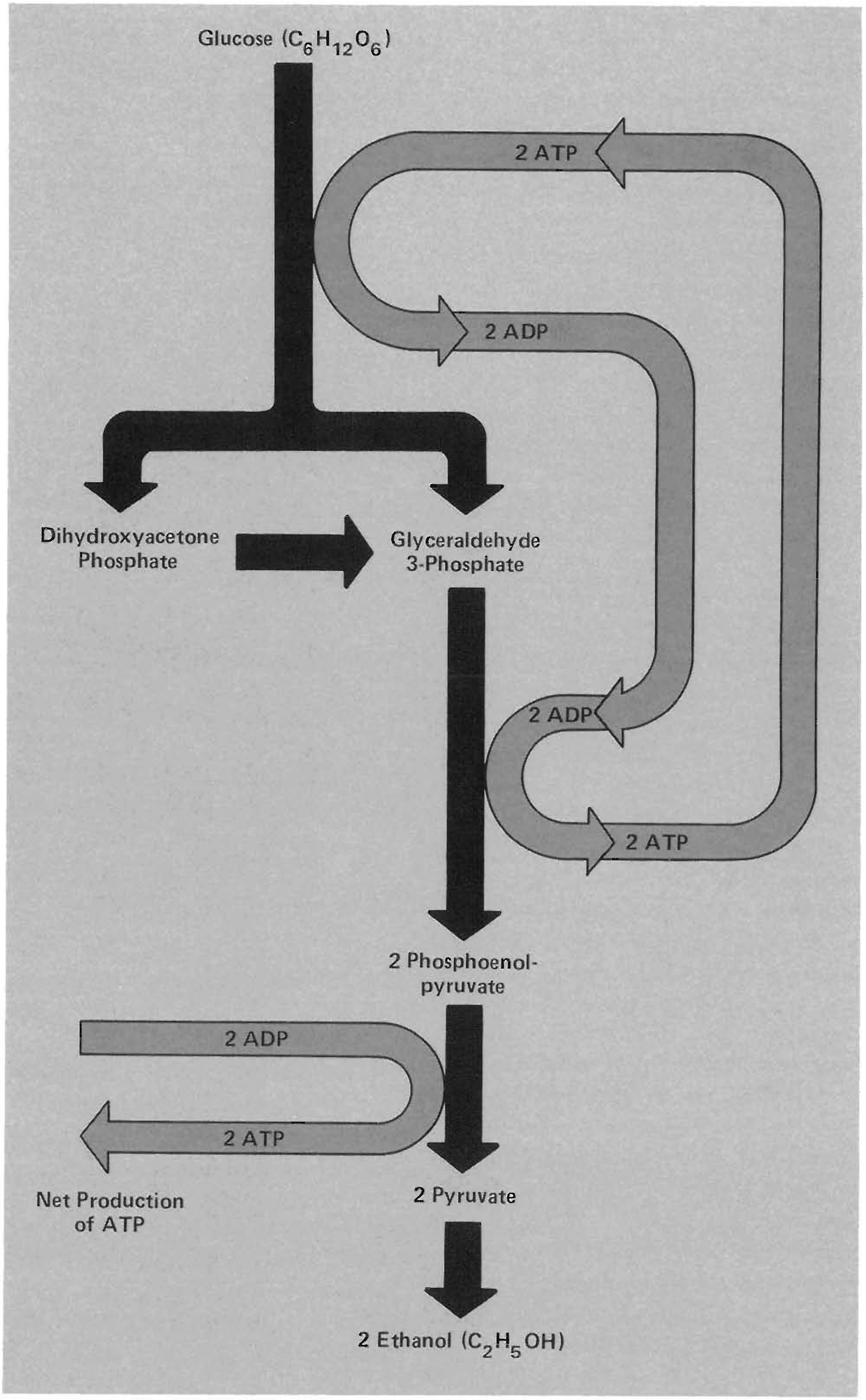


Fig. 3. Glycolysis in yeast cells under anaerobic conditions. This pathway, which occurs in many types of cells, produces ATP by converting glucose to pyruvate. ATP provides the energy required for many cellular processes. The 6-carbon sugar glucose is first converted (in four steps) to two 3-carbon carbohydrates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) with the consumption of two molecules of ATP. These ATP molecules are regenerated, however, as two molecules of phosphoenolpyruvate are formed from the 3-carbon carbohydrates. Subsequent hydrolysis of the phosphoenolpyruvate molecules to pyruvate is accompanied by the production of two molecules of ATP. Consequently, glycolysis yields a net production of ATP. In yeast cells under anaerobic conditions, the pyruvate is converted to ethanol.

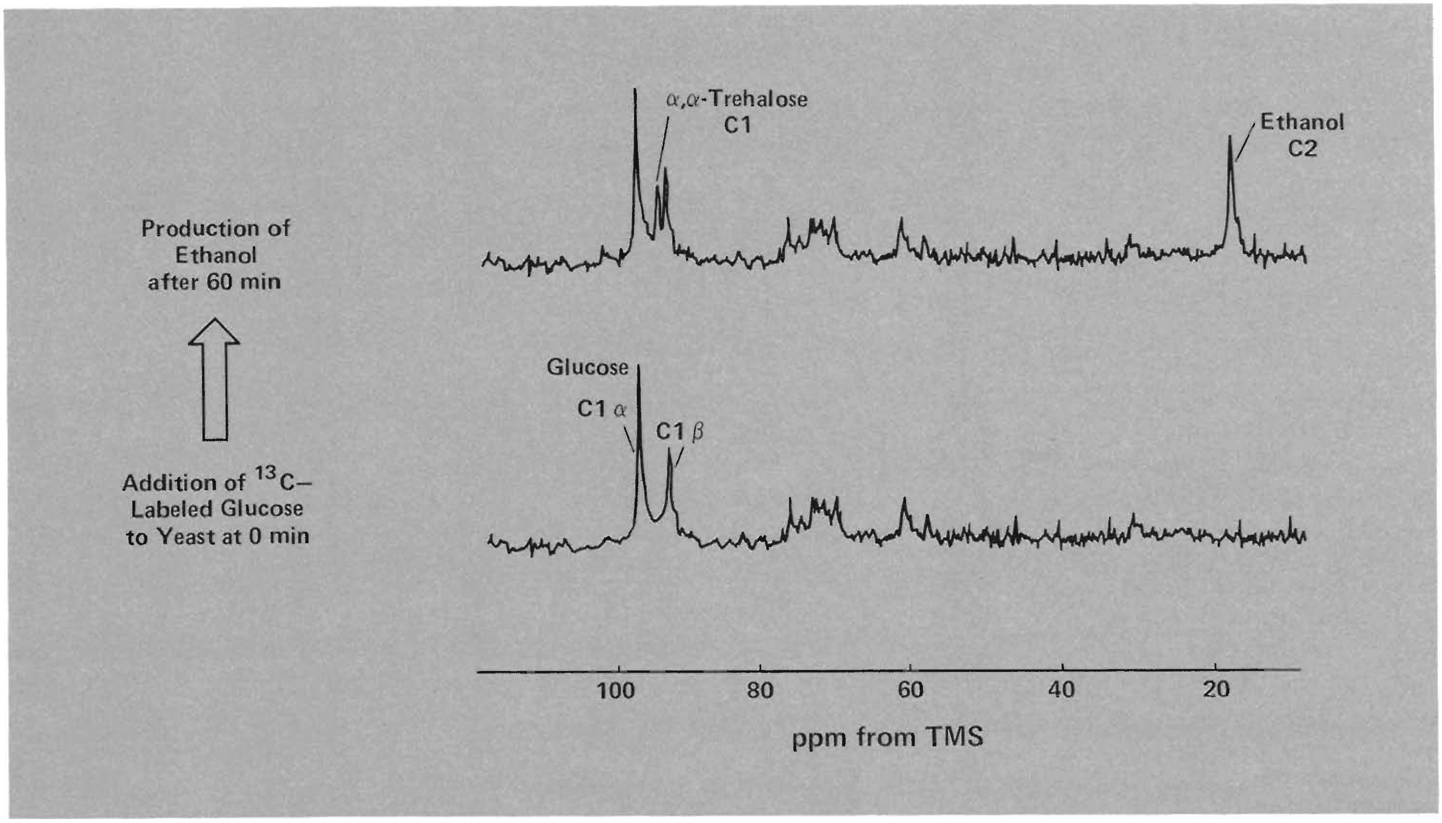


Fig. 4. Proton-decoupled carbon-13 NMR spectra of a suspension of yeast cells after addition of 5.5-millimolar [1-^{13}C]glucose as a substrate for glycolysis. The spectrum taken immediately after addition of [1-^{13}C]glucose shows resonances only from the labeled carbon of glucose in its two anomeric forms. The spectrum taken after 60 minutes of metabolism shows, in addition, resonances from ethanol and α,α-trehalose, a storage form of glucose in yeast.

The first *in vivo* study of metabolism monitored the conversion of glucose to ethanol in yeast cells. Glucose, an energy-rich molecule, is broken down by living systems in a sequence of chemical reactions known as the glycolytic pathway (Fig. 3). The function of this sequence is to produce ATP (adenosine triphosphate), a molecule whose high chemical energy is used to drive many biochemical reactions.

Glucose labeled with carbon-13 (90 atomic percent) at carbon 1 ([1-^{13}C]glucose) was added to a suspension of yeast cells, and the course of its conversion to ethanol was followed with NMR spectra (Fig. 4). The initial spectrum, obtained shortly after the addition of [1-^{13}C]glucose, showed only two intense resonances, those from the α and β configurations of the labeled carbon atom. After about an hour additional intense resonances appeared. One of these resonances was assigned to ethanol and the other to the disaccharide α,α-trehalose, which serves in yeast as a storage form of glucose. This experiment demonstrated that metabolism could be studied noninvasively and in real time within living organisms. Furthermore, it showed that the yeast cells converted a portion of the glucose to ethanol to meet their immediate energy requirements and converted another portion into a storage form of carbohydrate.

From our early studies of metabolism in yeast and related studies of the structure of abnormal hemoglobin molecules in intact red blood cells, we were convinced that labeled metabolites and NMR spectroscopy could be used with minimal invasiveness to study biochemical reactions as they occur *in vivo* under normal and pathological conditions. There has been slow but sustained progress toward this end in a number of laboratories, largely paralleling developments in NMR instrumentation. In this article we will emphasize the experimental strategies and information content of this methodology, using examples from ongoing research at Los Alamos.

Why Study Gluconeogenesis in the Liver?

In 1981 we acquired a high-field NMR spectrometer with a wide-bore magnet that allowed us to make the transition from the study of cells to the study of perfused organs, that is, organs removed from the body but kept viable by circulation of an oxygenated blood-like medium through the organ. The study of perfused organs is a necessary next step toward applying the NMR technique to live animals. Although experiments on perfused organs preclude investigating relationships between different organs, they do allow us to change the composition or flow rate of the perfusion medium at will and thereby to study a wide variety of alterations in metabolism.

We chose to study the liver because of its central role in mammalian metabolism, and the primary question we addressed was whether carbon-13 NMR spectra could be used to detect changes in metabolism when the oxidation-reduction state or oxygen supply to the liver was changed. Correspondingly, could carbon-13 NMR metabolic profiles be used to predict the oxidation-reduction state and oxygen supply? We focused on one particular metabolic process, namely gluconeogenesis, or resynthesis of glucose. This process was chosen for several reasons. First, it has been well studied by classical methods, and the available data would provide a good check of our developing methodology. Second, the synthesized glucose accumulates in the liver and could therefore be easily detected by carbon-13 NMR. Finally, gluconeogenesis acts in competition with, parallel to, or synergistically with other metabolic pathways for the use of glucose and products of glucose metabolism. The other pathways include synthesis of glycogen, of fatty acids and other lipids, and of amino acids and glycoproteins. Thus a detailed understanding of gluconeogenesis under normal and abnormal oxidation-reduction states or oxygen supplies might provide insight into the regulation of many metabolic processes.

The body normally stores only enough glucose to provide fuel to the brain for twelve hours, and therefore the liver's unique ability to resynthesize glucose is extremely important for maintaining energy homeostasis. The glucose metabolized by the body supplies the precursors for gluconeogenesis. For example, muscle and brain cells oxidize glucose to pyruvate and lactate. Frequently, the pyruvate combines with an amino group ($-NH_3^+$) to form the amino acid L-alanine, which is delivered by the blood stream to the liver where it is converted to glucose through gluconeogenesis.

This gluconeogenesis from alanine was the subject of our study. Figure 5 depicts its basic features and its relationship to oxygen supply. The figure shows the two components of the liver cell that participate in gluconeogenesis: the cytosol, or soluble portion of the cell, and the mitochrondria, structures about the size of a bacterium surrounded by a double membrane. (The mitochondria use most of the oxygen supplied to the cell and are the sites of the principal energy-yielding reactions.)

The first step of gluconeogenesis takes place in the cytosol. There alanine, a 3-carbon amino acid, loses its amino group. (How the liver disposes of this amino group is an important question and will be considered later.) Alanine's 3-carbon skeleton then enters the mitochondria and undergoes one of two reactions (shown in Fig. 6). Products of these reactions enter the Krebs cycle and a 4-carbon skeleton emerges from the mitochondria. Two 4-carbon skeletons are then used in the cytosol to form glucose.

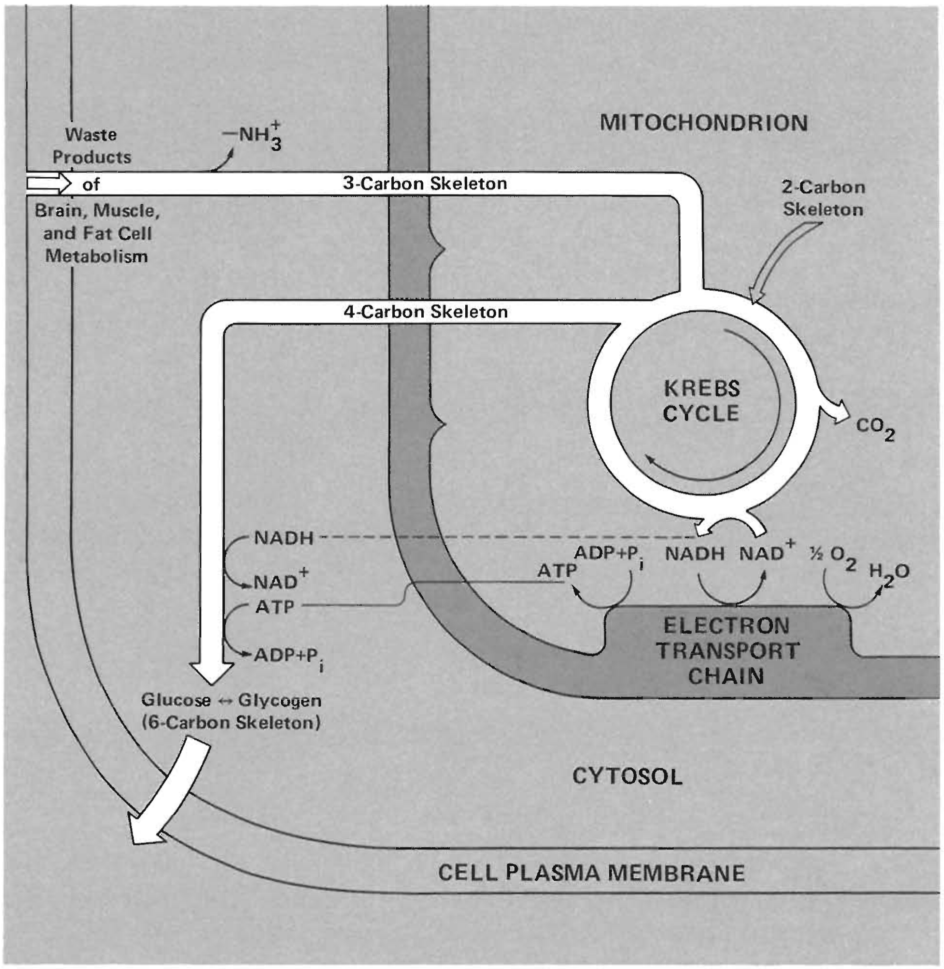


Fig. 5. Gluconeogenesis, oxygen supply, and the Krebs cycle. The white pathway traces gluconeogenesis: waste products of metabolism in muscle, brain, and fat cells enter the liver cell and glucose exits. This synthesis of glucose requires ATP and the reducing agent NADH. ATP is supplied by the electron transport chain in the mitochondrial membrane through a complex set of reactions that reduce oxygen. These reactions also regenerate the oxidizing agent NAD^+, which in turn is used in reactions in the Krebs cycle that yield NADH. Proper functioning of the electron transport chain depends on an adequate oxygen supply. The 4-carbon skeleton for gluconeogenesis is supplied by intermediates of the Krebs cycle. This cyclic sequence of enzymatic reactions in aerobic organisms serves primarily to convert chemical energy from carbohydrates, fatty acids, and amino acids into reduced pyridine nucleotides such as NADH, which are then used to produce ATP. Intermediates of the Krebs cycle form the building blocks for a variety of anabolic processes in addition to gluconeogenesis, and one of these intermediates, malate, transports reducing equivalents of NADH across the mitochondrial membrane. Such a mechanism is required since this membrane is impermeable to NADH itself.

Although gluconeogenesis does not involve oxygen directly, it does require ATP and NADH (reduced nicotinamide adenine dinucleotide). The supply of these two substances depends on the supply of oxygen to the electron transport chain in the mitochondrial membrane (see Fig. 5). NADH, a reducing agent, is produced from the oxidizing agent NAD^+ by the Krebs cycle, and NAD^+ is regenerated by the electron transport chain. Note that ATP can cross the mitochondrial membrane directly, but NADH cannot. Instead the reducing equivalents of NADH are transported to the cytosol indirectly by "shuttle" mechanisms

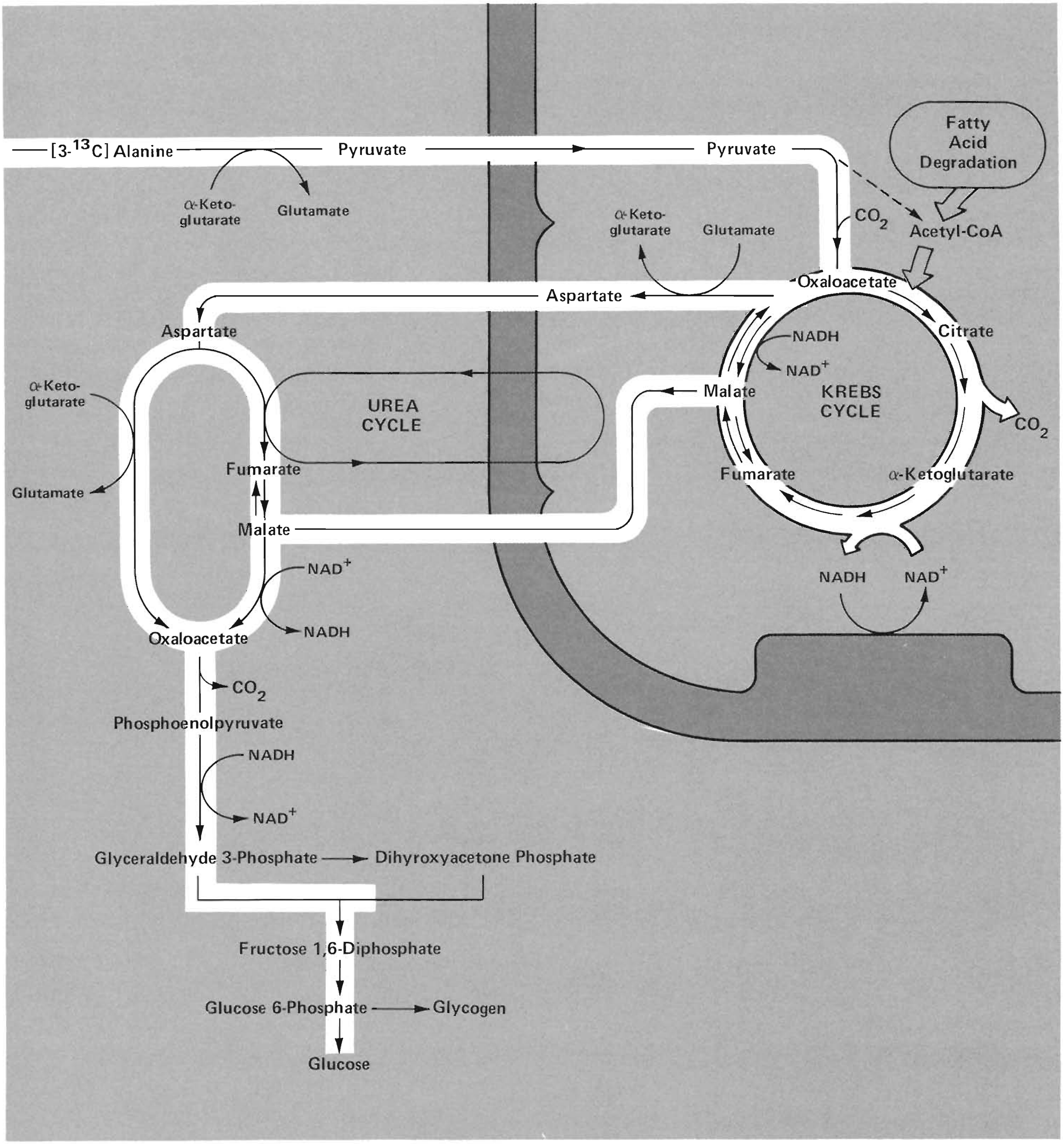


Fig. 6. Gluconeogenesis in detail. The white pathway traces the key reactions in gluconeogenesis from the 3-carbon amino acid alanine. Alanine loses an amino group to form pyruvate, which is converted in the mitochondria to oxaloacetate, the molecule required in the cytosol for production of glucose through phosphoenolpyruvate. But oxaloacetate cannot cross the mitochondrial membrane and instead is converted in the mitochondria to malate and aspartate, which cross the membrane and are reconverted to oxaloacetate. The pathway through malate, which consumes NADH in the mitochondria and regenerates it in the cytosol, in effect transports NADH across the mitochondrial membrane. A comparison of gluconeogenesis and glycolysis (see Fig. 3) is interesting. In glycolysis the high-energy molecule phosphoenolpyruvate is converted to the low-energy molecule pyruvate in one step. The reverse of this reaction in gluconeogenesis, however, requires several steps that involve participation of mitochondrial and cytosolic enzymes and energy in the form of ATP and the related energy-containing molecule GTP.

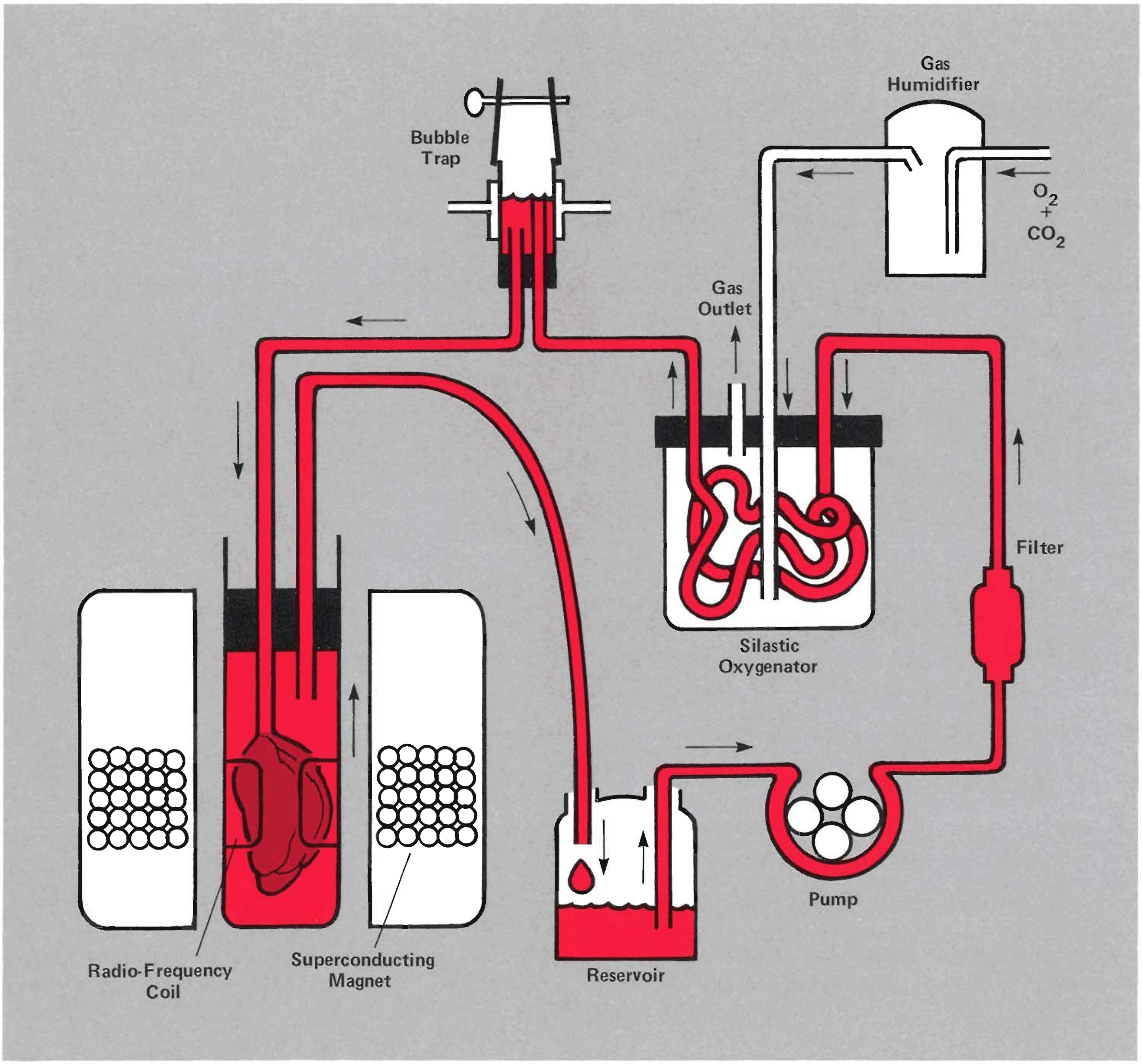


Fig. 7. Apparatus for carbon-13 NMR spectroscopy on perfused Syrian hamster livers. The hamster is anesthetized, and a cannula is sutured into the portal vein of the liver. The liver, still in the hamster, is then supplied with oxygen and nutrients by the flow of perfusate into the vein. The liver is then surgically removed from the animal and placed in a cylindrical sample tube, which is positioned in the bore of a 7.0-tesla superconducting magnet. The perfusate (a buffered saline solution containing 7 percent hemoglobin for increased oxygen-carrying ability) is recycled during the experiment.

such as the malate shuttle shown in Fig. 6, a more detailed picture of gluconeogenesis. Figure 6 also shows that gluconeogenesis from alanine is coupled to the urea cycle (since the cell must dispose of amino groups) and as we shall see, to fatty acid degradation. Variations in the functioning of gluconeogenesis may thus provide a marker for a wide variety of metabolic disorders.

What happens to gluconeogenesis when the oxygen supply is reduced? For drastic decreases we know that the rate of gluconeogenesis is suppressed. But with carbon-13 NMR as a diagnostic tool, we can also detect subtle changes in metabolism caused by subtle changes in oxygen supply or in the oxidation-reduction state of the liver as defined by the NADH/NAD$^+$ ratio.

There is much clinical interest in understanding the regulatory effects of NADH/NAD$^+$ ratios because elevated NADH/NAD$^+$ ratios seem to be associated with fatty and cirrhotic livers. Elevated ratios can occur through the intake of alcohol or as a consequence of inadequate oxygen supply. If carbon-13 NMR studies of gluconeogenesis could enable us to assess the oxidation-reduction state of the liver under normal and stressful conditions, such as those induced by a dose of alcohol, we would be able to help identify those individuals at risk even in "social" drinking.

The Baseline Study of Gluconeogenesis

Before attempting to understand the regulation of gluconeogenesis by NADH/NAD$^+$ ratios and to identify signs of disorder, we needed to monitor and understand in detail how gluconeogenesis proceeds under normal conditions. This in itself is a surprisingly intricate story that illustrates well the power of the carbon-13 NMR technique. We chose to study the liver from a small animal, the Syrian hamster, since it would easily fit into the 3-inch-bore magnet of our NMR spectrometer. Figure 7 is a diagram of the perfusion apparatus used to keep the liver viable and metabolically active after it has been surgically removed. The hamster was starved for 24 hours before the experiment to activate the enzymes involved in gluconeogenesis.

We monitored gluconeogenesis in the perfused liver with NMR spectra taken before and after addition of L-[3-^{13}C]alanine

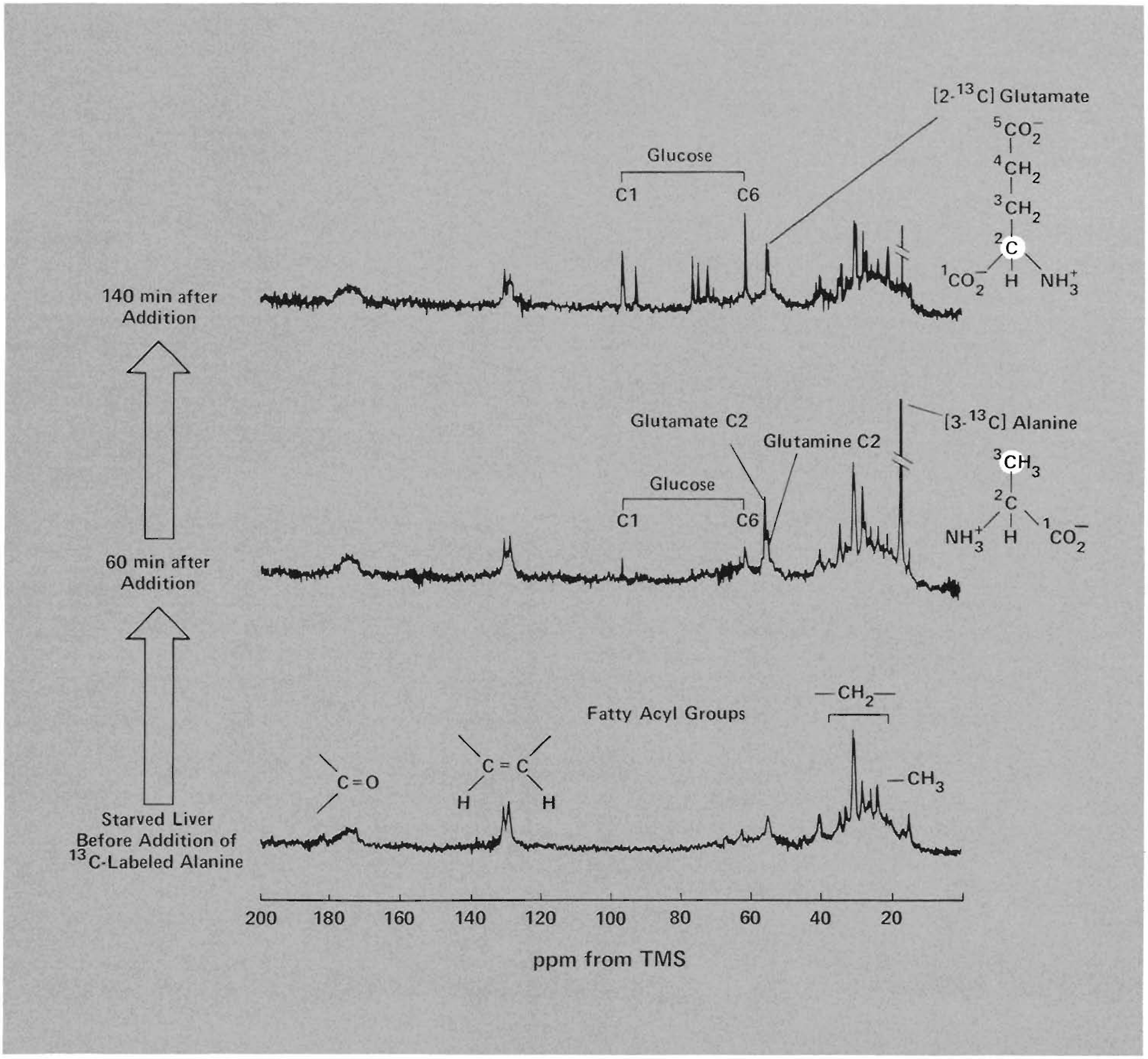


Fig. 8. Carbon-13 NMR spectra from a perfused Syrian hamster liver performing gluconeogenesis from [3-^{13}C]-alanine. The background spectrum, taken before addition of labeled alanine to the perfusate, shows resonances from lipids in the liver containing naturally occurring carbon-13. The spectrum taken 60 minutes after addition of labeled alanine shows resonances from the amino acids glutamate and glutamine and from carbons 1 and 6 of glucose. By 140 minutes all carbon positions in glucose produce detectable resonances.

to the perfusate (Fig. 8). The background spectrum, measured before addition of the labeled alanine, contains resonance peaks from the naturally occurring carbon-13 in lipids in the liver. The spectrum taken 60 minutes after addition of alanine contains a very intense resonance from the labeled carbon atom of alanine as well as less intense resonances from products of alanine metabolism in the liver. The most prominent products we detect are glutamate (which is formed as alanine transfers its amino group to α-ketoglutarate) and glutamine (which is formed subsequently from glutamate). These amino acids provide a temporary sink for the amino group brought in by alanine. Glucose is also being produced but only in small quantities. After 140 minutes of perfusion, gluconeogenesis is in full swing and resonances from all carbon positions in glucose are detectable.

Since the background spectrum of the liver might obscure resonances from metabolites that have acquired carbon-13 labels during the experiment, we subtract the background spectrum and examine the resulting "difference" spectra (Fig. 9). In these spectra we can identify resonances from carbons 2 and 3 of glutamate and glutamine, but a resonance from carbon 4 of glutamate is conspicuously absent.

The specific positions of the carbon-13 label in glutamate are extremely informative

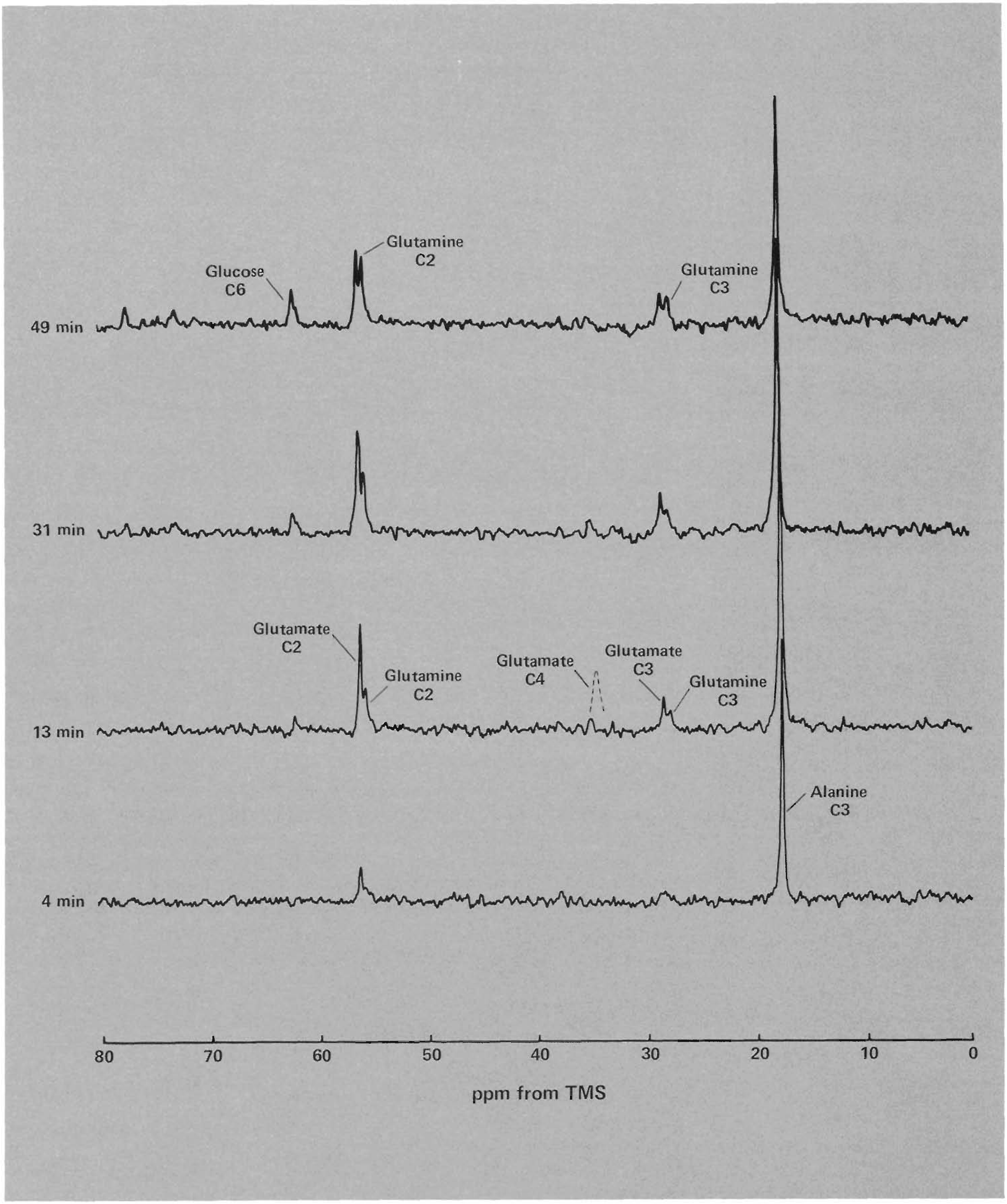


Fig. 9. Carbon-13 difference spectra of perfused Syrian hamster liver during the first hour of gluconeogenesis from 8-millimolar [3-^{13}C]alanine. These spectra are obtained by subtracting the background spectrum from each measured spectrum. The dashed peak occupies the position of the nonexistent resonance from carbon 4 of glutamate. The presence of resonances from carbons 2 and 3 of glutamate and the absence of a resonance from carbon 4 show that label from alanine enters the Krebs cycle through oxaloacetate rather than acetyl-CoA (see Fig. 10).

because they enable us to pinpoint the pathway by which glutamate was formed. To do this we use detailed information about the possible pathways to glutamate and the stereochemistry of the reactions involved (Fig. 10). This information has been accumulated from investigations with radiolabeled metabolites.

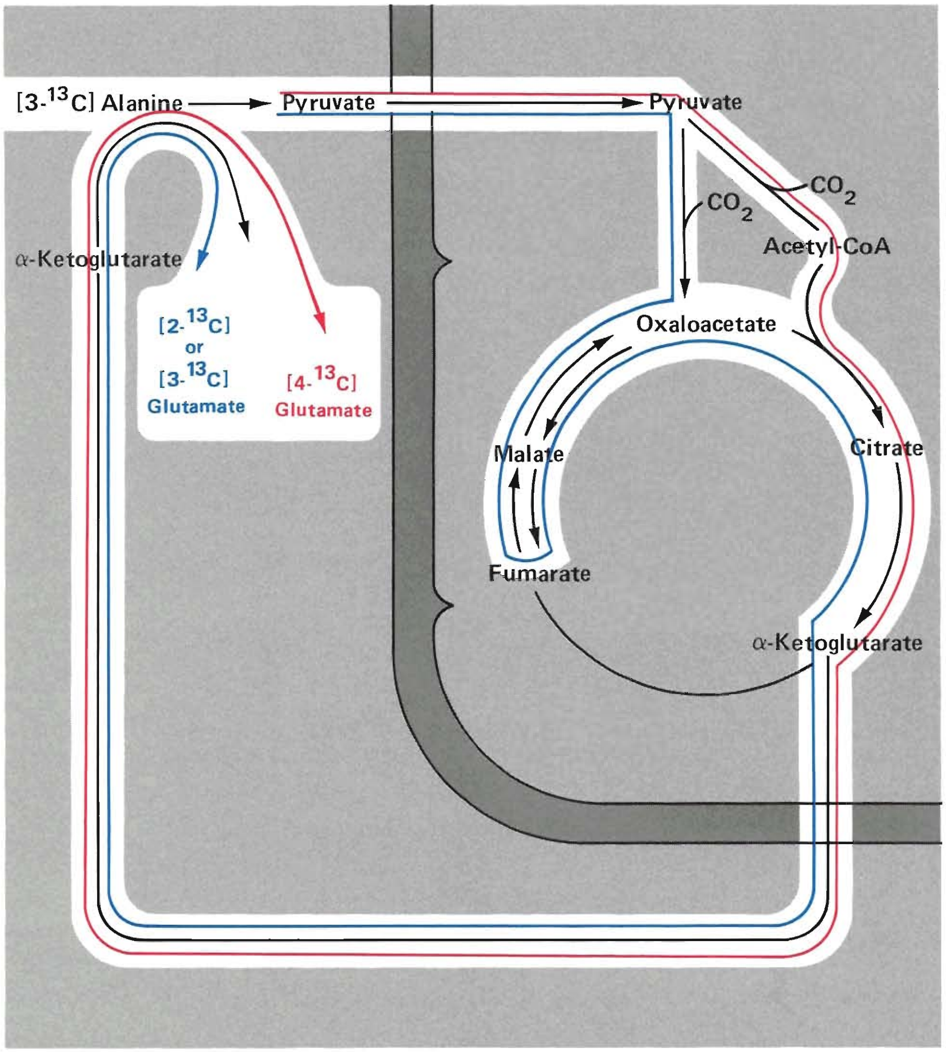


Fig. 10. Possible pathways for synthesis of glutamate during gluconeogenesis from [3-^{13}C]alanine and the stereochemistry of the reactions involved. Label from alanine may enter the pool of Krebs cycle intermediates through a pathway involving conversion of pyruvate to acetyl-CoA, which yields [4-^{13}C]glutamate, or through a pathway involving conversion of pyruvate to oxaloacetate, which yields a mixture of [2-^{13}C]glutamate and [3-^{13}C]glutamate. This "scrambling" of the label between carbons 2 and 3 (indicated in the figure by blue spotlights on each of the two possible carbon positions) is due to the reaction converting malate to the symmetric Krebs cycle intermediate fumarate. Since carbon-13 NMR spectra taken during gluconeogenesis by the liver from [3-^{13}C]alanine (Fig. 9) do not reveal a resonance from carbon 4 of glutamate, we conclude that essentially all of the label from alanine enters the pool of Krebs cycle intermediates as oxaloacetate rather than acetyl-CoA.

Figure 10 shows that glutamate acquires its carbon-13 label from α-ketoglutarate made in the Krebs cycle. The figure also shows that α-ketoglutarate can acquire its label in two ways depending upon how it is formed from the labeled pyruvate derived from [3-^{13}C]alanine:

$$\text{pyruvate} \rightarrow \text{oxaloacetate} \xrightarrow{\text{Krebs cycle}} \alpha\text{-ketoglutarate}$$

or

$$\text{pyruvate} \rightarrow \text{acetyl-CoA} \xrightarrow{\text{Krebs cycle}} \alpha\text{-ketoglutarate}\ .$$

The pyruvate that enters the Krebs cycle as oxaloacetate will yield a mixture of [2-^{13}C]glutamate and [3-^{13}C]glutamate because of the equilibria among oxaloacetate, malate, and the symmetric Krebs cycle intermediate fumarate, which "scrambles" the label between carbons 2 and 3. On the other hand, the pyruvate entering the Krebs cycle as acetyl-CoA will yield only [4-^{13}C]glutamate. Since the spectra of Fig. 9 exhibited glutamate resonances only from carbons 2 and 3, we conclude that essentially all the alanine enters the Krebs cycle as oxaloacetate and that acetyl-CoA (which is required for Krebs-cycle activity) is derived, not from alanine, but from oxidation of lipids stored in the liver.

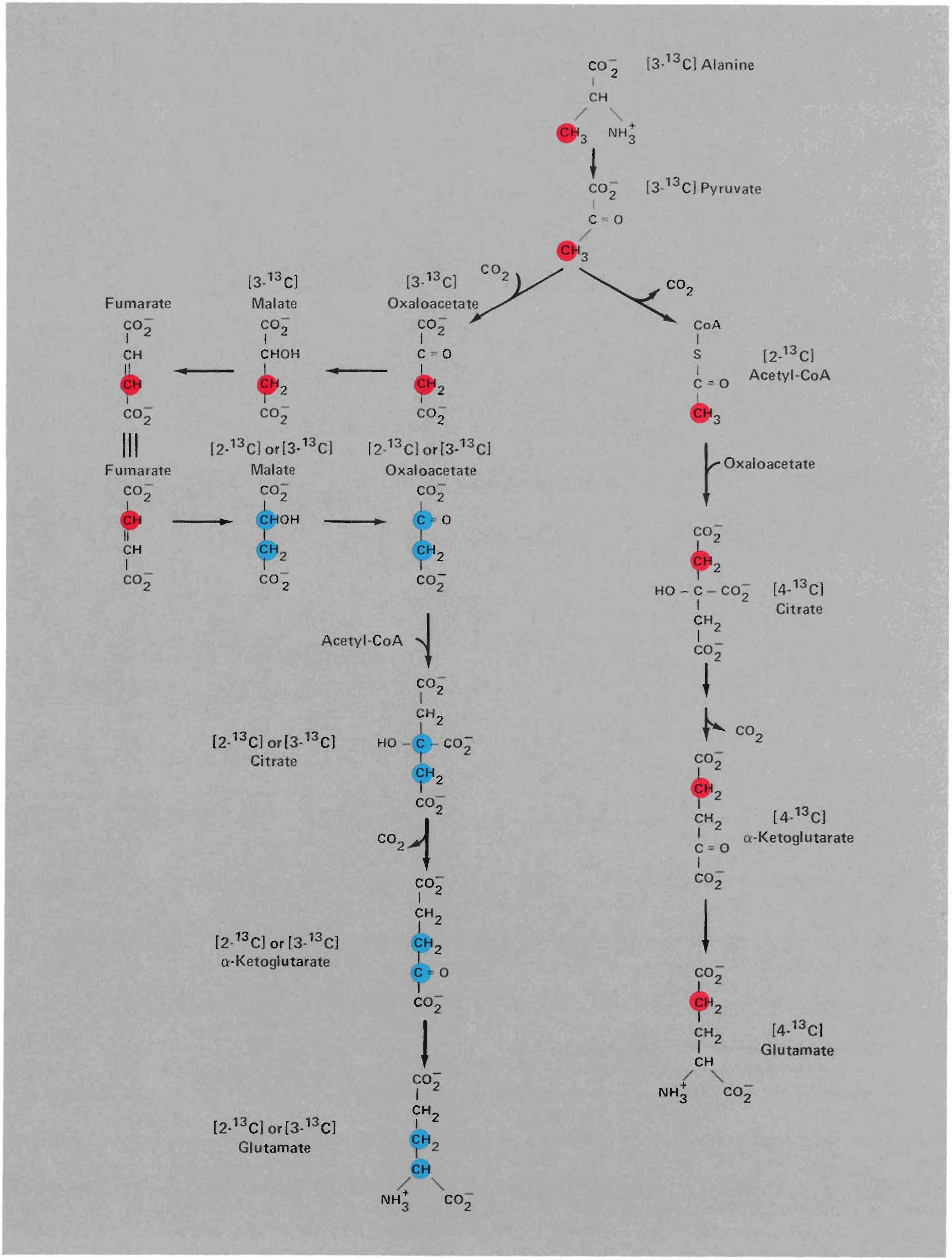
Before leaving the discussion of glutamate, we note another point of interest in the spectra of Fig. 9: after 60 minutes the glutamate resonances decrease rapidly in intensity but the glutamine resonances continue to increase. These two trends are not unrelated. Excess production of glutamate is disadvantagous to liver metabolism because it removes a key intermediate, α-ketoglutarate, from the Krebs cycle and thus reduces the ATP-generating capability of the mitochondria. Therefore the liver regenerates α-ketoglutarate through the reaction

$$\text{glutamate} \rightarrow \alpha\text{-ketoglutarate} + NH_4^+\ .$$

This use of glutamate explains the observed decrease in intensity of the glutamate resonances. Since the ammonium ion (NH_4^+) produced in this reaction is very toxic to the liver, its concentration is in turn controlled by the reaction

$$\text{glutamate} + NH_4^+ \rightarrow \text{glutamine}\ ,$$

which explains the increasing intensity of the glutamine resonances. The formation of glutamine provides a sink for NH_4^+ during gluconeogenesis from alanine. The formation of urea, another nitrogen-containing metabolite, also contributes substantially to maintaining proper NH_4^+ concentrations and will be discussed later.


[3-13C] Alanine
[3-13C] Pyruvate
CO2
CO2
[3-13C] Oxaloacetate
[3-13C] Malate
Fumarate
[2-13C] Acetyl-CoA
Fumarate
[2-13C] or [3-13C] Malate
[2-13C] or [3-13C] Oxaloacetate
Oxaloacetate
[4-13C] Citrate
Acetyl-CoA
[2-13C] or [3-13C] Citrate
CO2
CO2
[4-13C] α-Ketoglutarate
[2-13C] or [3-13C] α-Ketoglutarate
[4-13C] Glutamate
[2-13C] or [3-13C] Glutamate

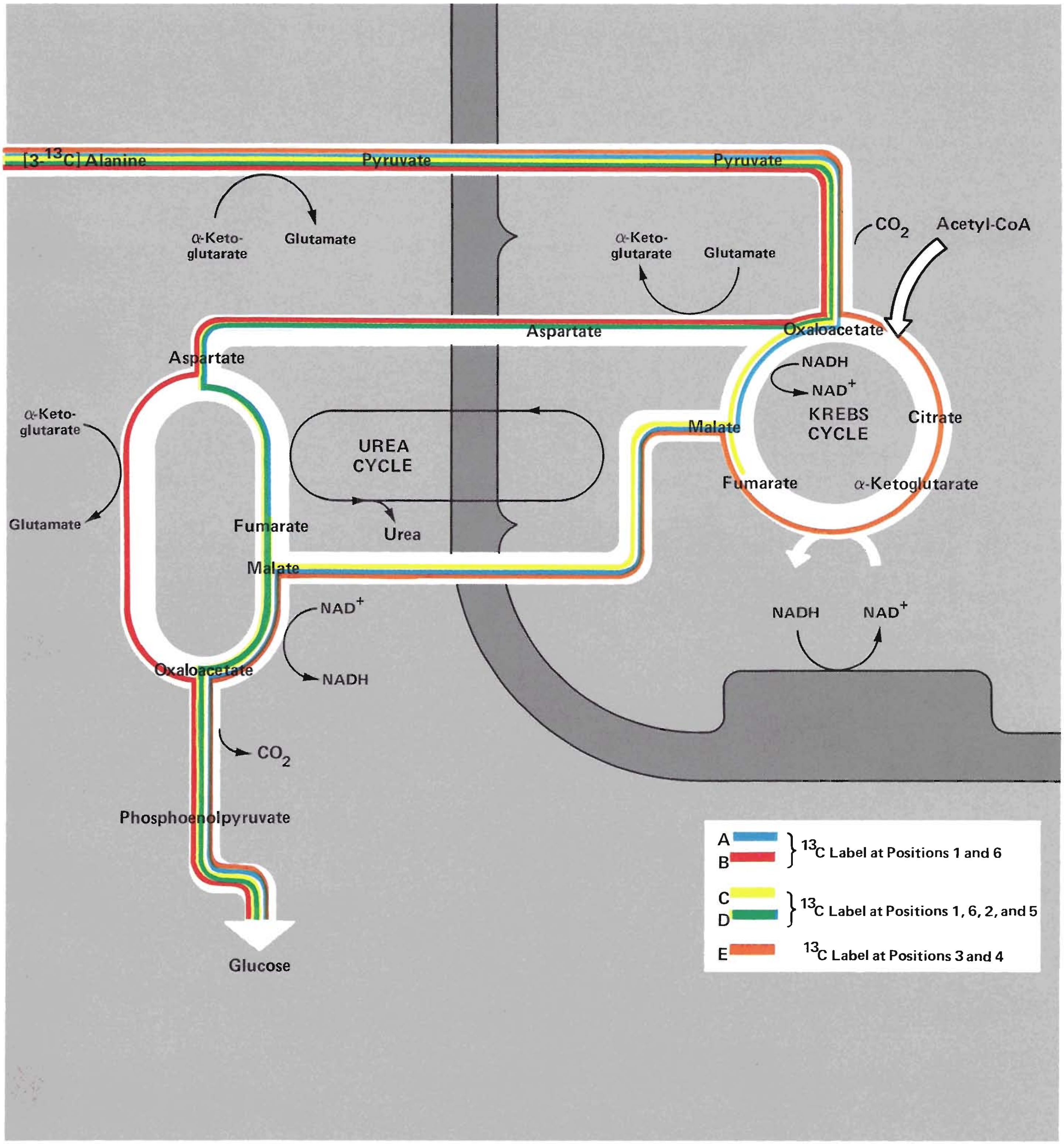


Fig. 11. Pathways for synthesis of glucose during gluconeogenesis from [3-^{13}C]alanine. Pathway A (reduction of oxaloacetate to malate in the mitochondria and the reverse of this reaction in the cytosol) and pathway B (conversion of oxaloacetate to aspartate in the mitochondria and the reverse of this reaction in the cytosol) yield glucose labeled at positions 1 and 6. Pathway C is identical to pathway A except for involvement of fumarate, which scrambles the label. Pathway D involves conversion of oxaloacetate to aspartate in the mitochondria (as in pathway B), but then, instead of being directly converted back to oxaloacetate, aspartate participates in the urea cycle. Consequently, the label is scrambled through fumarate. Pathways C and D both yield glucose labeled at positions 1, 2, 5, and 6. Pathway E, in which oxaloacetate is converted to citrate and continues around the Krebs cycle, yields glucose labeled at positions 3 and 4. The fractions of glucose produced by the combination of pathways A and B, the combination of pathways C and D, and pathway E can be determined from the relative intensities of the resonances assigned to the various carbon positions in glucose.

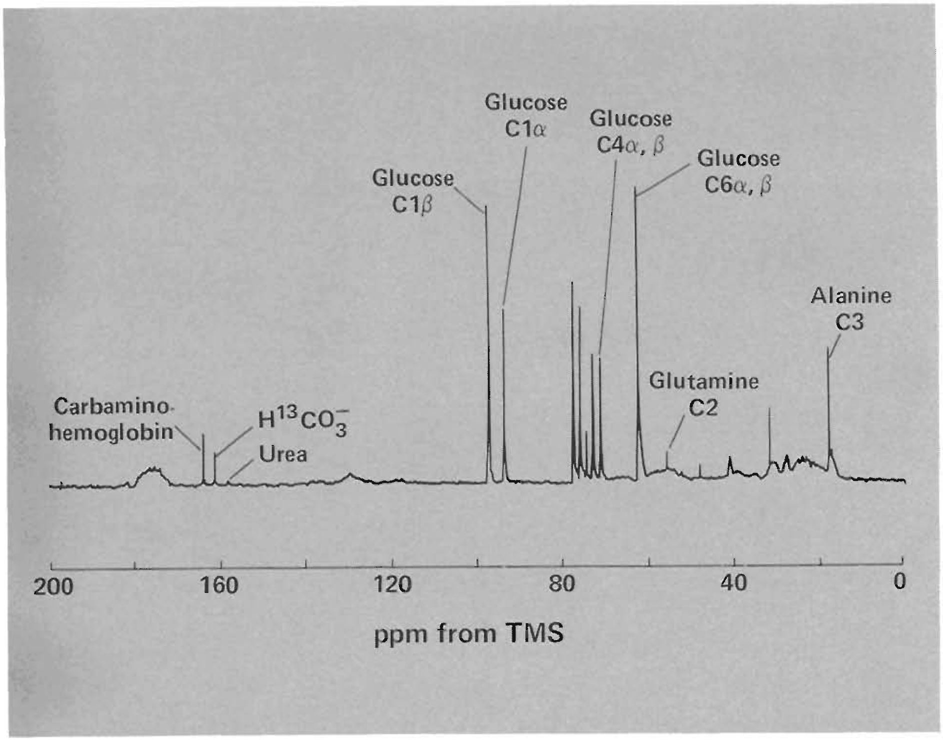


Fig. 12. Proton-decoupled carbon-13 NMR spectrum of the medium used to perfuse the liver during gluconeogenesis from $[3\text{-}^{13}C]$alanine. This perfusate spectrum exhibits better resolution of the glucose resonances than does the liver spectrum (Fig. 9) and is therefore used to determine the relative fluxes of carbon-13 through the pathways of gluconeogenesis. This spectrum also shows resonances from labeled glutamine, bicarbonate, carbaminohemoglobin, and urea. The last three substances are formed from carbon dioxide, some of which acquires carbon-13 through oxidation of the ^{13}C-labeled alanine and some through oxidation of fatty acids containing naturally occurring carbon-13.

TABLE I

RELATIVE FLUX OF CARBON-13 THROUGH PATHWAYS OF GLUCONEOGENESIS

	Pathway		
	A+B	C+D	E
Relative Flux (%)	12-15	62-67	20-21

Let us now return to the synthesis of glucose. The liver spectrum taken after 140 minutes of perfusion (see Fig. 8) shows detectable resonances from all the carbon positions in glucose. This labeling pattern indicates that, in contrast to glutamate, more than one metabolic pathway contributes to the formation of glucose. Figure 11 shows five possible pathways. (Other pathways exist but yield very little glucose.) Note that two pairs of these pathways (A and B, C and D) are degenerate in the sense that they yield identically labeled forms of glucose.

What fraction did each pathway contribute to the total amount of synthesized glucose? Or equivalently, what relative amount of carbon-13 passed through each pathway? We can obtain this information from the relative intensities of the glucose resonances, although for a pair of degenerate pathways (such as A and B in Fig. 11), we obtain only a combined relative flux.

The relative flux of label through the pathways could be determined from the liver spectrum itself (Fig. 8), but the accuracy would be compromised by the poor resolution of the closely spaced resonances. Since some glucose diffuses out of the liver and into the perfusate, we used instead a spectrum from the isolated perfusate (Fig. 12), which exhibits better resolution because the perfusate is considerably more homogeneous. This spectrum also shows the presence of other labeled metabolites, such as bicarbonate ion (HCO_3^-) and urea, that readily diffuse out of the liver.

Table I lists the values calculated from the perfusate spectrum for the relative fluxes of label through the pathways of Fig. 11 when the liver is well oxygenated and $[3\text{-}^{13}C]$alanine is the sole substrate. The combination of pathway A (the conversion of oxaloacetate through malate to glucose) and pathway B (the conversion of oxaloacetate to aspartate in the mitrochondria and the reversal of this reaction in the cytosol) yields about 13 percent of the observed glucose. The combination of pathway C (fumarate scrambling of the malate pathway) and pathway D (through aspartate and its participation in the urea cycle) yields about 64 percent. About 21 percent of the glucose is derived from conversion of oxaloacetate through citrate and malate to glucose (pathway E).

Note that the flux through pathway E draws on the total mitochondrial pool of oxaloacetate and is therefore a measure of Krebs cycle activity. However, we measure the *relative* flux through pathway E, that is, the flux through pathway E divided by the total flux through all the gluconeogenesis pathways. Thus we can determine whether Krebs cycle activity is increasing or decreasing relative to gluconeogenesis by monitoring changes in relative flux through pathway E.

Regulation of Gluconeogenesis by NADH/NAD$^+$ Ratios

It is relatively easy to probe the regulatory mechanisms of gluconeogenesis by adding other substrates to the perfusate and determining their effects on the relative fluxes through the pathways and on the accumulation of various intermediates. We have focused initially on substrates designed to alter the ratio of the reducing agent NADH to the oxidizing agent NAD$^+$. As mentioned earlier, these coenzymes participate in many of the key reactions of metabolism and the NADH/NAD$^+$ ratio is believed to have an important regulatory effect on metabolism. This ratio changes with variations in oxygen supply and substrate availability.

What happens to the relative fluxes through the pathways when NADH levels are raised in the mitochondria alone, in the cytosol alone, or in both? How, under these various conditions, does the liver dispose of the amino groups lost by alanine? What happens to Krebs cycle activity? Might any of the observed effects be used as clinical markers of disorder? To determine the effects of altered NADH/NAD$^+$ ratios, we monitored gluconeogenesis from labeled alanine in perfused livers before and after adding, separately, the following substrates to the perfusate:

- ○ β-hydroxybutyrate, which produces NADH only in the mitochondria through action of the mitochondrial enzyme β-hydroxybutyrate dehydrogenase;
- ○ ethanol, which produces NADH first in the cytosol through the reaction ethanol + NAD$^+$ $\xrightarrow{\text{cytosolic enzyme}}$ acetaldehyde + NADH and then in the mitochondria through the reaction acetaldehyde + NAD$^+$ $\xrightarrow{\text{mitochondrial enzyme}}$ acetate + NADH;

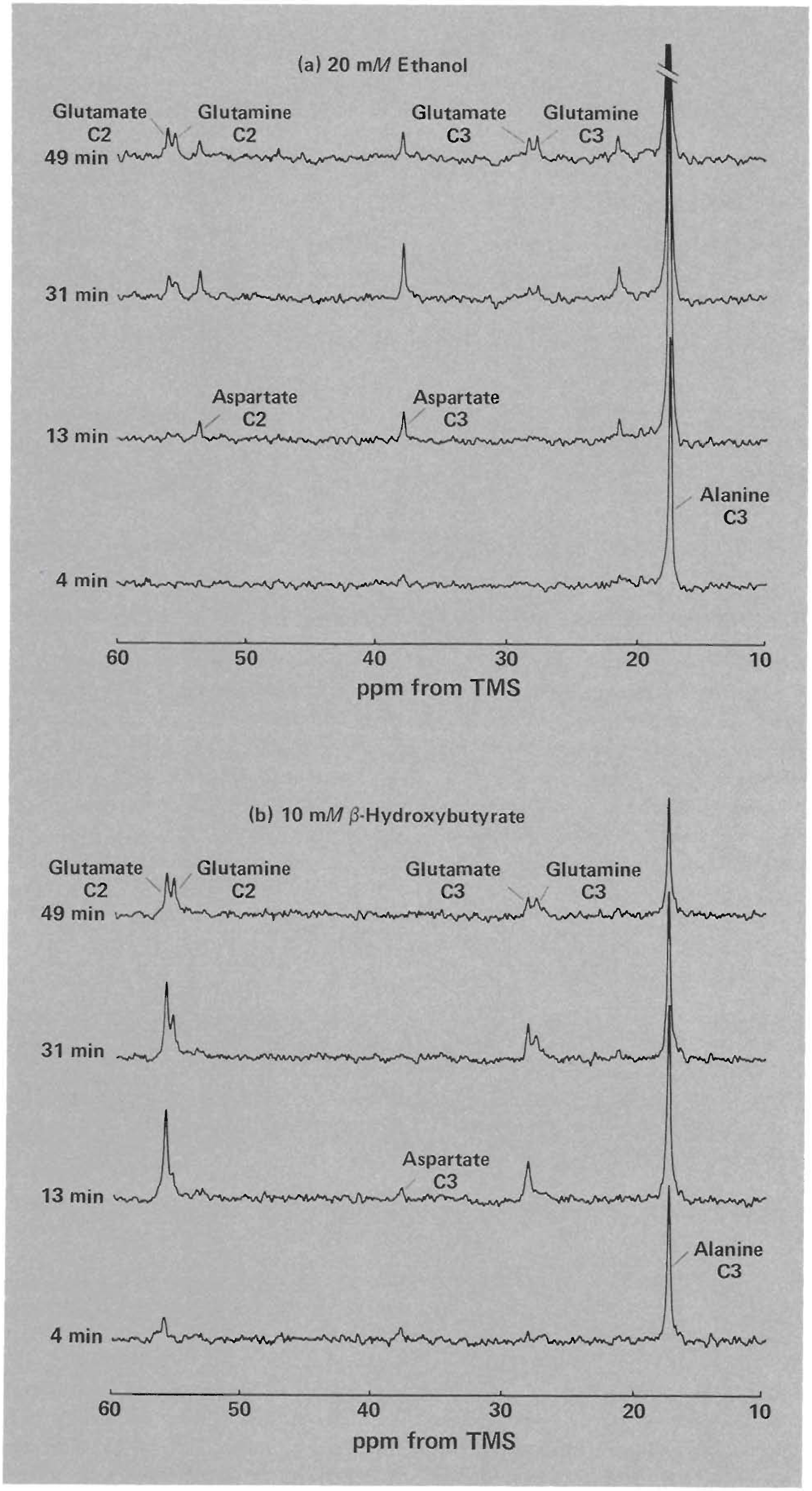


Fig. 13. Carbon-13 difference spectra of perfused liver during gluconeogenesis from 8-millimolar [3-^{13}C]alanine in the presence of (a) 20-millimolar ethanol and (b) 10-millimolar β-hydroxybutyrate. Both these substances cause transient accumulation of aspartate, but the effect is much more pronounced in the case of ethanol.

TABLE II

EFFECTS OF ETHANOL, β-HYDROXYBUTYRATE, AND REDUCED OXYGEN ON GLUCONEOGENESIS IN PERFUSED LIVER

	Relative Carbon-13 Flux through Pathways (%)			Amino Acid Accumulated during First Hour
	A+B	C+D	E	
Ethanol				
Control	12	67	20	Glutamate
Ethanol	5	83	11	Asparatate, then glutamate
Ethanol plus disulfiram	7	79	14	Glutamate
β-Hydroxybutyrate				
Control[a]	20	60	20	Glutamate
β-Hydroxybutyrate	11	62	25	Very little asparate, then glutamate
Reduced Oxygen				
Control	15	62	21	Glutamate
One-ninth oxygen	21	66	12	Glutamate

[a] Calculation of the relative fluxes in this experiment was complicated by the appearance of some label at carbon 4 of glutamate.

○ ethanol plus the drug disulfiram, which specifically inhibits the mitochondrial reaction above and therefore produces NADH only in the cytosol.

In addition, we also monitored gluconeogenesis from labeled alanine in a liver perfused with a medium containing one-ninth the usual supply of oxygen.

Table II summarizes the effects of these changed conditions on the relative fluxes of carbon-13 from alanine through the pathways of gluconeogenesis. The relative fluxes given in the table represent averages during each three-hour experiment. The table also indicates which amino acid intermediates accumulated during the first hour of each experiment.

CHANGES IN RELATIVE FLUXES. We do not have a definitive interpretation of all the data. In particular, the data for reduced oxygen supply is puzzling. As expected, alanine utilization and glucose production are reduced markedly. However, the increase in flux through pathways A and B relative to pathways C and D has no simple explanation. The decrease in the relative flux through pathway E is even more surprising since this indicates that Krebs cycle activity is decreased more than gluconeogenesis. Since the Krebs cycle must produce the ATP needed for gluconeogenesis, where does the ATP come from? A plausible hypothesis is that when the oxygen supply is reduced, other processes requiring ATP slow down much more than gluconeogenesis so that ATP is available despite the reduced activity of the Krebs cycle. But this hypothesis needs further investigation.

Addition of ethanol to the perfusate also results in a lower relative flux through pathway E. Again this indicates a decrease in Krebs cycle activity relative to gluconeogenesis, but in this case the decrease is to be expected. Ethanol oxidation generates the NADH required for gluconeogenesis and for ATP production and thus reduces the requirements for NADH production by the Krebs cycle. These data are consistent with our understanding that the primary function of the Krebs cycle during gluconeogenesis is to supply NADH rather than to provide a pathway for conversion of the alanine skeleton into glucose. Futher, these data demonstrate that Krebs cycle activity during gluconeogenesis is regulated by the cell's requirements for ATP and NADH.

The other changes in the relative fluxes of label give additional information about the interrelationships between gluconeogenesis and the urea cycle and the role of various metabolite shuttles across the mitochrondrial membrane. However, a full discussion of these related topics is well beyond the intended scope of this article.

ACCUMULATION OF ASPARTATE. An interesting phenomenon is the transient accumulation of large amounts of aspartate in the presence of ethanol (Fig. 13a). Only small amounts accumulated in the presence of β-hydroxybutyrate (Fig. 13b) and none was seen in the other experiments. We were particularly interested in understanding this result because accumulation of an intermediate like aspartate might turn out to be a significant clue that the liver is under stress.

As shown in Fig. 6, the amino acid aspartate is formed from the Krebs cycle intermediate oxaloacetate. Like malate, it provides a 4-carbon skeleton for gluconeogenesis. Aspartate accumulates during the first hour of perfusion, acting as a sink for alanine's amino group, and then gradually disappears. While aspartate is accumulating, almost all of the label from alanine is incorporated into aspartate, and very little synthesis of glucose is detected. Eventually, glutamine and urea serve as sinks for the amino group, the aspartate (and later, the glutamate) resonances decrease, and the label from alanine is incorporated into glucose.

Aspartate accumulates when NADH/

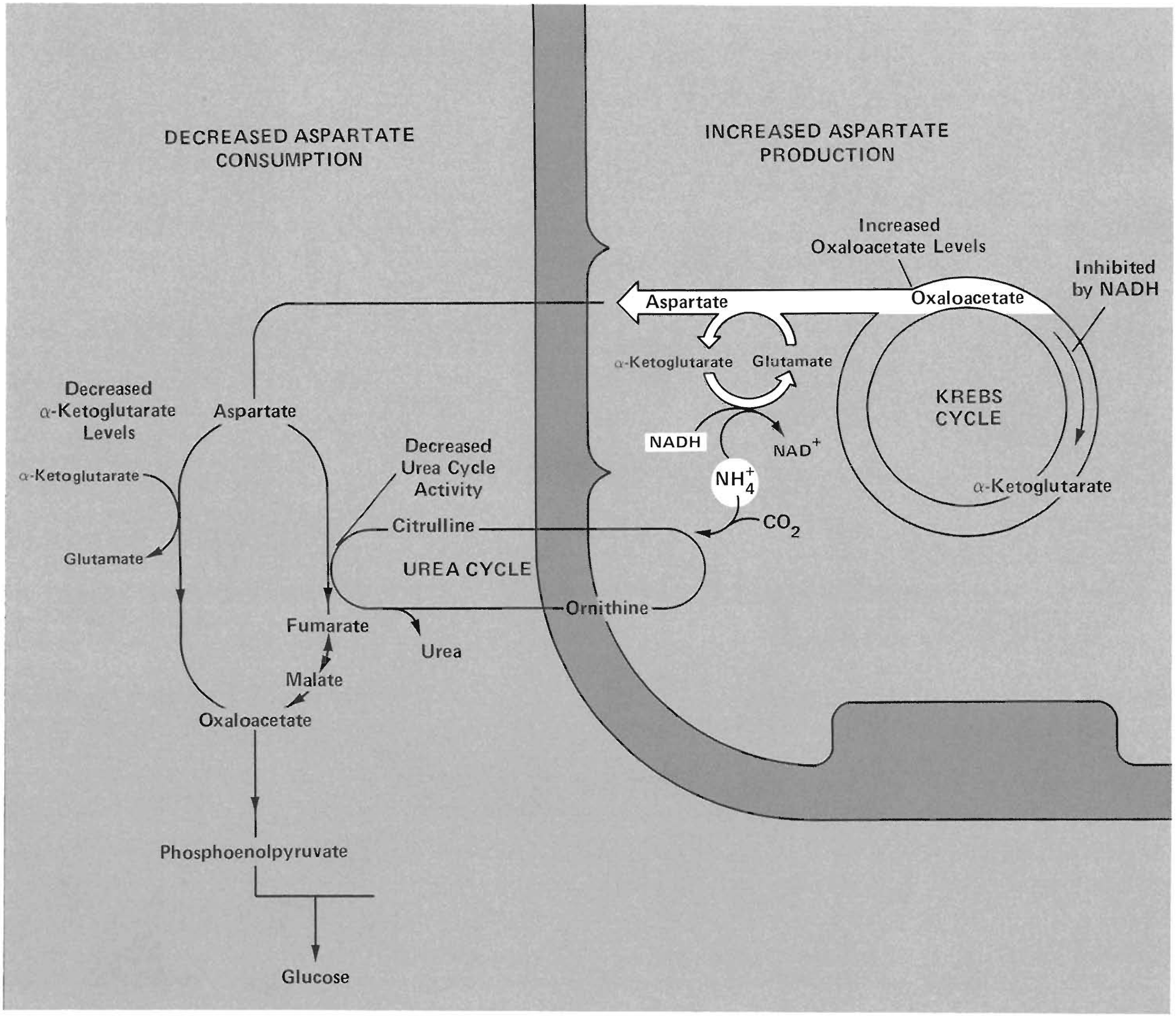


Fig. 14. Proposed mechanism for aspartate accumulation during gluconeogenesis from [*3*-^{13}*C*]*alanine in the presence of ethanol.*

NAD^+ ratios are increased by ethanol in both the cytosol and the mitochondria. Why? We propose one possible mechanism, which is illustrated in Fig. 14. The accumulation of aspartate suggests that its rate of production in the mitochondria is increased relative to its rate of consumption in the cytosol. We suggest that the increased concentration of NADH in the mitochondria resulting from oxidation of ethanol is responsible not only for increased production of aspartate in the mitochondria but also for its decreased consumption in the cytosol.

Increased $NADH/NAD^+$ ratios affect the production of aspartate by altering the concentration of α-ketoglutarate relative to that of oxaloacetate. (These two α-keto acids serve as acceptors for alanine's amino group through a reaction known as transamination. Transamination of α-ketoglutarate, a 5-carbon α-keto acid, yields glutamate, whereas transamination of oxaloacetate, a 4-carbon α-keto acid, yields aspartate.) A high $NADH/NAD^+$ ratio drives the reaction

$$\text{NADH} + \text{NH}_4^+ + \alpha\text{-ketoglutarate} \longrightarrow \text{glutamate} + \text{NAD}^+ \tag{1}$$

far to the right and thus lowers the concentration of α-ketoglutarate. In addition a high $NADH/NAD^+$ ratio inhibits several of the enzymes responsible for conversion of oxaloacetate to α-ketoglutarate in the Krebs cycle. These two effects increase the concentration of oxaloacetate relative to that of α-ketoglutarate, and consequently oxaloacetate, rather than α-ketoglutarate, serves as the acceptor for alanine's amino group.

After the aspartate has crossed the mitochondrial membrane to the cytosol, it is consumed by transamination to oxaloacetate or by reactions involving the urea cycle. The first of these pathways is the reaction

$$\text{aspartate} + \alpha\text{-ketoglutarate} \longrightarrow \text{oxaloacetate} + \text{glutamate}\ . \tag{2}$$

But the concentration of α-ketoglutarate in the cytosol is not sufficient to promote reaction 2 because reaction 1 is occurring in the mitochondria. (Reaction 1 decreases the concentration of α-ketoglutarate in the mitochondria, and since α-ketoglutarate can cross the mitochondrial membrane, its concentration in the cytosol is also decreased.) Thus increased NADH/NAD$^+$ ratios in the mitochondria inhibit the first pathway for aspartate consumption. We also suspect that the other pathway, which involves the urea cycle, may be inhibited by increased NADH/NAD$^+$ ratios in the cytosol.

We emphasize that in arriving at this mechanism for aspartate accumulation in the presence of ethanol, we considered only a few of many interrelated processes and in particular neglected charge balance across the mitochondrial membrane. Charge balance is maintained by countertransport of an anion when aspartate or malate is shuttled across the membrane.

Is the proposed mechanism for aspartate accumulation consistent with our results for β-hydroxybutyrate? This substrate, which increases the NADH/NAD$^+$ ratio only in the mitochondria, caused only a small accumulation of aspartate. We suggest that high NADH/NAD$^+$ ratios in the mitochondria relative to the cytosol tend to activate the transport of NADH from the mitochondria to the cytosol via the malate shuttle. This transport lowers the NADH/NAD$^+$ ratio in the mitochondria, and little aspartate accumulates. In this case the ammonium ion concentration is controlled by glutamate and glutamine production as it is for gluconeogenesis from alanine alone.

The data we have obtained are an insufficient basis for firm conclusions about the regulation of metabolism. They do, however, suggest a number of interesting hypotheses and questions. For example, the transient incorporation of label from alanine into the amino acids glutamate, aspartate, and (somewhat later) glutamine rather than into glucose indicates that the perfused liver, when first presented with alanine as a substrate for gluconeogenesis, uses not the urea cycle but the Krebs cycle intermediates α-ketoglutarate and (in the presence of ethanol) oxaloacetate as acceptors for the amino group derived from alanine. The continued depletion of Krebs cycle intermediates is bothersome in light of continued energy production by the Krebs cycle. Oxaloacetate in particular occupies a central role in the Krebs cycle, and accumulation of aspartate in the presence of ethanol may markedly alter already low (less than 0.2 millimolar) oxaloacetate concentrations.

Conventional bioanalytical techniques have revealed accumulation of glutamate, glutamine, and, in the presence of ethanol, aspartate in liver cells during gluconeogenesis from alanine. These conventional measurements were time-averaged, sometimes over periods as long as 90 to 100 minutes, and they suggested that the amino acid accumulation is a steady-state phenomenon. Our carbon-13 NMR measurements on perfused hamster livers, and those of other workers on rat liver cells and perfused mouse livers, permit almost real-time observation of changing metabolite concentrations and show that amino acid accumulation is transient. After an initial period the liver adapts to a new steady state in which its supply of 4-carbon skeletons required in the Krebs cycle is brought under control and amino groups are disposed of in the urea cycle.

How does the liver accomplish this? Does it depend on decreasing the NADH/NAD$^+$ ratio to a critical level? Or on adjusting the levels and activities of the appropriate enzymes to stimulate the urea cycle and modulate gluconeogenesis and the Krebs cycle? We don't know. Will these adjustments be impaired in the case of liver disease? Again, we don't know but strongly suspect that they will. To answer these and other questions about metabolism in the liver, baseline studies must be extended to other substrates for gluconeogenesis, especially pyruvate and lactate, in the presence or absence of other substrates that affect regulation, including ammonium ion, aspartate, glutamate, glutamine, fatty acids, and ethanol.

The role of oxygen uptake by the liver will also have to be studied carefully under a variety of conditions. In the experiments we have performed thus far, we drastically reduced the supply of oxygen to the liver (one-ninth the normal amount) and, as expected, caused drastic reductions in the rates of gluconeogenesis and glutamate and aspartate production. To completely unravel the effect of oxygen, we must monitor metabolism as the oxygen supply is gradually reduced.

Metabolism in the liver is especially complex because of the many interdependent metabolic pathways. (Two important ones we have partially neglected here are lipid synthesis and degradation.) We have work under way, in collaboration with David E. Hoekenga of the University of New Mexico School of Medicine and the Albuquerque Veterans Administration Medical Center, on another vital but metabolically much simpler organ—the heart. In this organ oxygen supply to the tissue is of overriding importance because almost all (more than 90 percent) of its energy requirements are met through production of ATP by the electron transport chain, and the operation of this chain, in turn, is crucially dependent upon oxygen supply. Our work on the heart is currently focused on substrates thought to be involved in heart metabolism under anoxic conditions, such as glucose and amino acids. In addition, we plan to study the role of carnitine in heart metabolism. This molecule is a central control point in heart metabolism since it must link to fatty acids before they can enter the mitochondria and be oxidized. Carnitine has also been used to protect the heart against ischemic injury, but the mechanism through which this protection is conferred is uncertain. Clifford J. Unkefer of our group has recently devised a method for synthesizing ^{13}C-labeled carnitine, and we expect to learn a great deal about its functions during anoxia and ischemia from carbon-13 NMR studies on perfused hearts.

Future Prospects

Baseline carbon-13 NMR studies of the type described here are being carried out in several laboratories. Raymond L. Nunnally of the University of Texas Health Science Center in Dallas is studying metabolism in perfused hearts and in the hearts of live rabbits and dogs with the intent of using NMR to define the extent and location of myocardial infarctions, to assess the temporal evolution of irreversible tissue damage, and to monitor the course of drug therapy. Similarly, Robert G. Shulman of Yale University, Sheila M. Cohen of the Merck Institute of Therapeutic Research, and John R. Williamson of the University of Pennsylvania are carrying out baseline metabolic studies on perfused livers and hearts and on livers, hearts, and brains of live animals. In another Los Alamos study Laurel O. Sillerud is studying metabolism in adipose tissue as a prelude to studying insulin resistance in diabetes and fat-trapping lesions in chronic obesity. Concurrently, a great deal of progress has been made in applying phosphorus-31 NMR to studies of ATP production and utilization. (Since phosphorus-31 is a naturally abundant isotope, these studies do not require labeling techniques.) Phosphorus-31 NMR is in fact already being used in clinical applications.

The ultimate objective of our research, and that of others in the field, is the study, diagnosis, and treatment of disease. Realizing this objective requires the pursuit of a number of projects, including the following:

- ○ extend the baseline NMR studies on perfused organs and animals;
- ○ develop efficient, large-scale processes for synthesis of selectively labeled compounds;
- ○ design and construct magnets and radio-frequency coils with improved sensitivity and volume resolution;
- ○ assess more completely the biological effects of magnetic fields;
- ○ develop close cooperation among NMR spectroscopists, physiologists, biochemists, and physicians; and
- ○ extend the studies to human controls and to patients with clinically well-defined diseases.

As this article indicates, the first two projects are well under way, and we can expect their acceleration soon as more investigators enter the field. With regard to the third, we point out that the most severe limitation of NMR spectroscopy in general, and carbon-13 NMR spectroscopy especially, is its relatively low sensitivity. The magnet and radio-frequency technology available at present limit real-time kinetic studies of metabolism in NMR to small, soluble metabolites (like glucose and alanine) present at concentrations exceeding 0.5 millimolar (500 micromolar). By way of comparison, recently developed electrochemical methods based on microelectrodes inserted into living cells can detect certain metabolites at concentrations as low as about 0.1 micromolar. The sensitivity of NMR experiments can be increased to some degree by sacrificing time resolution, that is, by averaging the signal over longer periods. However, for many metabolic studies the loss in time resolution is unacceptable. Advances in the design of more sensitive radio-frequency coils offer the most promise for increasing the sensitivity of *in vivo* NMR measurements, perhaps by a factor of ten. To this end Eiichi Fukushima of Los Alamos and Stephen B. W. Roeder of San Diego State University have designed and constructed novel radio-frequency coils. They have also begun to design magnets that provide the high, homogeneous magnetic fields required for human studies but do not confine the subject as do conventional magnets. We would welcome industry's involvement in this project, since the supply of NMR equipment—especially appropriate, reliable superconducting magnets—will probably be the major bottleneck in the development of a national program for NMR in medicine.

A key issue in the use of NMR in human studies is whether static and oscillating radio-frequency magnetic fields are safe. Many years of experience with particle accelerators and with NMR instruments have produced no firm evidence of any biological hazard from static magnetic fields. However, anecdotal evidence is not very satisfactory. It could, for example, unfairly implicate static magnetic fields as an amplifying factor in the highly individualistic behavior of high-energy physicists and NMR spectroscopists when, in fact, it might be a neutral or moderating one! A review of the studies performed to date indicates that fields less than 2 tesla do not produce harmful cellular, biochemical, or genetic effects in humans. Continued study in this area is necessary because we will need static field strengths greater than 2 tesla to achieve higher sensitivity. At some field strength we expect biological effects to be observable. For example, a 20-tesla magnetic field should reduce the velocity at which nerves conduct signals.

The hazards associated with radio-frequency magnetic fields are more complex, but the principal hazard is radio-frequency heating of tissue. This should not be a major concern at the low frequencies we use, especially in light of the short duration of the radio-frequency pulses. Further, the new probe designs of Fukushima and Roeder may reduce this hazard by minimizing dielectric and ohmic heating effects.

The last two projects are closely related. Until now the rate of development in this field has been controlled largely by NMR spectroscopists who either learned cell culture and organ perfusion techniques themselves or who developed associations with cell biologists and physiologists on an as-needed basis. As we move from cells and perfused organs to animals and humans, the quality and rate of development of NMR spectroscopy in medicine will be determined by the strength of interdisciplinary teams that can not only obtain and interpret the

NMR data but also provide clinical, biochemical, and physiological expertise. The New Mexico state legislature recently provided funds for an NMR Center at the University of New Mexico School of Medicine. This center, which NMR spectroscopists and biochemists from Los Alamos are helping to develop, will be a unique national facility with respect to the breadth and depth of the interdisciplinary team that will be applying NMR to animals and humans. ■

Further Reading

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AUTHORS

James R. Brainard received his B.A. from Hope College in Holland, Michigan and his Ph.D. in chemistry from Indiana University. He spent two years as a National Institutes of Health postdoctoral fellow at Baylor College of Medicine investigating the structure of plasma lipoproteins using nuclear magnetic resonance methods. Since coming to the Laboratory in December 1981, his research interests have focused primarily on the application of NMR and stable isotopes to the study of metabolism in perfused organs. A former avid sailor, Jim is still waiting for the reservoir behind Cochiti Dam to fill up.

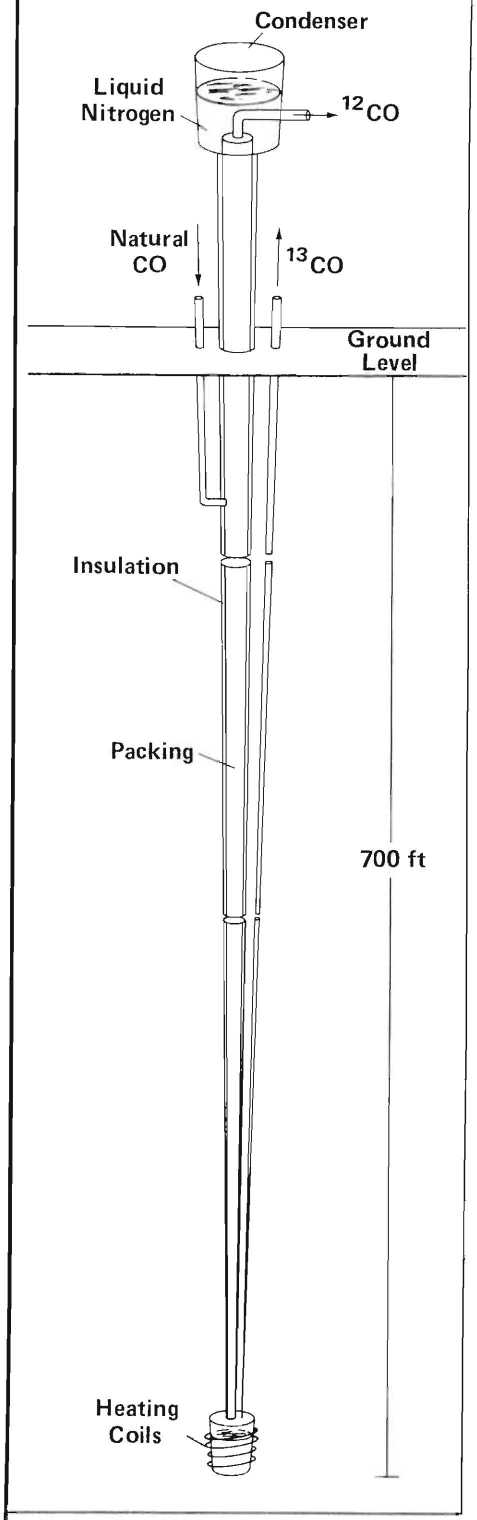
AUTHORS

Judith Y. Hutson. I received a B.A. (1958) and an M.A. (1961) in chemistry and a Ph.D. (1968) in biochemistry from the University of Colorado. From 1961 to 1963 I did research at the Massachusetts Institute of Technology on the “‘early enzymes” of T-even bacteriophage infection in *E. coli.* In the fall of 1967 I came to Los Alamos with my husband and one small child. The first winter here was spent writing my Ph.D. thesis. By the summer of 1968, the degree was finished and we had a second child. A few months later I began research at the Laboratory on mammalian cells, on teratology, and on microbial biosynthesis with stable isotopes. For the past two years I have been involved with the research discussed in this article.

Robert E. London, a native of Brooklyn, New York, obtained his graduate degrees in physics and biophysical chemistry from the University of Illinois. He joined the Laboratory in 1973 as a postdoctoral fellow sponsored jointly by the Theooretical and Chemistry-Nuclear Chemistry divisions and in 1975 became a staff member in the Inorganic and Structural Chemistry Group. He has an interest in both theoretical and experimental aspects of the study of biological systems using nuclear magnetic resonance methods and has authored or coauthored approximately sixty-five papers in this field. A dedicated and hard working scientist, London worked at the Laboratory for three years before noticing that it was situated in the mountains of New Mexico.

Nicholas A. Matwiyoff was born in Ann Arbor and raised in northern Michigan. He received his B.S. in chemistry from the Michigan College of Mining and Technology in 1959 and his Ph.D. in chemistry from the University of Illinois in 1963. Following a year as a National Science Foundation postdoctoral fellow at Stanford University, he joined the chemistry faculty at Pennsylvania State University. He moved to the Laboratory in 1968 to start an NMR project associated with a program on fluorine and actinide chemistry. As the stable isotope program at the Laboratory developed, his interests in NMR evolved to the study of biological systems labeled with carbon-13. He led the National Stable Isotopes Resource from its establishment in 1974 until 1982. At present he is Deputy Leader of the Isotope and Nuclear Chemistry Division.

RELATED WORK



Stable Isotope Production

—a distillation process

by Nicholas A. Matwiyoff, Berthus B. McInteer, and Thomas R. Mills

A 700-foot distillation column is far from ordinary, but the Laboratory was forced to this length to separate the rare but stable isotope carbon-13 from the common isotope carbon-12 by distillation of carbon monoxide. In somewhat shorter distillation columns the Laboratory also enriches the even rarer stable isotopes nitrogen-15, oxygen-17, and oxygen-18. Produced in the western hemisphere only at Los Alamos, these enriched stable isotopes are used as tracers in many research areas ranging from metabolism to agriculture to atmospheric circulation.

Distillation, one of the oldest and most effective methods for separating mixtures, exploits differences in the boiling points of the components. Two familiar examples of its beneficial applications are the separation of hydrocarbons from crude oil and the production of a beverage with a high alcohol content and a distinctive flavor from sour mash.

Schematic drawing of the Los Alamos distillation column for separating the naturally occurring mixture of ^{12}CO and ^{13}CO. The 700-foot column is supported over most of its length within a hole in the ground. The packing (spherical or saddle-shaped pieces of ceramic or metal) provides surface area for contact between liquid and vapor phases. The column is cooled initially to liquid nitrogen temperature as natural carbon monoxide enters, rises to the condenser, and liquefies. The liquid flows downward and cools the column as it vaporizes. When liquid carbon monoxide collects at the bottom, it is vaporized by carefully regulated heat. At equilibrium a downflow of liquid repeatedly contacts upstreaming vapor, and the more volatile component accumulates at the condenser.

Distillation is most often applied to mixtures whose components have boiling points that are above 50 degrees Celsius and that differ from one another by more than 10 degrees. Separation of such mixtures can then be carried out in relatively short (10- to 30-foot) columns. In contrast, the boiling points of the various isotopic forms of carbon monoxide or nitric oxide are very low [only slightly higher than the boiling point (−196 degrees Celsius) of liquid nitrogen] and, more important, differ by only tenths of a degree. Small as these differences may be, they are larger than those exhibited by most other compounds of carbon, oxygen, and nitrogen.

The boiling points of ^{12}CO and ^{13}CO differ by less than 0.1 degree Celsius (more precisely, their vapor pressures differ by less than 0.8 percent). To separate the two, the Laboratory built a 700-foot distillation column—probably the longest in existence. This engineering marvel, developed by B. B. McInteer, T. R. Mills, and J. G. Montoya, produces 20 kilograms per year of 99+ atomic percent carbon-13. The problem of supporting such a long column was solved economically by lowering it into a cased, 15-inch-diameter hole in the ground, but this method of support prevents access for repair. Design and construction of the system therefore required the greatest care. The length changes (about 2 feet) that occurred when the system was initially cooled to the temperature of liquid nitrogen for operation (or that will occur should shutdown be necessary) demanded particular accommodation. The welds joining the thirty-five 20-foot sections were thoroughly tested for soundness, and the gas feed lines were fitted with expansion joints. Proof that the system met the highest standards of design and construction is its continuous operation without incident since 1978.

Since the vapor pressures of the various isotopic forms of nitric oxide differ more from each other (2.7 percent in the case of $^{14}N^{16}O$ versus $^{15}N^{16}O$) than do those of carbon monoxide, the two distillation columns at Los Alamos for separating them are "only" 150 and 270 feet long. These systems are complicated, however, by the large number of product streams ($^{14}N^{16}O$, $^{14}N^{17}O$, $^{14}N^{18}O$, $^{15}N^{16}O$, $^{15}N^{17}O$, and $^{15}N^{18}O$) and by the necessity, since liquid nitric oxide is a high explosive, for barricades and remote controls. Nonetheless the two columns have been operated routinely since 1975 with an annual production capacity of 18 to 20 kilograms of nitrogen-15 at enrichments of up to 98 percent and 1 kilogram of oxygen-17 and 13 kilograms of oxygen-18 at enrichment of better than 40 and 95 atomic percent, respectively.

Under the guidance of McInteer and Mills, research has been directed recently to development of distillation methods for separating stable isotopes of heavier elements that are used as targets in the production of short-lived radioisotopes for nuclear medicine. ■

Synthesizing Labeled Compounds

by Robert E. London, Nicholas A. Matwiyoff, Clifford J. Unkefer, and Thomas E. Walker

RELATED WORK

Among the applications for the Laboratory's harvest of carbon monoxide enriched in carbon-13 are metabolic studies based on NMR spectroscopy. But first the isotope and its nuclear magnetic moment label must be incorporated at chosen sites in biochemical substances such as sugars and amino acids. The Laboratory has pioneered in developing chemical and biochemical methods for accomplishing this often intricate task.

For example, T. W. Whaley and R. M. Blazer of Los Alamos and Robert Barker and coworkers of Cornell University devised a chemical process (Fig. 1) for preparing a labeled form of the most common sugar, D-glucose. The essential reactant is ^{13}C-labeled hydrogen cyanide ($H^{13}CN$), which is obtained from ^{13}C-enriched carbon monoxide by the reactions $^{13}CO + H_2O \xrightarrow{Ni} {}^{13}CH_4$ and $^{13}CH_4 + NH_3 \xrightarrow{Pt} H^{13}CN$. The labeled hydrogen cyanide is then reacted with the 5-carbon sugar D-arabinose to form a mixture of D-[1-^{13}C]gluconitrile and D-[1-^{13}C]mannononitrile, which is then converted to a mixture of D-[1-^{13}C]glucose and D-[1-^{13}C]mannose.

Since D-mannose is of limited utility and yet forms the bulk (75 percent) of the product, considerable effort has been directed to investigating various means for catalyzing its conversion to D-glucose. Barker and coworkers found recently that molybdate ion (MoO_4^{-2}) is an effective catalyst, but NMR studies showed unequivocally that this catalyst converts D-[1-^{13}C]mannose to D-[2-^{13}C]glucose rather than the expected D-[1-^{13}C]glucose. This result is but one example of the unique insights into the course of chemical reactions provided by isotopic labeling and NMR spectroscopy.

Chemical syntheses such as the Barker process are sometimes the only practical route to a particular labeled compound but are often beset by difficulties: many are multi-step and hence lengthy, labor-intensive, and costly, and the yield of the labeled

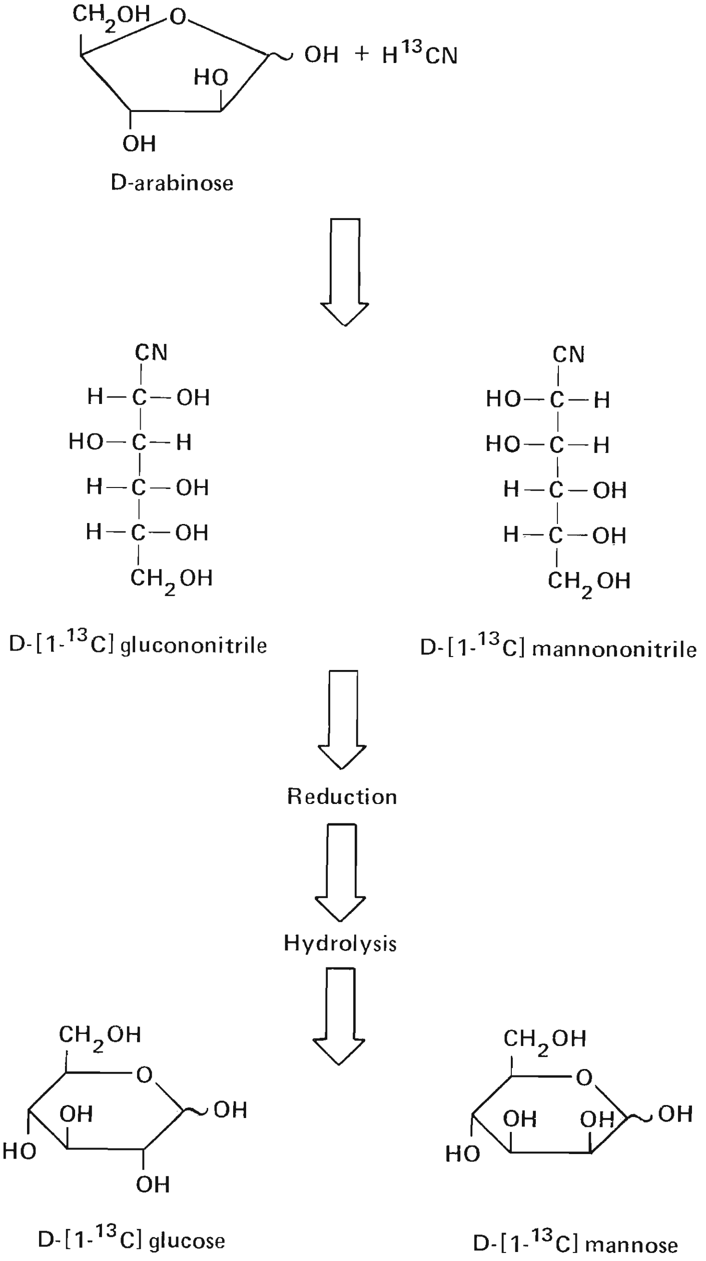


Fig. 1. Synthesis of ^{13}C-labeled D-glucose, a 6-carbon sugar, involves adding a labeled nitrile group to the 5-carbon sugar D-arabinose by reaction with labeled hydrogen cyanide. The product of this reaction, a mixture of labeled nitriles of D-glucose and D-mannose (another 6-carbon sugar), is then reduced and hydrolyzed to a mixture of the labeled sugars. The two sugars are separated by absorption chromotography.

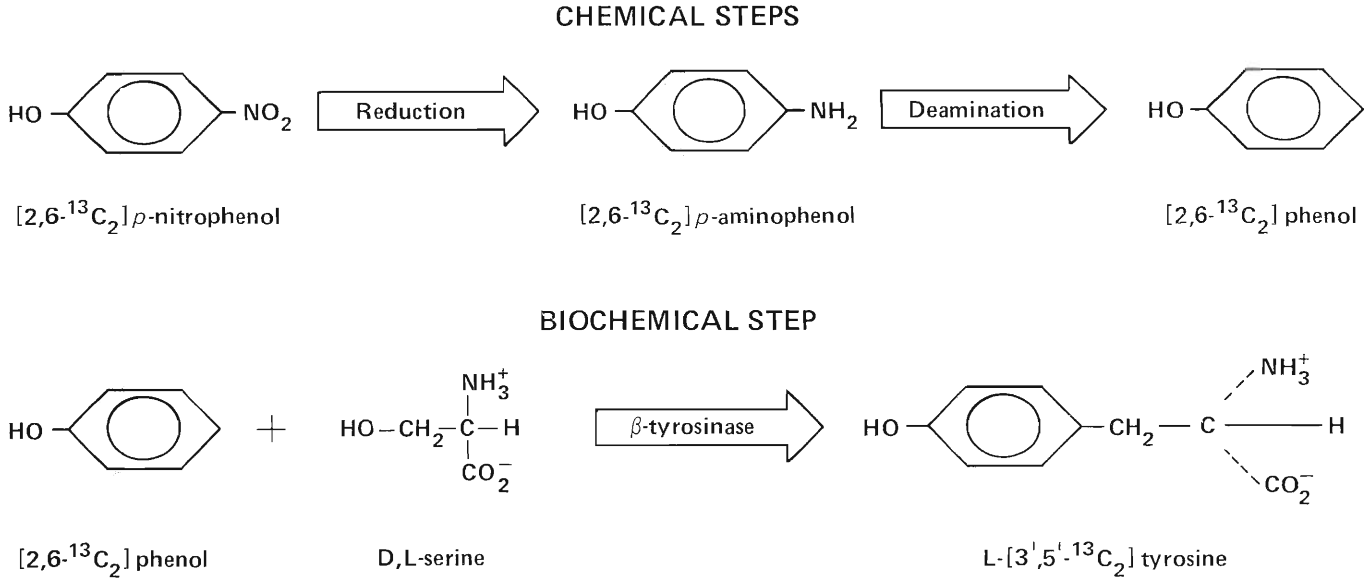


Fig. 2. Synthesis of ^{13}C-labeled L-tyrosine, an amino acid, is accomplished in only three steps. The third and simplifying step, the reaction between labeled phenol and the amino acid serine, capitalizes on the ability of the bacterium Erwinia herbicola *to produce large quantities of the necessary enzyme catalyst. Note that the reaction yields L-serine irrespective of whether the enzyme acts on D-serine, L-serine, or a mixture of the two isomers.*

product may be disappointingly low because of diversion of the isotope into undesired products or positions. Since biochemical syntheses are often less subject to these difficulties, they are being pursued at Los Alamos—and with noteworthy success.

To illustrate, consider the synthesis of a labeled form of the amino acid L-tyrosine, which is present in nearly all proteins and is a precursor of the hormones epinephrine (adrenalin), norepinephrine, and thyroxine. V. J. Hruby of the University of Arizona has developed a ten-step chemical process for synthesizing a mixture of the D and L isomers of $[3',5'\text{-}^{13}C_2]$tyrosine from labeled *p*-nitrophenol. Based on the carbon-13 contained in the *p*-nitrophenol, the yield of the isomeric mixture is about 30 percent; separating the biologically active L isomer reduces the yield to 14 percent.

In contrast, T. E. Walker of Los Alamos and C. B. Storm of Howard University developed a combined chemical-biochemical method for preparing L-$[3',5'\text{-}^{13}C_2]$tyrosine from the same labeled reactant in three steps with a yield of 80 percent. As in the synthesis of D-glucose, the labeled reactant, in this case *p*-nitrophenol, has its origins in ^{13}C-enriched carbon monoxide: $^{13}CO + O_2 \longrightarrow {}^{13}CO_2 \xrightarrow{Cu,Cr,Zn} {}^{13}CH_3OH$; $^{13}CH_3OH + CO \xrightarrow{HI,Rh} {}^{13}CH_3COOH$; $^{13}CH_3COOH + Ba(OH)_2 \longrightarrow Ba(^{13}CH_3COO)_2 \xrightarrow{pyrolysis} {}^{13}CH_3CO^{13}CH_3 \xrightarrow{condensation} [2,6\text{-}^{13}C_2]$*p*-nitrophenol. The tyrosine synthesis (Fig. 2) involves two chemical steps that convert *p*-nitrophenol to phenol followed by a key biochemical step that converts phenol directly to L-tyrosine with the aid of the bacterium *Erwinia herbicola.* Under suitable conditions this microorganism manufactures high levels (up to 10 percent of its cell protein) of the enzyme β-tyrosinase. (This property was discovered in the course of Japanese research and development directed toward the use of microorganisms to produce large quantities of amino acids as food supplements.) After filling themselves with a surfeit of the enzyme, the bacteria are placed in a medium containing the labeled phenol and D-serine, L-serine, or a mixture of both, and the enzyme catalyzes the reaction

$$[2,6\text{-}^{13}C_2]\text{phenol} + \text{D,L-serine} \xrightarrow{\beta\text{-tyrosinase}} \text{L-}[3',5'\text{-}^{13}C_2]\text{tyrosine}.$$

E. herbicola's generous supply of β-tyrosinase yields other labeled forms of L-tyrosine from variations on this reaction: L-[1-, 2-, or 3-^{13}C]tyrosine from unlabeled phenol and D,L-[1-, 2-, or 3-^{13}C]serine; and ^{15}N-labeled L-tyrosine from unlabeled phenol, unlabeled pyruvate, and ^{15}N-labeled ammonia. (Nitrogen-15 is another isotope useful in metabolic studies based on NMR spectroscopy.)

Another example of biochemical synthesis is the use of the metabolically defective microorganism *Brevibacterium flavum* to produce L-$[2,4\text{-}^{13}C_2]$glutamate in high yield from D-$[1\text{-}^{13}C]$glucose. The route to this amino acid (a salt of which, monosodium glutamate, is well known as the seasoning MSG) involves α-ketoglutarate, one of the intermediates in the Krebs cycle. In normal organisms α-ketoglutarate progresses through the Krebs cycle to succinate by action of the enzyme α-ketoglutarate dehydrogenase. *B. flavum,* however, produces very little of the enzyme, and when these bacteria are grown in a medium containing glucose, α-ketoglutarate accumulates and is converted, in the presence of ammonium ion, to L-glutamate. A sick but kindly organism, *B. flavum* suffers from the additional disorder of a leaky cell membrane, which allows passage of the glutamate into the medium where it can be recovered readily.

On the horizon for the biochemical synthesis program at Los Alamos is the use of recombinant DNA techniques to genetically engineer microorganisms with optimal properties for the production of labeled biochemical substances. By concurrently probing these microorganisms *in vivo* with NMR techniques, the metabolic consequences of the genetic engineering can be ascertained. This approach, being taken by C. J. Unkefer of Los Alamos in collaboration with J. K. Griffith of the University of New Mexico, may be one of the most significant research directions in stable isotope technology in this decade. ■

Enzyme Structure and Interaction with Inhibitors

by Robert E. London

RELATED WORK

The metabolic studies described in the previous article represent a relatively straightforward application of NMR spectroscopy. The positions of the various resonances and their heights allow one to determine the presence and amount of metabolic intermediates and products and, in turn, the specific metabolic pathways involved. These tracer studies with stable-isotope labels are thus directly analogous to studies with radiolabels such as carbon-14.

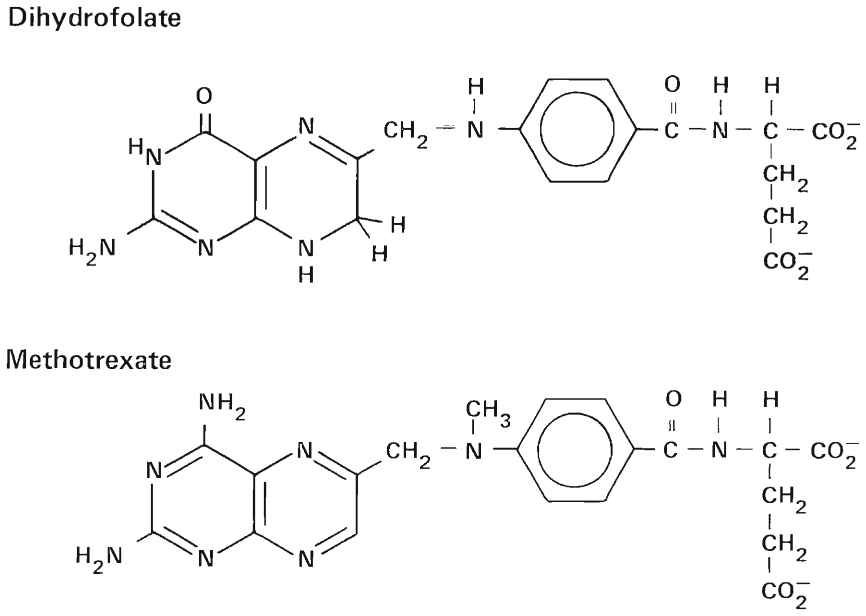
But NMR spectra contain a wealth of additional information. Changes in resonance position and shape under various conditions can reveal important structural and dynamic features of complex biological macromolecules. Here we will review some results of an extensive series of studies on the ^{13}C-labeled enzyme dihydrofolate reductase, or DHFR. The idea was to explore how much we could learn about structure and dynamics using NMR techniques in combination with isotopic labeling.

Enzymes are particularly interesting to study from this point of view since both their structure and dynamics may be important to their function of catalyzing biochemical reactions. In the familiar "lock and key" model of enzyme catalysis, a structurally rigid enzyme "lock" can bind only the structurally complementary substrate "keys." Extensive crystallographic data have also fostered this picture of enzymes as rigid structures. But recent evidence suggests that enzyme dynamics is also at work in recognition and catalysis.

We chose to study the enzyme DHFR because of its clinical relevance. Its function is to "activate" the vitamin folic acid by catalyzing the oxidation-reduction reaction

$$\text{dihydrofolate} + \text{NADPH} \overset{\text{DHFR}}{\rightleftharpoons} \text{tetrahydrofolate} + \text{NADP}^{+}\,, \qquad (1)$$

where dihydrofolate is an inactive form of folic acid, and tetrahydrofolate is its activated form.*


Dihydrofolate

Methotrexate

Fig. 1. Substrate dihydrofolate and inhibitor methotrexate of the enzyme dihydrofolate reductase (DHFR). Note that the leftmost regions of the substrate and the inhibitor are similar in structure. Because these regions presumably interact strongly with the enzyme, they were labeled with carbon-13 (at the locations indicated by gray circles) for the purpose of studying enzyme/substrate and enzyme/inhibitor interactions.

Tetrahydrofolate is required for the synthesis of thymidine, a component of DNA. Inhibiting the production of tetrahydrofolate therefore retards DNA synthesis and cellular growth. In fact, one treatment for the unregulated cellular replication that characterizes cancerous growth is to inhibit the production of tetrahydrofolate by administering drugs that bind with very high affinity to DHFR. These drugs, so-called antifolate inhibitors, are structurally quite similar to dihydrofolate (Fig. 1) and can bind at DHFR's "active site" for dihydrofolate, thereby preventing DHFR from catalyzing reaction 1. One goal of our studies was to understand, at the molecular level, why these inhibitors have a very high affinity for DHFR. Such information can be of value for the design of even more potent and specific inhibitors of the enzyme.

DHFR is a medium-sized enzyme with a molecular weight of about 20,000, and its NMR spectrum in the absence of labeling would show only a low-intensity "background," due to naturally occurring carbon-13, of nearly 800 carbon resonances. Labeling specific portions of the enzyme with carbon-13 was therefore essential to

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**NADPH (nicotinamide adenine dinucleotide phosphate), a derivative of the vitamin niacin, is an enzyme cofactor involved in biological oxidation-reduction reactions.*

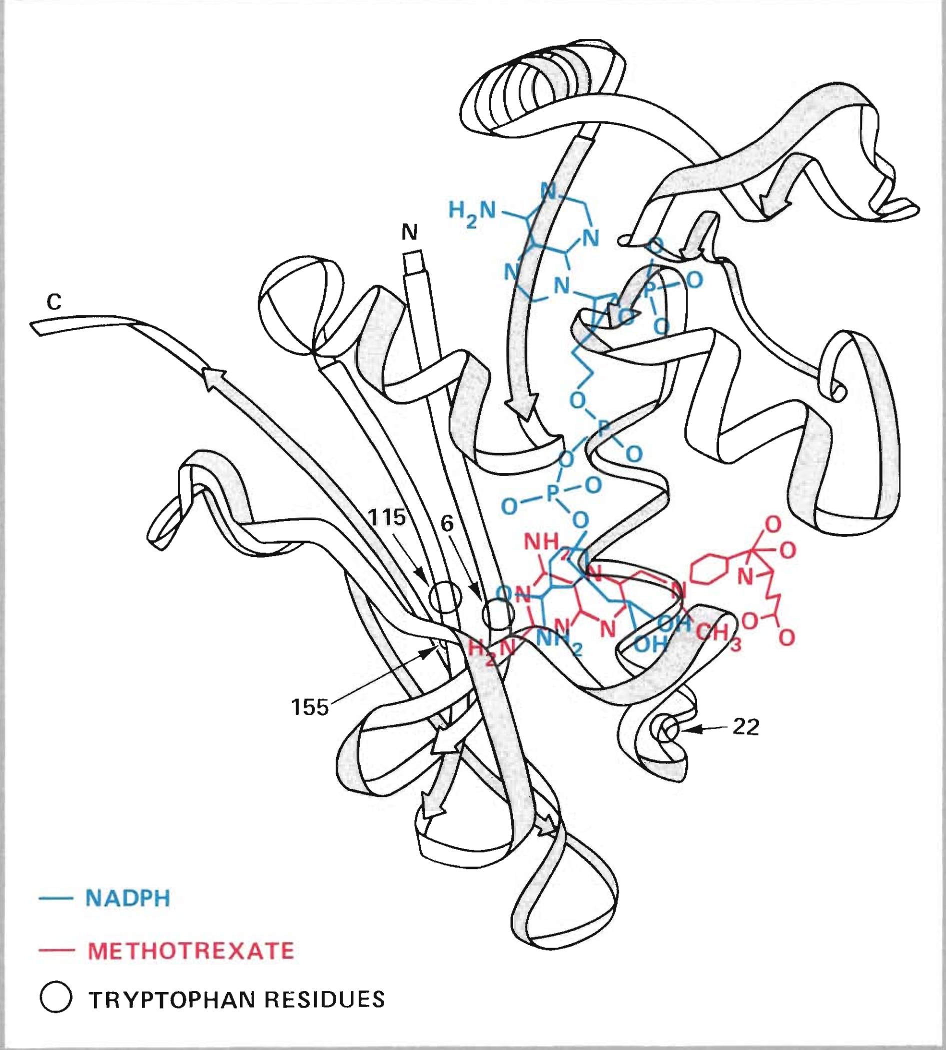


Fig. 2. Backbone ribbon drawing, showing the locations of the enzyme cofactor NADPH and of the inhibitor methotrexate, of the enzyme DHFR (a linked chain of 167 amino acid residues) derived from the microorganism Lactobacillus casei. *[Adapted from J. T. Bolin, D. J. Filman, D. A. Matthews, R. C. Hamlin, and J. Kraut, Journal of Biological Chemistry 257, 13650 (1982).] By occupying the enzyme's active site for dihydrofolate, the inhibitor prevents DHFR from catalyzing the reaction that converts this inactive form of the vitamin folic acid to its active form. NMR spectra for DHFR derived from the microorganism* Streptococcus faecium *and containing the ^{13}C-labeled residue of the amino acid tryptophan provided information about the dynamic behavior of the enzyme and the interaction between enzyme and inhibitor. The approximate positions of the four tryptophan residues in DHFR derived from* S. faecium *are those indicated in the drawing, if it is assumed that DHFR derived from* S. faecium *and* L. casei *are homologous.*

enhance selected peaks and thereby reduce the complexity and increase the sensitivity of these studies.

X-ray crystallographic studies have shown that enzymes are long strings of peptide-linked amino acids (that is, the amino group of one acid residue binds to the carboxylic acid group of the next). These long strings of amino acids fold in a complex way to form a globular structure (Fig. 2). To study the sensitivity of NMR spectra to structure, we labeled selected amino acid residues of DHFR with carbon-13 and measured the spectra of the labeled enzyme in its globular form and again after its structure had been changed into a random coil by the addition of urea.

The carbon-13 labeling was accomplished by first labeling the amino acids methionine, arginine, and tryptophan and then growing the microorganism *Streptococcus faecium* in media containing one of these labeled amino acids. *S. faecium*, which is a good source of the enzyme DHFR, incorporates the labeled amino acids into the DHFR molecules. The labeled DHFR was then isolated from the microorganism, and its NMR spectra were obtained for the globular and random coil configurations. Figure 3 shows the results for DHFR labeled with [3-^{13}C]tryptophan. The spectrum for the globular form of DHFR shows several carbon-13 resonances corresponding to different positions along the polypeptide backbone and therefore to different chemical environments of the individual tryptophan residues within the enzyme. Note that most of these so-called chemical shifts disappear when the enzyme structure is disrupted into a random coil. Thus NMR spectra are sensitive to structure.

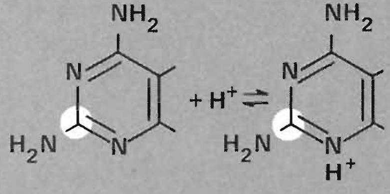
Looking more closely at the spectrum for the globular structure, we note that it has five resolved resonances although there are only four tryptophan residues in each enzyme molecule. Evidently a single tryptophan residue is responsible for the two adjacent peaks near 110 ppm. This splitting probably indicates that the enzyme takes on two different configurations in the region of that particular residue.

The resonance at 106 ppm is also noteworthy because it is much broader than the others, a fact suggesting that the residue responsible for the resonance is located in a portion of the enzyme that undergoes conformational changes with time. Spectra taken at different temperatures (Fig. 4) confirmed this suggestion. The resonance at 106 ppm (resonance 4) exhibits a strongly temperature-dependent linewidth believed to be associated with "breathing" of the enzyme. That is, the enzyme adopts an ensemble of molecular conformations leading to temperature-dependent dynamic behavior. Figure 4 also shows that this "breathing" phenomenon disappears when the enzyme is complexed with the inhibitor 3′,5′-dichloromethotrexate. (The structure of this inhibitor is like that of methotrexate in Fig. 1 except that chlorine replaces hydrogen at positions 3 and 5 on the benzene ring.) The resonance then becomes sharp, indicating that a part of the binding energy between enzyme and inhibitor stabilizes a particular subset of enzyme conformations. For DHFR derived from *S. faecium* we found that the substrate dihydrofolate and each of the inhibitors studied lead to sharpening of resonance 4. In contrast, NADPH, the enzyme cofactor in reaction 1, does not significantly sharpen the resonance. Presumably, the NADPH binds to a portion of the enzyme molecule more remote from the particular tryptophan residue responsible for resonance 4.

The addition of substrate or inhibitor molecules can also lead to changes in resonance position, or chemical shifts. For example, the spectra at 15 degrees Celsius in Fig. 4 show that dichloromethotrexate causes a slight shift of resonance 1 to the right. Such chemical shifts reflect interactions between the enzyme and the bound molecules. In general, our results demonstrate the sensitivity of NMR spectra to the precise folded enzyme structure. Interactions among specific pairs of residues are important in producing chemical shifts, and these shifts are altered when the enzyme binds other molecules. Dynamic behavior such as "breathing" is also observable, but whether this behavior observed in DHFR is significant for catalysis (for example, whether it helps the enzyme to "recognize" the substrate dihydrofolate) is still an open question.

A second set of studies with DHFR was designed to investigate the basis for the high affinity between the inhibitor methotrexate and DHFR. Rather than labeling the enzyme, we chose to label the inhibitor. The label was placed in that portion of methotrexate thought to interact strongly with DHFR (see Fig. 1, where the position of the carbon-13 label is marked by a gray circle). We found that the NMR spectrum of a solution containing labeled methotrexate and DHFR exhibits two carbon-13 resonances, one corresponding to inhibitor molecules that are free in solution and one corresponding to inhibitor molecules that are tightly associated with the enzyme. The fact that the single carbon-13 label exhibits two resonances is a reflection of the very high affinity of methotrexate for the enzyme.

We then varied the pH of the solution containing the enzyme and the labeled inhibitor and found that the two resonances behave very differently (Fig. 5). The resonance ascribed to the uncomplexed inhibitor undergoes a large shift in position at a pH of about 5.7. This so-called titration behavior indicates that the uncomplexed inhibitor accepts a proton (H^+) in the following reversible reaction:



(2)

The pH at which the curve's large shift is centered (5.7) is called the pK of this proc-

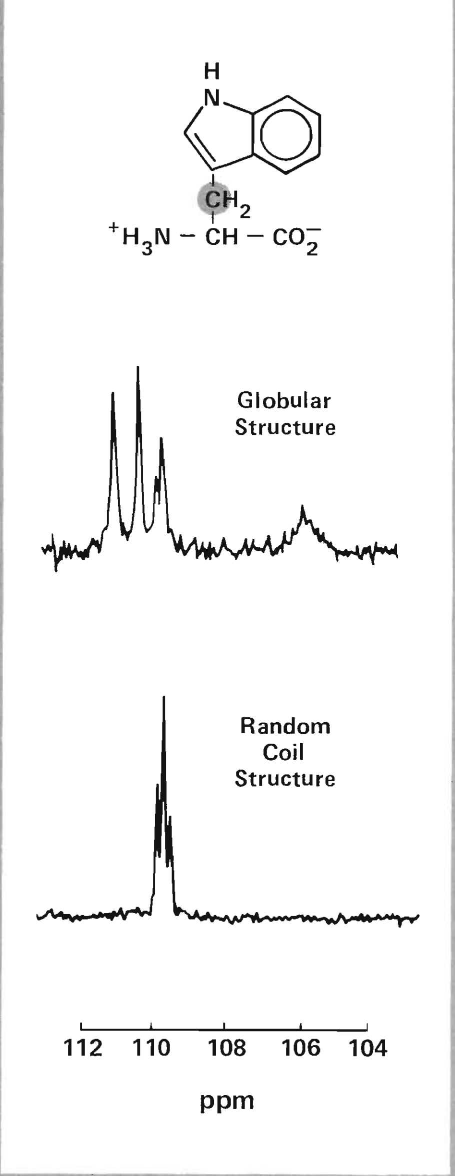


Fig. 3. Carbon-13 NMR spectra for [3-^{13}C]tryptophan-labeled DHFR in its active globular form and in its random coil form. The structure of tryptophan is also shown, with the position of the carbon-13 label indicated by a gray circle. Note that the globular structure produces many more distinct resonances than does the random coil structure.

ess. It represents the pH value at which half of the molecules are protonated and half unprotonated. Thus the one resonance observed for the uncomplexed inhibitor is actually an average produced by its protonated and unprotonated forms. In contrast, the resonance ascribed to the enzyme-complexed methotrexate remains fixed near the resonance of the protonated form of the uncomplexed inhibitor. These data indicate that the protonated form of methotrexate is the potent enzyme inhibitor; in other words, a strong interaction between protonated methotrexate and the enzyme must be the critical factor in making methotrexate an effective inhibitor. This conclusion is supported by the x-ray crystallographic data of Matthews and coworkers at the University of California, San Diego. They found that in the crystalline state the enzyme-complexed methotrexate is protonated and hydrogen-bonded to a negatively charged amino acid (aspartate-26) of the enzyme.

Figure 5 shows that the pK for protonation of the enzyme-complexed inhibitor is well above 10, the highest pH value used in the study. Such a large difference between the pK of the enzyme-complexed inhibitor and that of the free inhibitor is a measure of the binding energy between the inhibitor and the enzyme. The strength of binding was not accurately known prior to these studies and had in fact been incorrectly determined using conventional ultraviolet spectroscopic techniques. It is an impressive achievement of the isotopic labeling/NMR method that of all the interactions among amino acid residues and between amino acids and solvent molecules that stabilize the enzyme structure, we can probe a single one and quantify its strength. ■

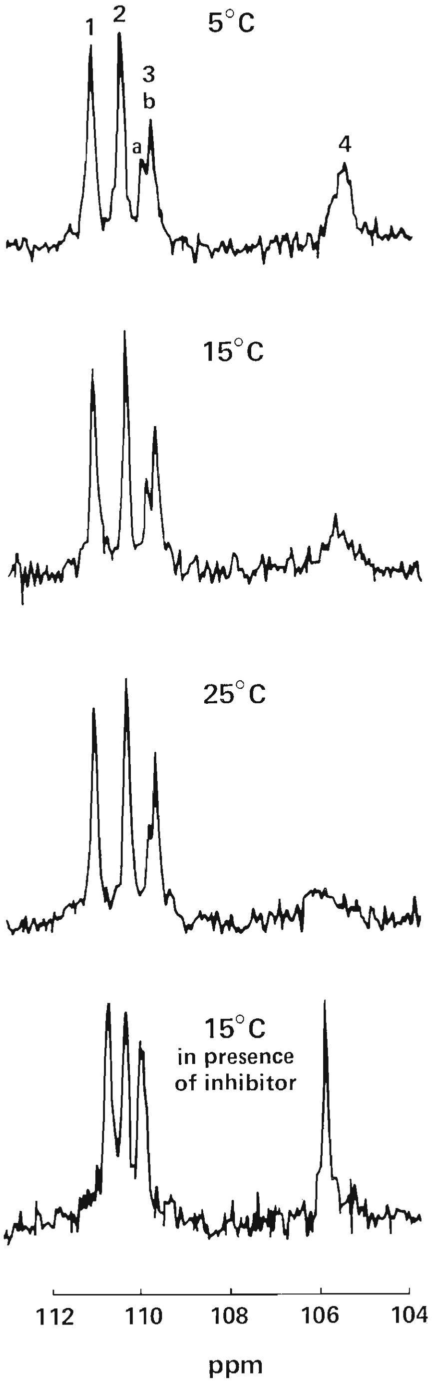


Fig. 4. Carbon-13 resonances observed for [3-^{13}C]tryptophan-labeled DHFR at various temperatures and at 15 degrees Celsius in the presence of the inhibitor 3′,5′-dichloromethotrexate. Resonance 4 exhibits a temperature-dependent linewidth that becomes a sharp resonance in the presence of the inhibitor. The tryptophan residue thought to be responsible for resonance 4 is labeled 6 in Fig. 2.

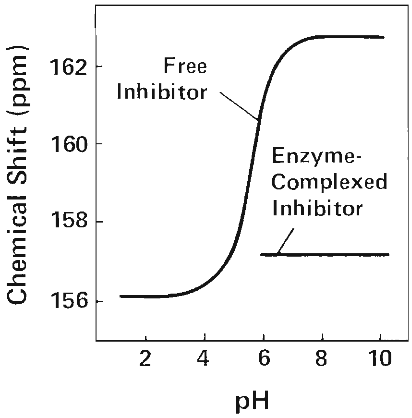


Fig. 5. Chemical shifts of the carbon-13 resonance for the inhibitor methotrexate (labeled as in Fig. 1) as a function of pH. When the enzyme DHFR is present (that is, at pH values above 5.5), two sets of resonances are observed, one corresponding to free methotrexate and one to the enzyme-complexed inhibitor. These results show that methotrexate in its protonated form has an extremely high affinity for the enzyme.

Acknowledgment

These studies were carried out at St. Jude's Research Hospital in Memphis,Tennessee in collaboration with Raymond L. Blakley, Lennie Cocco, and John Groff. Groff is currently on the staff of Abbott Laboratories.

What Lies Ahead

by Darleane C. Hoffman

I have always enjoyed the intriguing mix of fundamental research and applied programs in our division. There is a synergistic effect in which the research sparks new applications, while the needs of our diverse interdisciplinary programs stimulate the research. And, as in the past, future challenges for the division are being generated from both our current applied programs and our research.

A particularly exciting challenge that promises to test the full range of our capabilities is how to attach radionuclides to monoclonal antibodies. Such tagged antibodies could be used both to help diagnose tumors with a technique known as tumor imaging or to deliver radiation to a specific diseased site in the body with minimal damage to healthy tissue. The trick will be to keep from destroying the biological activity of the antibody while, at the same time, "caging" the radionuclide securely enough so it won't get loose and go to the wrong place.

Jumping to another exciting area, that of heavy-element and fission research, I think we'll be able to produce many neutron-rich isotopes of the heavy actinide and transactinide elements by heavy-ion transfer reactions, that is, transfer of a neutron-rich portion of an accelerated ion to a heavy target nucleus. More importantly, we will have developed the techniques required to identify these short-lived isotopes as well as to measure their spontaneous fission properties. These advances should also permit resolution of the controversies surrounding the discovery of elements 104, 105, 106, and 107. Perhaps they'll even facilitate the discovery of element 108 which is expected to decay only by spontaneous fission.

We further predict that some of these new isotopes will live long enough to be separated by ingenious chemical techniques, such as centrifugally separated solvent extraction, gas transport, or thermochromotography. It should then be possible to study a number of exciting features that help refine our basic understanding of the atom. In particular, we may be able to observe marked deviations in chemical properties and reactions because of the greater influence of relativistic effects in these new very heavy elements compared to lighter homologs. We wish to compare the observed chemical behavior of the transactinide elements 104, 105, and 106 with their expected behavior as Group IV, V, and VI elements. These experiments will certainly not be easy, but understanding the architecture of the periodic table and its ultimate limits are worthy of our best efforts.

We will also not neglect the light-element region and plan to construct a time-of-flight isochronous spectrometer at LAMPF. We will measure the masses of two to five new isotopes of each of the elements from nitrogen to zinc produced in fragmentation and spallation reactions induced by 800-MeV protons. These measurements will enable us to explore the limits of nuclear stability in the light element region and will critically test the atomic mass models.

I foresee a renaissance of interest in separating isotopes by chemical exchange instead of by more sophisticated techniques such as electromagnetic, gaseous diffusion, or laser separation. Underdeveloped countries will be particularly attracted to such processes because they require only current chemical engineering rather than more sophisticated technology.

The recent progress in artificial intelligence, including robotics and microelectronics, could revolutionize how we handle radioactivity. In fact, techniques developed for radioactive materials will surely carry over into other laboratory operations for any type of hostile environment.

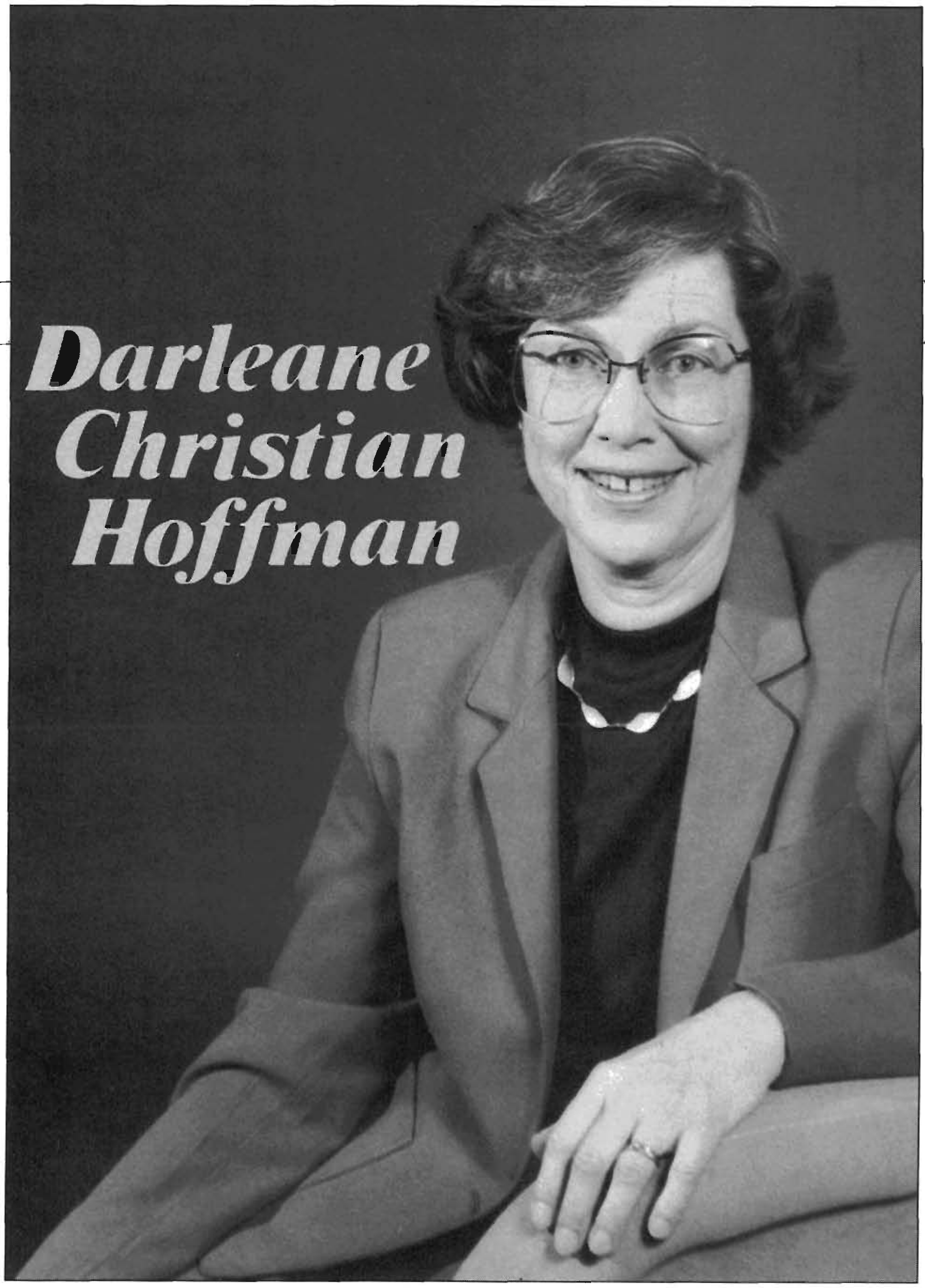
I expect recently developed very sensitive analytical techniques to have a far-reaching impact on many disciplines. For example, we have recently used accelerator-based mass analysis to investigate geochemical processes at much lower concentrations and rates than ever before (see "Migration of Radioisotopes in the Earth's Crust"). We have detected some atoms, such as chlorine-36 in groundwater, in concentrations of only 10^7 atoms per liter—one atom in 10^{19}! We are already applying nuclear microprobe techniques to analyze for surface and trace impurities in materials critical to our nuclear weapons, and we are developing resonance-ionization mass spectrometry for our weapons radiochemical diagnostics program. We anticipate applying these techniques to more fundamental problems, such as to study variations in the solar neutrino flux—or to examine rare modes of nuclear decay such as double beta emission—or to search for quarks and the very heavy X particles that have been postulated.

We are using our expertise in nuclear magnetic resonance to develop methods to follow the progress of metabolism and biochemical processes. We label biologically active compounds with stable carbon, oxygen, and nitrogen isotopes that have nuclear magnetic moments and then follow these compounds with NMR techniques (see "Metabolism As It Happens"). Similarly, NMR techniques can be applied to a variety of other problems, such as elucidating the structure of zeolites and how these minerals bind and hold various chemical species including the radioactive nuclides in nuclear waste. Or, when combined with x-ray and neutron diffraction and time-resolved spectroscopy, NMR can help show why one explosive is very sensitive to detonation while another, superficially very similar, is not.

The techniques we've developed to assess how well a geologic and hydrologic system will isolate radioactive waste will surely find use for nonradioactive waste, such as toxic chemicals. Our studies on rock-water interactions and the migration and fixation of elements in rocks are relevant to production of geothermal energy, ore body formation, and the exploration and development of mineral resources.

I've mentioned only a few of the things I can see for the future. If it sounds as though our division has a wide diversity of interests, that's true, it does. It keeps me hopping trying to keep up. But that's also what keeps it interesting and lets us make contributions that cross traditional, narrow disciplinary lines. ■

Darleane Christian Hoffman



Darleane Hoffman has been Division Leader of Isotope and Nuclear Chemistry at Los Alamos since her return from a Guggenheim Fellowship at Lawrence Berkeley Laboratory in 1979. Despite more than twelve years in management and generous service in community, state, and international organizations, she remains preeminently a scientist. At Los Alamos since 1953, her research includes rapid separation of short-lived fission products, separation chemistry of lanthanide and actinide elements, nuclear spectroscopy, transuranium element research, studies of the fission process, the search for heavy elements in nature, and studies of radionuclide migration in geologic media.

She has testified before Congress on the problems of nuclear waste isolation, has helped arbitrate international claims to the discovery of elements, is chairperson of the Committee on Nuclear and Radiochemistry of the National Research Council,[*] and is coordinating the program of the 1984 Pacific Basin Chemical Conference of the American Chemical Society.

She is the 1983 recipient of the American Chemical Society Award for Nuclear Chemistry.

Do what you think is right and what you want to do.

The Iowa State freshman listened to her art adviser's vigorous protests regarding a change in major. What could she do with chemistry? Didn't she know it would be a lonely field for a woman? What about her talent in art? The student was Darleane Christian, and she could feel herself becoming more determined with each and every protest.

"It all happened that spring of '45 because of Professor Nellie Naylor's beginning chemistry class. I had lots of math in high school (it was my favorite subject) and some physics, but no chemistry. When I decided to study applied art, the university still required me to have one year of chemistry. It turned out that I liked Dr. Naylor's class best of everything I was taking, and I attribute that to her teaching skills. After two quarters of chemistry with her, there was no longer any doubt in my mind."

Darleane majored in physical chemistry and even dared, in her junior year, to apply for a research assistant's job at the Institute of Atomic Research at Iowa State. "I started working right away in nuclear and radiochemistry with young Professor Don Martin, with whom I later did my graduate study. My very first task was to construct Geiger counters. The tricky part was splitting mica into very thin yet airtight pieces for the windows."

Three years after her Bachelor's degree, Darleane acquired her Ph.D. and married Marvin Hoffman. She then worked a year at Oak Ridge while her husband completed his Ph.D. in physics at Iowa State. The underlying principle she followed for this and the rest of the professional career is

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[*] The National Research Council, the principal operating agency of the National Academy of Sciences and the National Academy of Engineering, serves both government and educational organizations.

expressed in the advice she later gave her children: "I don't care what other people are doing. What I do care about is what you do—and that you think about what you do—and that you do what you think is right and what you want to do."

Certainly there was one thing Darleane didn't want to do in her career. "I determined when I decided to go into chemistry that I wasn't going to allow myself to become the stereotyped woman scientist of that day. At the university I saw spinsters who had essentially given up everything to pursue their professions. After all, if men could marry, have families, enjoy other things, I saw no reason why women couldn't, too. And that doesn't mean I didn't work hard, because in those days graduate students worked days, nights, and weekends. But I always took time for the things I enjoyed, things like tennis and music. As for marriage, both partners have to agree on what they are doing. If a woman is going to have a career and a family and so forth, she has to have a very supportive husband, or she might as well forget the whole thing."

... her scientific intelligence ... her dedication

An unexpected result of Mike, the first thermonuclear test at Eniwetak in the summer of '52, was the discovery of new elements. It may have been one of the few times in Darleane Hoffman's life when her determination failed to overcome obstacles. "Marvin was hired and in Los Alamos by October '52. I terminated at Oak Ridge and joined him in December. For a while I couldn't even locate the job allegedly available to me. Once I found the job, the security office couldn't find my clearance, which had been stashed in New York. For three frustrating months I sat and waited while others were discovering einsteinium and fermium in the test debris."

Also discovered in the Mike debris was plutonium-244. Searching the earth's crust for postulated residual plutonium-244 was to become one of Darleane Hoffman's most serious and best known efforts. "Because even the most sensitive mass spectrometric measurements require $\approx 10^7$ atoms ($\approx 4 \times 10^{-15}$ g) of plutonium for positive detection, it is necessary to process very large samples of ores which are highly enriched in plutonium" [*Nature* 234: 132 (1971)]. After two years of laborious work, Darleane and her co-workers found the 244 isotope in plutonium isolated from 260 kg of Precambrian bastnäsite.

Francine Lawrence of the nuclear and radiochemistry group worked with Darleane on that project and remembers many others. "For thirty years I've been privileged to watch a gifted, hard-working scientist in action. Darleane's work on decay schemes for some of the rare earths, on heavy ion investigations, on the discovery of plutonium-244 in nature are all indications of her scientific intelligence and of long hours of effort. Many times she worked through the night and well past dawn. She earned the recognition that has come to her, and science has benefited from her dedication."

In the 1960s both Livermore and Los Alamos had success making heavy element isotopes in nuclear tests. "But we never found anything beyond fermium-257, even though predictions based on its 100-day half-life indicated that fermium-258 and -259 should also be detectable. However, we were able to isolate the fermium-257 from the debris, and it provided us material for study of the fission properties of fermium. We discovered something we hadn't known, namely that spontaneous fission could occur by fission into two nearly equal parts. In this isotope both symmetric and the more usual asymmetric fissioning were occurring—we had come upon a new mode of spontaneous fissioning."

Next, scientists at Livermore and Berkeley discovered a different piece of the puzzle. "They produced fermium-258 at the Savannah River reactor and measured its half-life at 380 microseconds instead of the predicted 45 days. So a disaster in the half-life for spontaneous fission occurs right there at fermium-258."

Los Alamos and Livermore later combined their expertise in a project that in 1976 produced fermium-259 at the Los Alamos Van de Graaff. Jeremy Wilhelmy, who is now a Fellow at Los Alamos, took part in the effort. "The project was very much headed by Darleane in a coordinating role. Not only did she negotiate and coerce whatever was needed, her enthusiasm was very contagious. The eventual success of the project was probably largely due to her warm concern for everyone and to the mechanical genius of Josef Weber, our post doc from Munich. (Josef was also extremely hard working and couldn't believe people went home from the Lab at 5 or 6 p.m.)

"We decided it might just be feasible to produce fermium-259 via the (t, p) reaction on fermium-257. We obtained from the Transplutonium Production Program at Oak Ridge the largest amount of fermium available ($\approx 10^9$ atoms) and formed a liaison with Ken Hulet's Livermore group, which provided us the talents of Ron Lougheed to prepare the target as well as a collection wheel and a microprocessor system. All in all we had ten days of beam-on-target time at extremely high average currents of 10 to 15 microamps of 16-MeV tritons. A grand total of 497 events were recorded, but they showed us a spectacular symmetric fission distribution and that a new regime was with us—one that didn't fit the systematics of less heavy elements."

Perils of a woman scientist!

Of necessity Darleane Hoffman worked in a man's world. "In the early days of the Laboratory, the number of women holding Ph.D. degrees could be counted on the fingers of one hand, and I was very conscious of the need to do my best so that other women would be accepted. If a woman

Darleane Christian Hoffman

wants to be treated equally, especially in the work place, then she has to do certain things she may think are men's work. I feel fairly strongly about that. You can't expect to be treated as an equal one minute and then demand special consideration the next."

Colleagues like Charles I. Browne remember that Darleane insisted on doing her share, whatever the work. "I wonder if she's forgotten the time we used the CPC (card programmed calculator) computer? We were just beginning to use multichannel analyzers and were overwhelmed with data. In the evenings we would all head north over the 'bridge of sighs' (the overpass across Trinity) to the theoretical building. There we took turns feeding batches of IBM cards carefully into the machine and then just as carefully retrieving them. The input hopper was about four feet above the floor, and Darleane is not exactly what one would call tall. I can still see her stubbornly climbing up on a chair to put those cards in that hopper when she felt it was her turn."

George Cowan, long her division leader, recalls trips to Nevada. "We treated her with a special consideration which nowadays would probably be regarded as chauvinistic. But although she was accustomed to the admiration of men, she obviously wanted to be accepted as one of the boys. She worked twenty-four hour days whenever the testing program required them.

"Darleane and I shared a fondness for potato pancakes. I will never forget the night at a favorite Las Vegas restaurant when she set what must be the all-time record for potato pancakes demolished at one sitting. It was awe-inspiring; she must have trained by skipping meals for days."

You can stretch yourself too thin.

"I did not take the job of division leader reluctantly," Darleane Hoffman says, "but I'm glad I didn't become a manager at an early age, I really am. And I feel sorry for some of the men who have to make that career decision so early. After all, we are trained as scientists, we have a profession. On the other hand, I think it's important for scientists to become managers, because most scientists don't want to be managed by someone they feel is scientifically incompetent, someone who tries to solve scientific problems by edict."

Darleane has no complaints of discrimination in her current position. "To most of the people in my division, I'm just a colleague." She also feels lucky to have her children already grown. "The current demands of a management job are not compatible with raising a family—or with doing research, for that matter. The idea of a five-year rotation of management jobs from group leader up is not without merit. When I am not traveling, I spend all day on the care and feeding of the division, sometimes until 8 p.m. Then at night and on the weekend I try to do my research. Science has become my avocation.

"When I had children at home, I was much more careful of my time. I always had help and someone to start the evening meal. I didn't travel nearly so much as now, and my evenings were spent with the children." She encourages young women to seek education for careers that will be "more than just a job" and that will also enable them to afford help as needed. "Women should organize their careers thoughtfully. For example, I've always been glad I chose to work at the Laboratory rather than at a university. I think discrimination against women in universities has been severe."

Although Darleane enjoys many aspects of her present responsibility and especially the opportunity to help develop the potential of her division and of the Laboratory, she nevertheless finds the multiple layers of today's management burdensome both to her and to her people. "When a proposal goes somewhere these days, the group leader looks at it, I look at it, a program manager looks at it, and people from various of the associate directors' offices look at it. I'm not sure all that processing helps in the end—not that much. Now, I think policy-level decisions do need to be made and are crucially important: 'Are we going to work in this field, in that field, or in some other?' But once you've set the stage and hired good people, then you ought to let them do it."

Doing science.

Doing science to Darleane Hoffman can mean either basic or applied research, as long as the work is excellent. "In applied research projects you bring all your intelligence, all your knowledge, all the experimental data to bear on solving a problem—and that's stimulating. On the other hand, in basic research you are discovering fundamental truths of nature—which is very exciting.

"To my mind, one of my division's most important projects in the applied area has been nuclear waste isolation, because I happen to believe very strongly that we have to have nuclear power and that we will one day want retrievable storage. We began in 1977 with only a $30,000 part in the Nevada Nuclear Waste Storage Investigations. (It wasn't called that then; the name keeps changing.) When we got into it, it was still being regarded simply as an engineering problem. All you had to do was decide how big the shaft had to be and how big the tunnels had to be and how close you could place the spent fuel. There was not much thought being given to the possible breaching of the repository, possible leakage, or how the radioactivity that was released might interact with the geochemical environment. I feel our principal contribution has been to look at the interaction of the various nuclides in spent fuel or other nuclear waste with the geochemical environment and to try to devise ways for ascertaining what will happen to them under various conditions. We have methods for finding which elements will sorb under which conditions, which ones won't, and what forms they might be in,

Darleane Hoffman at the 88-inch cyclotron at Lawrence Berkeley Laboratory. With her is co-worker Diana Lee of Professor Seaborg's group. The wheel is the "MG" [merry-go-round] system, an "economy model" for measuring properties of short-lived spontaneously fissioning isotopes.

from minute details to gross calculations.* It's a problem we can solve and are solving, and I want to see us continue to do it—in an orderly fashion. We are beginning to amass the information to make people comfortable about storage. I'm a lot more optimistic about the common sense of the general public than many people are."

Darleane Hoffman vigorously supports, and takes part in, the fundamental research done by her division. Much of this work involves international funding and international cooperation. "One of the benefits I've greatly enjoyed throughout my career is the opportunity to meet and sometimes to work with scientists from many countries. The year Marvin and I spent in Norway in 1964-65 meant a great deal to me professionally and personally. Since then I've been able to visit and work with scientists from many countries."

Darleane cites as an example a collaborative effort of last March. She and others from Los Alamos, along with members of Glenn Seaborg's group at Lawrence Berkeley and a Swiss colleague, all traveled to the GSI [Gesellschaft für Schwerionenforschung] accelerator at Darmstadt. There they joined a host of Germans under Günter Herrmann, director of the Institute for Nuclear Chemistry at the University of Mainz. "Although the major portion of the effort was to try to produce superheavy elements via compound nucleus reactions (the complete fusion of projectile and target), we also measured yields of actinides produced in calcium-40 and -48 bombardments of curium-248. We provided our expertise in actinide separations and rare heavy-element target isotopes from the U.S. Transplutonium Production Program. The Germans helped fund our stay. In fact, Professor Herrmann's Institute has regularly supported several of our people there for three-month intervals."

Whether fulfilling her role as a scientist or her role as an administrator, Darleane Hoffman says she tries always to remember advice given by I. I. Rabi at a Los Alamos colloquium: "You need to know what is important to do. Otherwise you can work your whole life away on unimportant experiments. It's not a matter of knowledge or competence so much as the recognition of the important things to do."

Those who work with and for Darleane Hoffman reveal her special qualities in the anecdotes they tell.

"She's so dedicated she once ran a guard gate to get to an experiment. . . ."

"She'll do whatever needs doing. I've seen her shoveling snow from the sidewalk. . . ."

"Unlucky people are said to be snakebit. Well, one night last summer Darleane daintily skipped right past a rattlesnake coiled at her doorstep—that's the kind of luck she has. . . ."

But she is characterized most fully in the simple words Kurt Wolfsberg spoke when he introduced her at the award ceremony of the American Chemical Society: "Darleane is a friend, a colleague—and last and least—my boss." ■

**See "Migration of Radioisotopes in the Earth's Crust" in this issue.*

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Errata for the 40th anniversary issue (Number 7):

Since we are now labeling successive issues by sequential numbers, "Volume 4" should be deleted from the banner on page 1.

We apologize for the misspelling of "Bettmann" in the photo caption on page 41 and for the omission of credit, to Frederick H. Rick of the Laboratory's Public Affairs Office, for the photo on page 55.

The "unidentified guide" in the photo on page 48 has now been recognized as Livermore test director Phil Randolph.

The photograph on page 115 is not that of the Cockcroft-Walton accelerator requisitioned during the war from the University of Illinois but of one developed at the Laboratory about ten years later.

In the discussion on page 121, column 2, about measuring the cross section for fusion of tritium and deuterium, the roles of target and projectile were interchanged; tritium ions, not deuterons, were used to bombard a target of deuterated, not tritiated, water. The measurements were performed in 1945 rather than 1944, and the cross section for fusion of tritium and deuterium was found to be greater than that for fusion of deuterium and deuterium by as much as a factor of 100 rather than a factor of 10.

On page 161 the Mark 7 and the Mark 8 were misidentified; captions (b) and (c) should be interchanged. In caption (d) on the same page, the Mark 17 is incorrectly described as the first deliverable thermonuclear weapon; that distinction belongs instead to the Mark 14.