New Window on Biochemistry

An analytical tool long used by chemists now reveals complex, coordinated biochemical reactions of living cells

BY JULIE ANN MILLER

With flows and fluxes, cycles and shunts, cells process the chemicals of life. More than a thousand coordinated chemical reactions extract energy from foodstuffs and synthesize materials essential to a cell's functions. During the last century painstaking biochemical analyses have mapped arrays of enzyme-catalyzed reactions and produced complex charts of metabolic pathways. Like train cars on an extensive railway system, the atoms of molecules entering the system are uncoupled and redistributed according to what routes they follow, current demands and traffic along the other lines.

Simplification, then complication, is the research tactic that has been applied in these biochemical studies. Isolating an enzyme from cells and examining its performance under simple, controlled conditions is the strategy that first revealed the basic components of metabolism. But now biochemists are turning back to nature to describe the timing, control and interaction of the many cellular pathways. It is as if, after having examined a sample train traveling through each switching yard, scientists want to step back and observe the entire network in full motion.

An analytical tool long used by organic chemists to identify single compounds in pure solution is now coming to the forefront for viewing the coordinated chemical activity of cells. It is called nuclear magnetic resonance (NMR) spectroscopy and is based on the magnetic properties of atoms. The nuclei of some atoms—including hydrogen, phosphorus and a minor isotope of carbon — resemble miniature bar magnets; they will align along a strong magnetic field. When energy of radio frequencies is provided to the oriented nuclei, some will absorb enough energy to flip 180 degrees and reach the other stable position in the magnetic field. An NMR spectrophotometer graphs the energy absorbed at each radio frequency.

The profile that results is characteristic of a nucleus and also of its environment in a molecule. Organic chemists, who generally look at the energy absorbed by hydrogen nuclei, can distinguish, for instance, between a hydrogen attached to a carbon that is attached to an oxygen and a hydrogen attached to a carbon that is attached to other hydrogens.

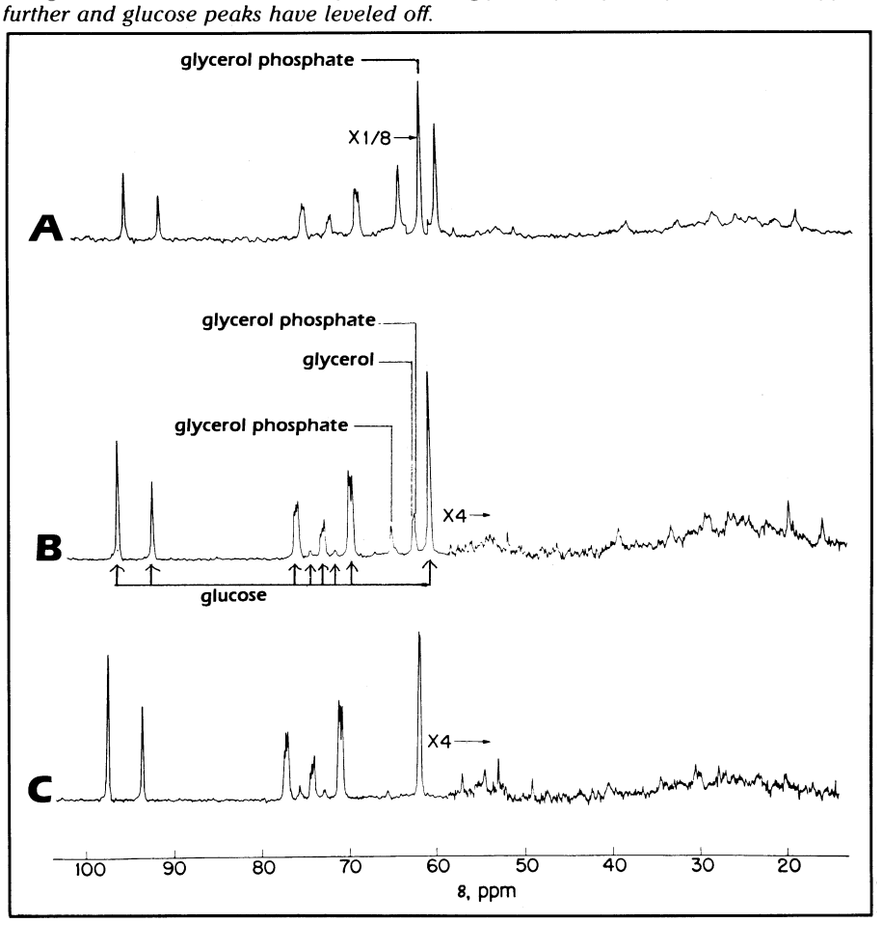
Recently the sensitivity of this organic chemistry technique has increased 100-fold. The improvement is due to use of superconducting magnets, which create a stronger magnetic field, and to more sophisticated techniques for data collection and analysis. Compounds inside cells, as well as chemicals in solution, can now be resolved into a tracing of peaks, dips, shoulders and valleys.

Biochemists using NMR spectroscopy examine the absorption of phosphate and carbon, instead of the hydrogen absorption spectrum most popular with organic chemists. The naturally occurring phosphate atom ^{31}P has the nuclear properties required to give a clear NMR signal. Because phosphate is a key atom in biological energy storage and release, NMR spectroscopy has been a valuable tool for examining economics of muscle contraction and deficiencies in muscle disease (SN: 1/29/77, p. 71). NMR spectroscopy with phosphate in rat liver cells provided the first direct measure of a pH difference between mitochondria and cytoplasm.

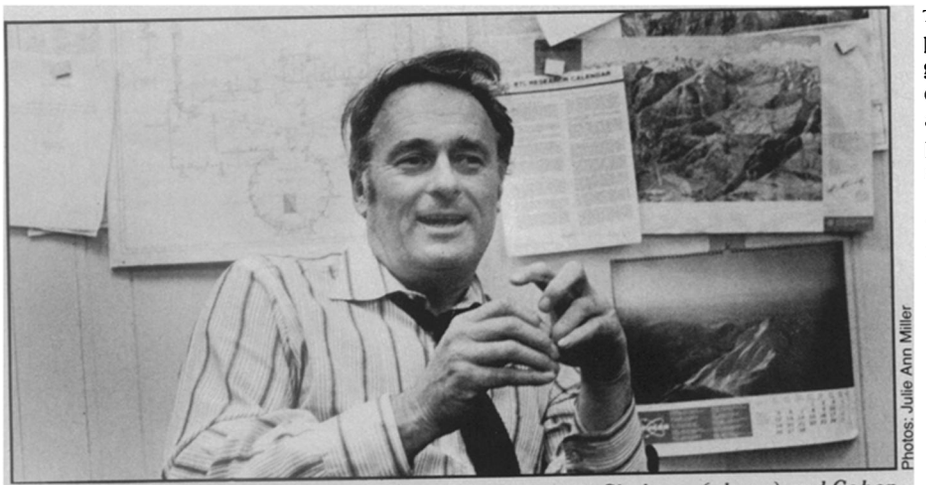
Carbon, however, is the backbone of metabolism; rearrangement of chains of carbon is the basic metabolic action. But because the most abundant form of carbon, ^{12}C, does not have magnetic characteristics suitable for NMR spectroscopy, a slightly heavier isotope, ^{13}C, is observed with the method. To follow a carbon atom in a living cell, chemists deliberately make a compound containing a high concentration of ^{13}C at one position and introduce the compound into cells. Reactions involving the ^{13}C-"enriched" position are apparent against an invisible background of reactions involving the many other carbons.

In concept, the approach resembles radioactive tagging techniques that have been widely used to study metabolism. When most biochemists want to observe the path of a carbon atom in a cell, they feed the cell a compound labeled with radioactive carbon, ^{14}C. After a time, they extract an array of carbon-containing compounds from the cell and locate the

Traces illustrate path of carbon atoms inside rat liver cells. The cells were "fed" glycerol containing ^{13}C in two positions. At first, glycerol was responsible for most of the NMR signal. (In trace A, the major peak is drawn one-eighth its true height.) After 35 minutes (trace B), the ^{13}C atoms are found less in glycerol and more in the intermediate glycerol phosphate and glucose. Trace C shows that by 85 minutes glycerol phosphate peaks have dropped further and glucose peaks have leveled off.



after Cohen, Ogawa and Shulman



Photos: Julie Ann Miller

By analyzing the jagged peaks and valleys of NMR spectra, Shulman (above) and Cohen (below) have tracked chemicals in cells and have come upon unexpected components.

radioactivity. Once the compound is identified, it still must be degraded to determine which of its carbon atoms is radioactive.

In contrast to the radioactivity tracing, scientists can follow atoms of ^{13}C without killing the cells. Thus, a carbon atom's journey through the metabolic network can be traced for hours. One research group in England is even beginning to look at the energy absorption by both carbon and phosphate atoms in the same sample — a strategy parallel to a double label experiment with radioactivity.

Robert G. Shulman of Bell Laboratories in Murray Hill, N.J., recounts, "We thought we were exaggerating in the past year or so when we said, 'Look, this one [NMR] spectrum gives you information that by normal chemical degradation and ^{14}C biochemistry would take two months.' But the last time I talked and showed a spectrum, a guy came up to me with a pained look on his face and said, 'You know how long it would take us to do that?' I said, 'A month, two months,' trying not to be insulting, and he said, 'A year.'"

Shulman and colleagues were the first biochemists to have a spectrophotometer with a superconducting magnet and are among NMR spectroscopy's most enthusiastic proponents. Although the superconducting spectrometers are expensive instruments, there are now several hundred in the world, and about a dozen laboratories are using them for studying the complex biochemistry of living cells.

The expense of the equipment is not the only barrier to biochemists contemplating tossing out their radioactivity detectors. "It's not as easy to get an NMR signal of a ^{13}C compound as to get radioactive counts from a ^{14}C-labeled compound," Shulman explains. The NMR technique is less sensitive, limited to compounds present at concentrations greater than one part in 100 million, and works only for molecules tumbling freely in solution or in a cell. Therefore, the researchers cannot record NMR signals from molecules embedded in membranes or from DNA, RNA and other molecules that are larger than a few thousand daltons in molecular weight.

But that leaves open a wide range of biological problems for study. Choosing which to investigate is a major dilemma.

In a typical experiment using NMR spectroscopy, a dense suspension of cells is placed in a clear tube in the spectrometer. Shulman says that the cells comprise about a quarter of the suspension volume. Thus a cubic centimeter of sample contains 100 million liver cells or 100 billion bacteria. After the carbon compound enriched with ^{13}C is added to the suspension, its absorption is measured repeatedly. Each observation takes 1 to 20 minutes.

The scientists analyze the jagged profile traced by the spectrometer. They compare the position of peaks with the profiles of known compounds. The library of spectra — actually a packet of microfiche — includes about 10,000 carbon compounds studied by organic chemists. Even so, in the course of their studies, Sheila M. Cohen, who works with Shulman, has had to identify profiles of compounds not previously cataloged.

One of the greatest strengths of the technique is that it allows scientists to find unexpected components of the system under study. "The NMR method has the advantage of simultaneously detecting all metabolites that have been adequately labeled," Cohen, S. Ogawa and Shulman say in the April PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. For example, in studying the effect of a hormone on glucose production in rat liver cells, they discovered a simultaneous increase in amino acids being made by a different process. "To some extent you are at the mercy of the system," Shulman says.

Shulman's work has concentrated on the pathways of glucose breakdown in different cells. The pathway is used to make ATP for heart muscle and, in the absence of oxygen, for skeletal muscle, and provides most of the energy for many microorganisms. That pathway was the first sequence to be completely charted by studies of the test-tube interaction of pure enzymes, substrates and cofactors. With ^{13}C NMR, the biochemists have observed the path of carbon atoms from glucose through numerous intermediates, as well as the end products. In addition, they have observed actual rates of reaction and the distribution of material between different pathways.

The effect of pH on the rate of the glucose breakdown reactions is another new observation made with the NMR technique. Researchers had previously measured the pH dependence of individual enzymes, but no one had measured simultaneously the pH of cells and the reaction rates, Shulman says. Making those measurements in the bacteria *Escherichia coli*, he and collaborators find that as the cell becomes more acidic (pH below 7.2), the glucose breakdown rate drops to a quarter of its maximum. The researchers are now examining other cell types; Shulman expects pH to be a general method for a cell to control metabolic rates.

The breakdown and production of glucose are the trunklines of cellular biochemistry, but NMR spectroscopy is also charting reactions in the outer districts of the metabolic map. A. Ian Scott of Texas A and M University is examining cells' patterns of synthesizing some complicated molecules. For example, he, Gerardo Burton and Paul E. Fagerness applied NMR technique to molecules on the metabolic pathways leading to heme, chlorophyll and vitamin B_{12}. The results indicated reaction intermediates that had not been detected previously. Scott expects practical benefits to accrue from identification of such fleeting go-betweens. He and colleagues hope to map in detail how cells synthesize penicillin and other antibiotics. That information may allow scientists to boost cells' productivity in pharmaceutical production.

The biggest problem to the scientists now is just what questions in the wide array of biochemistry to examine first with the powerful new technique. They make decisions, Shulman says, by self-examination, continual questioning and self-doubt. "We go around all the time saying, 'Is this the right thing to do?'" he says. "We may not be doing the right thing, but we can't think of any 'righter' thing to be doing — right now." □