Synchrotron Radiation (I): A Light for All Seasons

Synchrotron radiation is the light given off by a charged particle orbiting in a curved trajectory. Although scientists have used the ultraviolet radiation emitted by electrons in synchrotrons and storage rings for at least 15 years, the recent availability of synchrotron radiation in the x-ray region of the spectrum has spurred an increasing interest in the use of such radiation for all manners of spectroscopy in atoms, molecules, solids, and biological materials, and for x-ray diffraction. Researchers confidently predict that altogether new types of experiments or those previously possible only with great difficulty will result from the use of synchrotron radiation, a light source second only to the laser in its brightness, but which has many features that lasers do not yet possess.

Synchrotron radiation is not exactly a novel phenomenon. Understanding why orbiting electrons in atoms did not emit such radiation was one of the major steps in the development of quantum mechanics. Synchrotron radiation from electrons spiraling around magnetic fields in space has figured strongly in astrophysicists' models of stars and galaxies. And for the electron accelerator designer, synchrotron radiations had been more and more of a nuisance as the energies of electrons in synchrotrons and storage rings increased because the energy lost in emitting synchrotron radiation (proportional to the fourth power of the electron energy) must be replenished and because water cooling must be provided to accommodate the hundreds of kilowatts dissipated in high energy storage rings. Since the power radiated is also proportional to the inverse fourth power of the particle's rest mass, synchrotron radiation is negligible in proton accelerators.

The success of the Stanford Synchrotron Radiation Project (SSRP) which opened a year and a half ago at the Stanford Linear Accelerator Center (SLAC), as well as a demonstrable commitment on the part of overseas governments to support research with synchrotron radiation, seems to have given a strong boost to American scientists' interest in using this radiation. One reason for this interest is that, as the energy of the electrons increases, they emit shorter and shorter wavelength synchrotron radiation. The radiation extends over a continuous spectrum from this minimum wavelength to a wavelength corresponding to the orbital frequency of the electron in the accelerator (about 1 megahertz). The most intense and thus the most useful radiation, however, ranges in wavelength from the ultraviolet (4000 angstroms or shorter) to the so-called hard x-rays (1 angstrom or less).

The SPEAR electron-positron storage ring at SLAC stores electrons up to nearly 4 Gev, and the shortest useful wavelength of the synchrotron radiation is about 0.3 angstrom. An older storage ring (Tantalus I) at the University of Wisconsin, Stoughton, in contrast, stores electrons at a maximum of 240 Mev, and only wavelengths of 40 angstroms or longer are obtainable in useful intensities. Not surprisingly, then, x-ray studies occupy three of the five experimental stations available at SSRP, and new facilities now under construction there will be devoted entirely to x-rays.

Researchers at Stanford also point to the usefulness of the radiation from their high energy storage ring. Storage rings are more desirable sources of synchrotron radiation than synchrotrons because they emit more intense radiation and because the beam, which circulates for many hours at a constant energy in a storage ring, is much more stable than that in a synchrotron, where the electrons are injected, accelerated, and extracted 60 or more times a second. With the exception of the short-lived Cambridge Electron Accelerator, previous sources of synchrotron radiation in the x-ray region were all synchrotrons.

An Intense, Tunable Light Source

As one example of the usefulness of storage rings, researchers at Tantalus I and at the newly constructed, low energy SURF II storage ring at the National Bureau of Standards, Gaithersburg, Maryland, have used synchrotron radiation as a standard light source for calibrating atmospheric and satellite radiation detectors.

Several properties of synchrotron radiation make it an exceptionally useful light source, especially its high intensity and its tunability over the entire range of wavelengths emitted. Other types of sources cannot match the intensity of synchrotron radiation in the ultraviolet and x-ray regions of the spectrum; there are as yet no working x-ray lasers, and the few ultraviolet lasers operate only at certain discrete wavelengths or have low intensities. (In the visible, however, tunable lasers are available whose intensities easily exceed that of synchrotron radiation.)

One advantage of high intensities is that researchers can accumulate high-quality experimental data very quickly, in some cases with very simple instrumentation. For example, Keith Hodgson of Stanford University reports that, in x-ray absorption experiments with metalloproteins (enzyme proteins that contain metallic elements as parts of their essential structure), his group can obtain much higher quality data from SPEAR and about 50,000 times faster than it can with the use of the continuous background radiation from a conventional x-ray tube. Other researchers have a rule of thumb that 1 hour at SSRP is equivalent to 1 week in the laboratory.

Hodgson, James C. Phillips, and Alexander Wlodawer at Stanford have also been able to take x-ray diffraction data on crystals of such proteins as azurin, nerve growth factor, and L-glutaminase-asparaginase with the use of a tunable, focusing x-ray diffraction camera built for SSRP by a group from the California Institute of Technology headed by John Baldeschwieler. Normally such measurements require relatively large single crystals of the protein, but because of the high intensity of x-rays from SPEAR the Stanford researchers could make do with considerably smaller crystals.

The high intensity of synchrotron radiation structure will soon permit scientists to follow the diffraction patterns of biological samples with time and thus observe structural changes such as that in muscles during contraction. Such studies are now under way with the synchrotrons at the DESY laboratory in Hamburg, West Germany, and at Daresbury, England. And the Caltech investigators plan to use a similar technique to observe the change in structure of rhodopsin when it is irradiated with visible light. Rhodopsin is a pigment in the retinal rods (structures responsible for vision in dim light), which is rapidly depleted when subjected to bright light.

The high intensity of synchrotron radiation in the x-ray regions has also enabled researchers to do x-ray microscopy. Eberhard Spiller, Ralph Feder, and their coworkers at the IBM research center in Yorktown Heights, New York, have made x-ray micrographs of biological objects with a resolution of better than 1000 angstroms. The investigators, who first carried out their studies at DESY and have continued them at Tantalus I in Wisconsin, used an x-ray photolithographic technique to make impressions of the sample in an

organic polymer material and a scanning electron microscope to image the impression. Exposure times were reduced by factors of 100 or more as compared with the use of conventional sources of soft x-rays.

Synchrotron radiation can be tuned to any desired wavelength with the use of a monochromator, and this second feature of the radiation is at least as important to the researcher as the intensity. Scientists may be able to use the tunability of synchrotron radiation to more easily solve one of the long-standing difficulties in determining the structures of such complicated biological materials as proteins by x-ray diffraction. In order to solve these structures from experimental diffraction data, investigators must obtain both the intensities and the phases of the x-rays forming a diffraction pattern. A cumbersome technique known as heavy atom ismorphous replacement (*Science*, 6 December 1974, p. 913) must be used to derive the phase information. This method involves successively substituting several heavy atoms into the protein and making diffraction pictures for each substitution. The number of such substitutions can be reduced to one with the use of the phenomenon of anomalous scattering. In brief, by using the ability to select the wavelength of the synchrotron radiation in order to take diffraction data on both sides of an x-ray diffraction edge, researchers can deduce the phase angles from the differences in the diffraction patterns. A number of researchers are planning such experiments on metalloproteins.

Spectroscopy of Solid Surfaces

Investigators using the ultraviolet light from Tantalus I and from many other sources have long since established the value of the tunability of synchrotron radiation. In photoelectron spectroscopy, for example, researchers irradiate a sample with ultraviolet or x-ray photons and measure the kinetic energy of the photoelectrons that are emitted in order to learn the details of the electron quantum states. Because of certain scattering processes undergone by an electron migrating toward the sample surface, the photoelectrons tend to come predominantly either from the bulk or from the surface, depending upon the incident photon wavelength. Thus, by varying the photon wavelength, researchers can study bulk and surface electronic states separately.

William Spicer, Ingolf Lindau, Piero Pianetta, and Charles Garner at Stanford have used this technique to study the surface of gallium arsenide (a commercially important semiconductor) when it is exposed to oxygen. These investigators used a special monochromator built for SSRP by Frederick Brown of the University of Illinois, Robert Bachrach of the Xerox Palo Alto Research Center in California, and their co-workers. The Stanford scientists found that the electronic roles of gallium and arsenic are reversed on the surface, as compared to the behavior of atoms in the interior. This result may have implications for engineers trying to adapt silicon integrated circuit technology to gallium arsenide.

The high intensity and tunability of synchrotron radiation has contributed to a surge of interest in one promising technique that heretofore was relatively unused—extended x-ray absorption fine structure. In fact, the earliest results of great interest to researchers obtained at SSRP came from EXAFS, which is the name for the small oscillatory structure in the absorption spectra of x-rays that extends for several hundred electron volts above the x-ray absorption edge. Although the phenomenon was known to physicists for decades, interest in it lagged until 1971, when Dale Sayers and Edward Stern of the University of Washington, Seattle, and Farrell Lytle of the Boeing Company proposed that it was possible to convert these oscillations into the radial positions of neighbor atoms nearest to the atom absorbing the x-ray.

The basic reason for the oscillations in the absorption spectra is that the neighboring atoms scatter the photoelectron kicked out of an absorbing atom by an x-ray. When the photoelectron is scattered back toward the atom from which it came, a quantum mechanical interference effect comes into play which modulates the probability for the absorption of an x-ray. Since the interference depends on the radial location of the scattering atoms, the oscillations in the absorption spectra can be translated into radial positions. Theoretical treatments of this problem have been given by Stern, by Christopher Ashley and Sebastian Doniach of Stanford, and by Patrick Lee of Bell Laboratories, Murray Hill, New Jersey, and John Pendry of the University of Cambridge. Nonetheless, interpretation of EXAFS spectra is still regarded as a complex problem, and use of EXAFS is far from routine.

The EXAFS method is complementary to x-ray diffraction in that it determines only the local atomic environment around a specific constituent of a complex molecule or solid. Thus it would not be used to determine crystal structures. Sayers notes that distances out to perhaps the fifth or sixth shell of atoms can be obtained. EXAFS does not require single crystals, and thus such materials as biomolecules that are difficult to crystallize and noncrystalline solids are prime subjects for study. And in those biological materials that can be crystallized, points out Peter Eisenberger of Bell Labs, crystallographers doing x-ray diffraction can use information on local atomic arrangements obtained from EXAFS as a constraint which their models must fit.

One of the difficulties that limits the accuracy of the positions of atoms determined by EXAFS (typically 0.1 angstrom, but 0.01 angstrom in the best cases) is a quantity called a phase shift. This phase change manifests itself by shifting the apparent atomic positions relative to their true positions when a technique such as Fourier transforming or curve fitting is used to analyze the EXAFS spectra. Eisenberger, Paul Citrin, and Brian Kincaid (formerly of Stanford) have shown, however, that these phase shifts for a particular absorbing-scattering pair of atoms are often independent of the local chemical environment. Thus, investigators can measure the phase shifts in simple, model systems in which the atomic positions are known, and then use these phase shifts in more complex systems in which the atomic positions are unknown.

Looking at Dilute Samples

Eisenberger, Robert Schulman, and William Blumberg (who used an x-ray monochromator built for SSRP by Kincaid, Eisenberger, and Sayers) have applied this method to measuring the length of the iron-sulfur bond in rubredoxin, a small metalloprotein, with a molecular weight of about 6000, that occurs in bacteria. There are four such bonds in one protein molecule. The Bell Labs investigators found that, contrary to earlier evidence from x-ray diffraction that one bond was shorter than the others by 0.25 angstrom, all the iron-sulfur bonds were the same length to within 0.1 angstrom. They obtained this result both from samples in crystalline form and in solution. The University of Washington group has independently obtained the same result in their EXAFS studies.

In EXAFS, separate spectra exist for each chemical element that absorbs x-rays in the sample, thus the local environment of each element can be probed separately. Moreover, this selective focusing on one element means that EXAFS is ideal for studying elements present in only small concentrations in a sample. Lytle, for example, has been studying supported metal catalysts which are important in the oil industry (*Science*, 30 August 1974, p. 772). These catalysts consist of small particles of metal, such as platinum, on a porous ceramic support, such as alumina. The metal

is present in quantities of from 0.1 to 0.3 percent by weight.

For very dilute systems, however, the absorption due to the small number of atoms in a matrix of other atoms may appear as only a small perturbation against a large background. Researchers have increased the sensitivity of the EXAFS method by a factor of 100 to 1000 by measuring x-ray fluorescence instead of absorption. In this way, the only signal comes from the specific atoms being studied. The Bell researchers have used this technique to look at iron in a dilute solution of hemoglobin with about one iron atom for every million of other atoms. And Melvin Klein and his colleagues at the University of California, Berkeley, used the same technique to study molybdenum in the nitrogenase enzyme system. Nitrogenase enzyme is a component of the nitrifying bacteria that convert nitrogen from the atmosphere into ammonia. Few of these EXAFS studies would be practical without synchrotron radiation as a source.

A third feature of synchrotron radiation is the pulsed nature of the light. At SPEAR, for example, the pulses are 0.3 nanosecond wide and are separated by 780 nanoseconds. The pulsed nature of the radiation results because the electrons in a storage ring or synchrotron travel in bunches rather than in a continuous beam. This feature has been largely unexploited as yet, although Brown, Bachrach, and their colleagues have been able to use the pulsed time structure of the synchrotron radiation in their photoemission experiments. Nor-

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Neurochemistry: Unraveling the Mechanism of Memory

How a brain stores information has long been a puzzling problem, but in recent years advances in neurochemical techniques have provided scientists with some tools for exploring the small chemical events that occur when an individual learns something. Although the process of memory storage is still far from clear, many feel that it involves establishment of new nerve pathways or selective strengthening of old ones, probably within certain brain areas.

The field, however, is in some disarray; claims lie unproven and unrefuted, and disagreement as to the most fruitful approaches is common. Nonetheless, research into the mechanisms of memory continues to expand in scope and in importance. Because anatomical evidence is practically nonexistent and very difficult to obtain, the emphasis has been on the neurochemistry of memory. The central idea seems to be that the formation of new pathways is accomplished in part by neurochemical events, and it is the nature, location, and sequence of these events, particularly the synthesis of RNA and protein, that is the subject of many investigations.

In order to determine what changes result from learning, it is necessary to compare an animal that has learned a task to one that has not. One difficulty in making this seemingly simple comparison lies in the fact that any training experience will expose the animal to stressful situations involving such stimuli as flashing lights, shocks, handling, exercise, and frustration. Controlling for these other variables is particularly important because many of them appear to have effects on brain biochemistry similar to those believed to occur when an animal learns. Indeed, many believe that it may be impossible to design appropriate controls for these variables.

Another difficulty is that scientists do not always agree on what should be included under the term "learning." The traditional definition, modification of behavior by experience, is very vague, and might include behavior changes that may be primarily developmental. Some scientists argue that there may be different types of learning, each of which initiates different biochemical events in the nervous system.

A further problem is that the biochemistry of the brain is tremendously complex. It has recently become clear that it is very difficult, even with the techniques not available, to determine precisely what biochemical events occur during learning, and more difficult still to relate those events to memory storage. The enormous problems encountered by investigators in this field have led to the widespread belief that no easy solutions are forthcoming, but the consistent findings of many laboratories using many different kinds of learning situations suggests that progress is being made.

One of the groups that supports a broader definition of learning as almost any behavior modified by experience is a brain research group in England, including Steven Rose of Open University, Pat Bateson of Cambridge University, and Gabriel Horn of the University of Bristol. They have been investigating the biochemical changes associated with imprinting in chicks. A chick "imprints" upon, or learns to follow, its mother when it is exposed to her as a moving stimulus during a sensitive period of development within the first few days of life. Many stimuli, such as moving objects or flashing lights, can substitute for the mother, and the chick will imprint upon the substitute. Rose's group and others believe that imprinting can be considered a specific case of a more general phenomenon of learning, and thus that the neurochemical events which are triggered by exposure to an imprinting stimulus will be similar to those triggered whenever an animal learns something new.

Hypothesizing that increased RNA synthesis is associated with imprinting, Rose's group injected chicks with an isotopically labeled precursor for RNA after exposing them to an imprinting stimulus, a flashing light. Some of this labeled precursor would be used to synthesize new RNA. Later, when they extracted the RNA from the brain tissue, the amount of radioactivity incorporated into the RNA, relative to the free radioactivity, provided an estimate of how rapidly the cells were synthesizing the precursor into new RNA. The RNA from portions of the imprinted chicks' brains contained more radioactivity than that extracted either from birds exposed to plain light or from birds kept in total darkness, which suggests an increase in RNA synthesis.

Rose's group has further used this technique to investigate changes in protein metabolism. Imprinted birds incorporated more radioactive lysine into protein than did control birds, which suggests an increase in protein synthesis. This increase was observed in the same area of the brain in which the changes in RNA occurred. These results support the hypothesis that learning initiates RNA and protein synthesis.

The conclusions which can be drawn from any studies using the radioactive precursor technique are limited, however. One of the limitations is that it is very difficult to be certain that the labeled precursor is distributed evenly to all the metabolic compartments of the cells and throughout the tissue when it is injected into the animal. If the distribution is different because of the training—for example, because of changes in blood flow—then the proportion of radioactivity present in extracted fractions may be more a function of where the precursor tended to concentrate rather than where high rates of synthesis were under way. In addition, these effects might mean a change in the rate at which RNA is degraded rather than in the rate at which it

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mally researchers obtain a photoemission spectrum by scanning across a range of photoelectron kinetic energies with an electronic detector; that is, the detector is set at a particular energy and the number of photoelectrons with that energy is counted. Then the detector is reset to a higher energy, and the process is repeated. But, because of the long time between pulses at SPEAR, a method called "time of flight spectroscopy" can be used in which the time it takes a photoelectron to reach the detector can be converted into its kinetic energy. This method avoids the need for scanning the photoelectron energy, and is faster because all the photoelectrons are counted.

Other properties of synchrotron radiation include natural collimation of the light beam, polarization of the light, and a high vacuum environment. The light comes tangentially off the electron's orbit in a narrow cone. For a 2.5-Gev electron, for example, the apex angle of the cone is 2×10^{-4} radian, but because the electrons emit light continuously while orbiting, an observer sees a horizontal fan of radiation with strong vertical collimation. This property permits scientists to obtain bright light beams a considerable distance from the electron beam, a condition necessitated by considerations such as shielding from x-rays.

The extent of the polarization of the radiation depends on its wavelength and its position relative to the plane of the orbit. At the wavelength of maximum intensity and on the orbital plane, the polarization is nearly 100 percent with the electric vector of the light in the plane of the orbit. This polarization can be used to deduce the symmetry of quantum states in solids and to study the structure of anisotropic materials.

Storage rings must hold electron and positron beams for several hours. In order to avoid collisions between the electrons or positrons and residual gas molecules, the storage rings are evacuated to better than 10^{-9} torr. This high vacuum is ideal for certain types of experiments, such as those designed for studying solid surfaces that are free of contamination. The vacuum in synchrotrons can be much less (10^{-6} torr) because of the short lifetime of the beam.

For now, at least, researchers' enthusiasm about the future of synchrotron radiation is at a high level. Despite the fact that the average x-ray experiment at SSRP takes only a few hours, the facility has well over a year's backlog of experiments awaiting their chance at the synchrotron radiation beam.—ARTHUR L. ROBINSON