size was not "the result of a careful study in which someone found a curve with a dip in it," but a reaction to practical limiting factors. The administrator who is in charge of evaluating the competing design studies, Alan Skinrood at the Sandia Laboratories in Livermore, California, says that it is "fairly clear" that the optimum plant size for the United States is 50 to 200 Mwe.

The outcome of the design competition may shed more light on the thinking of those in the ERDA program, because one design (Martin Marietta) has a maximum modular size of 10 Mwe. The Martin scheme would be to build up a 100-Mwe plant from ten or more modules. Its technically limiting feature is the use of a narrow-angle cavity-type receiver in a north-facing tower.

Whatever the outcome of the aerospace competition, it is clear that the costs of power tower systems are far too high just now. The present price of collectors is about ten times the $70 price ERDA set as a goal, and the total pilot plant costs—which will be over $10,000 per kilowatt for the Barstow facility—are in excess of those in other energy technologies that have reached a similar stage of development. The ERDA plan is to reduce these costs—particularly for heliostats—by bulk manufacturing techniques and steadily improved designs.

But the economies of scale presumed to be possible with large power towers could prove illusory, and the benefits of systems on smaller scales may be overlooked and forgotten by the time the final answer to the power tower is known. In particular, the rule of thumb that "energy transport by light is more economical than by heat" may only be true for large systems, The heat losses in piping depend on the average distance in the heat transport system, so the economics of distributed collectors connected to a generator by heat (rather than light) transport should cross over and become favorable at some point. For much the same reason, total energy systems should be preferable on small scales. But the energy agency appears to be supporting such projects principally as a backup in case the power tower project should fail.

The history of the nuclear development program offers some pointed lessons in the dangers of overconcentration of effort on too few technologies. In a huge development program, the ideas of talented workers may be wasted because of the necessity of working within rigid management structures on programs with externally imposed goals. For a number of technologies there is very little choice. But for solar energy, even for the specific purpose of converting solar energy to electricity via thermal systems, there are many choices and new inventions are appearing rapidly. It would appear to be far too soon for the solar program to be discarding innovative options and sinking its research money into steel and concrete.

—WILLIAM D. METZ

Electron Probe Microanalysis: New Uses in Physiology

Elemental analysis can be performed on samples that are orders of magnitude smaller than samples that can be analyzed by conventional techniques. Elements within substructures of cells can be identified and quantified. Ionic movements within cells and across cellular membranes can be studied with ease. These are but a few of the many problems that can be solved with electron probe microanalysis (EPM), a 25-year-old technique that has only recently surfaced in biology.

EPM, also known as x-ray microanalysis or analytical electron microscopy, was developed by Raymond Castaing of the University of Paris in 1951 and its use was quickly adopted in metallurgy and mineralogy. Theodore A. Hall of Cambridge University pioneered in the use of EPM for study of hard biological samples, such as bone, but preparation of other types of biological samples proved much more difficult. Within the last 5 years, though, preparation of liquid samples has become almost routine, and preparation of tissue samples is nearing that stage, so that EPM is beginning to have significant application in biology. The results obtained thus far have been so impressive that many scientists now feel that EPM may be more valuable to physiology than electron microscopy has been to anatomy.

The heart of an EPM spectrometer is an electron beam with which the sample is bombarded. When an individual atom is struck by the beam, one of its inner-shell electrons is boosted into an outer shell of higher energy. Relaxation of the atom—refilling the inner-shell vacancy—is accompanied by either emission of a low-energy Auger electron or emission of x-irradiation at a wavelength characteristic of the atomic number of the element. Heavier elements are more likely to emit x-irradiation, whereas lighter elements are more likely to emit Auger electrons. The x-irradiation is collected for analysis of the sample in EPM, but elements lighter than magnesium emit so little radiation that they can be studied only with difficulty. Elements lighter than boron have no outer electron shells, and thus cannot be studied at all.

For many applications, the emitted x-irradiation is collected with what is known as a wavelength-dispersive spectrometer. In this device, each wavelength of interest is diffracted onto the detector by an appropriate crystal; several such crystals can be mounted on the probe simultaneously. The intensity of the radiation that reaches the detector is directly proportional to the quantity of the element in the sample. The characteristic wavelengths of the elements, furthermore, are generally well separated, and there is little overlap. It is thus possible to identify and quantify each element unambiguously. In most cases, it is possible to analyze a sample for as many different elements as desired.

The electron beam itself can be focused into a very small circle; routinely, a diameter of 50 Å can be obtained but, with special equipment, the diameter can be as small as 3 Å. It is thus possible to analyze small samples or small areas of a sample. The EPM spectrometer is frequently attached to an electron microscope, so that the user is able to identify visually the precise portion of the sample that is in the beam. Visualization can also be achieved with a light microscope. A good example of the wavelength-dispersive EPM spectrometer can be found in the Biotechnology Resource in Electron Probe Microanalysis at Harvard University. The Harvard facility, headed by Claude P. Lechene, was built to demonstrate the utility of EPM for studying biological materials.

Biological samples can be divided into two major categories—liquid droplets and intact cells or tissues. The easier of these to study is liquid droplets. With EPM, elemental analysis can be performed routinely on liquid droplets as small as 10 picoliters (10^{-12} liter) provided that the concentration of the desired element is at least 100 parts per mil-

lion. In absolute terms, this means that the minimum number of atoms of the desired element must be about 1.8×10^9; with sodium, for example, this corresponds to a mass of about 7×10^{-14} gram.

In practice, picoliter volumes are obtained with micropipettes controlled under a stereomicroscope with a micromanipulator. The liquid droplets are then deposited in precise positions on a support of highly purified beryllium and freeze-dried. Because the spots produced in this manner are geometrically comparable to each other, characteristic x-ray intensities can be compared from spot to spot. The intensities are proportional to the total amount of the element present in the dry sample, and thus to its concentration in the initial volume of liquid. Absolute concentrations can be obtained by comparing the sample counts to those from samples of known composition. The Harvard instrument has been completely automated so that it positions each sample under the electron probe, controls and monitors the electron beam current, and collects and processes all the data. Thus a large number of samples can be processed in a short time.

The chief advantage of EPM for liquid droplets is that elemental analysis can be performed on samples that are at least three orders of magnitude smaller than the smallest samples that can be studied with conventional ultramicroanalytical procedures. Many different elements can be analyzed, furthermore, whereas conventional techniques can be used for only one or two. One example of the application of the technique is a study by Lechene and Melvin L. Taymor of the Harvard Medical School of the elemental content of fluid from the follicles of human ovaries.

In previous studies with conventional techniques, it was possible to determine the concentration of sodium, potassium, and chlorine in follicular fluid from human and bovine ovaries, but this analysis could be achieved only with great difficulty. The two studies in which these elements were measured gave disparate results. One study, in humans, indicated that the concentration of the three elements in follicular fluid is about the same as their concentration in blood plasma; the second study, in cows, indicated that the concentrations in follicular fluid and blood plasma are different.

Lechene and Taymor determined the concentrations of seven elements—sodium, potassium, calcium, chlorine, magnesium, phosphorus, and sulfur—in a large number of samples from patients in various phases of the menstrual cycle. Their results showed that the concentrations of the elements are independent of the stage of the cycle and that they are very similar to the concentrations in plasma. This information should be valuable for, among other things, the development of a suitable culture medium for human oocytes.

The study of liquid droplets, Lechene says, shows great potential in many other areas of physiology. In reproduction research, for example, EPM makes possible the complete analysis of the fluids surrounding the ovum before discharge, during transit through the fallopian tubes, and after implantation. It can also be used to analyze the composition of the embryo. In renal research, EPM is being used to study the movement of ions within the kidney, the site and mechanism of action of kidney hormones, and specific methods of ion transport. Similar investigations are being conducted in many other organ systems.

Tissue Preparation More Difficult

The analysis of cells and tissues is much more difficult than that of liquid droplets because there are not yet standardized methods to prepare the samples for spectrometry. The lack of such methods, in fact, is one of the principal reasons why biological applications of EPM have lagged. The crucial problem in preparation of these samples is prevention of the displacement of dissolved elements during the procedure. Conventional preparative techniques such as fixation, staining, and embedding in polymers are thus generally not suitable because each involves a translocation of diffusible elements. The exact technique to be used depends on the nature of the sample.

Individual cells, such as erythrocytes and leukocytes, must be mounted in such a way that they are separate from each other, maintain their ionic content without any leakage or redistribution, and are not covered by ionic species from the suspending medium. One way that these conditions can be met, Lechene says, is to spray the cells onto a support by means of a jet of nitrogen gas. Perhaps a better way, developed by Raymond Kaufmann of the University of Dusseldorf, is to freeze the cells rapidly and maintain them frozen, but hydrated, in the electron probe. Studies with both techniques have made it possible, for instance, to quantify various elements in healthy blood cells. The investigators are now studying cells in diseased states to see whether they can detect differences that might be used to screen the diseases and to understand the underlying pathological processes.

The problem becomes much more difficult for the study of tissues because of the necessity to prevent translocation of diffusible elements between cells and between the cell and intercellular fluids. The solution to this problem depends, in part, on what element or elements are being studied. James R. Coleman, Ronald R. Warner, and Bernard Halloran of the University of Rochester School of Medicine have demonstrated that if only calcium analysis is required, for example, it is possible to precipitate the element onto the cellular matrix so that it can no longer be moved from its original location. Cellular water can then be removed with conventional techniques.

In this manner, Coleman and his associates have been able to study calcium flux in intestinal tissues and in model systems such as the chorioallantoic membrane in chick embryos. They have also made use of another facet of EPM. By operating the electron beam in a scanning mode and imaging only with x-irradiation characteristic of calcium, they are able to plot the distribution of calcium in the sample. (This can be done for any element if it is present in moderate to high concentrations.) Comparison of this image with that obtained by conventional electron microscopy, makes it possible to correlate calcium distribution with physical characteristics.

Coleman and his colleagues have in this way found, among other things, that only a small fraction of the cells in intestinal tissues actively transport calcium at any one time. These cells, furthermore, contain much more calcium than was previously thought. They have also shown that the calcium is sequestered within the cells, apparently in association with a protein. They are now investigating whether this protein plays a role in calcium absorption. Coleman and Jytte R. Nilsson of the University of Copenhagen have also studied calcium-rich lipid droplets in the protozoan *Tetrahymena pyriformis* and have used their results to develop a new hypothesis for biological control of intracellular calcium concentrations.

If it is necessary to study more than one element, precipitation is generally not possible. In this case, the best results are generally obtained by freezing the sample rapidly (to prevent formation of large crystals of pure water) and then either drying the sample or maintaining it in a frozen-hydrated state. Freeze-drying seems to work best when there is some type of intercellular matrix to limit translocation of dissolved elements. In the ab-

sence of such a matrix, it is generally necessary to keep the sample hydrated at temperatures below −130°C. At this temperature, the sample can be handled much like a rock.

Avril V. Somlyo, Henry Shuman, and Andrew P. Somlyo of the University of Pennsylvania have found that the best results are obtained in many cases by chilling the sample rapidly with supercooled chlorofluoromethane. The sample is then cut into ultrathin sections (about 100 nm thick) at −110°C and dried slowly at temperatures below −80°C. Muscle tissue prepared this way, they have found, retains both cellular ultrastructure and the original distribution of diffusible ions.

Shuman and the Somlyos have used this approach in many studies of muscle. They have, for example, determined the ionic composition of various portions of the sarcoplasmic reticulum of striated muscle, determined the distribution of chlorine throughout the muscle, and looked for intracellular compartments that had been thought to have an ionic composition similar to that of the extracellular space. They found such compartments in muscle incubated in solutions containing high concentrations of ions, but not in normal tissues.

Somlyo and his colleagues also observed that there is no compartmentalization of chlorine and sodium in the sarcoplasmic reticulum or in other cellular organelles, as had been suggested by bulk chemical analyses. And in smooth muscles from blood vessels they have measured the concentration of calcium in mitochondria and the sarcoplasmic reticulum, and have shown that the distribution of chlorine is not passive but is controlled by the cells. These findings, which should contribute greatly to the understanding of muscle physiology, could not have been obtained with conventional techniques, Somlyo emphasizes.

These results illustrate still another facet of EPM. Because the ultrathin sections produce only very small quantities of x-rays, Somlyo and his colleagues used a more sensitive detector known as an energy-dispersive spectrometer. This device, which many investigators believe will be the most used detector in the future, incorporates Si(Li) detectors that collect x-irradiation over a much larger solid angle than do crystals; they collect as much as 1 percent of the emitted radiation, compared to less than 0.01 percent for wavelength-dispersive spectrometers. The detectors collect radiation of all wavelengths simultaneously and convert it into electronic pulses whose intensities are inversely proportional to the wavelengths.

The energy-dispersive spectrometer introduces a 100- to 1000-fold increase in sensitivity in EPM. Somlyo and his colleagues have, for example, used it to detect the approximately 5000 iron atoms in the core of a single ferritin molecule, which corresponds to a mass of about 5×10^{-19} g of iron. With further improvements, Somlyo argues, it should be possible with an energy-dispersive spectrometer to detect about 10^{-22} g, or the equivalent of two atoms of iron.

Energy-dispersive spectrometry is not without its problems. The most severe of these is the low peak-to-background ratio that occurs because the detector picks up x-rays emitted when scattered electrons strike the specimen holder and other parts of the instrument. Furthermore, the Si(Li) detectors have a low energy resolution; in practical terms, this means that the signals for elements of low atomic weight overlap. These problems can be largely overcome, however, with a new computer program for data analysis adapted by Somlyo and his colleagues from a program developed by Fred Schamber of Tracor-Northern Inc. This multiple least-squares fitting program is able to subtract the background noise so that peaks can be identified; it also separates overlapping peaks. This program is generally necessary for quantitation of elements at low concentrations, Somlyo says; it also provides the only way to study low concentrations of an element in the presence of much higher concentrations of another.

Electron Energy-Loss Detectors

A complementary technique for providing high sensitivity is known as electron energy-loss spectrometry. When an electron strikes an atom and stimulates the emission of x-irradiation or an Auger electron, the incident electron loses an amount of energy that is characteristic of the atom it has struck. Since most of these electrons are scattered in the forward direction, it is possible—if the sample is not too thick—to collect them, measure the energy loss, and thereby identify and quantify the elements present in the sample. This effect is most useful for the lighter elements, which cannot be analyzed easily with the other two types of detector.

The electron energy-loss spectrometer collects the vast majority of the scattered electrons, according to David C. Joy of Bell Laboratories, so that it is at least as sensitive as energy-dispersive spectrometry. Michael Isaacson and Albert V. Crewe of the University of Chicago have demonstrated, for example, that the technique can easily detect the iron in ferritin. The theoretical limit for detection by electron energy-loss spectrometry, Joy says, is about 10^{-21}g. This technique is much newer than the other forms of detection, however, and not all the theory has been worked out yet. Some investigators thus feel that it is not yet as reliable as energy-dispersive spectrometry for quantitation.

Joy has used electron energy-loss spectrometry to study carbon impurities as small as 10^{-19} g in silicon semiconductors. But its most valuable application, he predicts, will probably be for tracing organic molecules in biological systems. Fluorinated organic molecules, for example, often demonstrate biological activity that is very similar to that of the parent compound. Fluorine cannot be detected easily with conventional EPM spectrometers, but it can be detected very efficiently by electron energy-loss spectrometry. Fluorinated analogs can thus be used to detect binding sites in cells. Joy and his associates have, for instance, used fluorinated serotonin to identify the sites within human blood platelets where the hormone binds and produces its effect.

EPM spectrometers are not cheap. High-resolution instruments for either wavelength-dispersive or energy-dispersive spectrometry can cost as much as $280,000. Electron microscopes can be adapted for EPM much more cheaply; an energy-dispersive detector and computer can be purchased for about $40,000. Joy built his electron energy-loss detector for less than $10,000, but others now think they will cost more. There does not seem to be much agreement about whether it is better to have a dedicated instrument or to adapt an electron microscope. For wavelength-dispersive spectrometry, the crystals are probably located more precisely in a dedicated instrument and there is less background noise. But for energy-dispersive spectrometry, Somlyo argues, it does not seem to make much difference which type of setup is used.

In summary, then, electron probe microanalysis gives the physiologist a new way to examine the composition of cellular subunits without having to try to separate those subunits from the rest of the cell. In this manner, it enables the investigator to examine the most elementary levels of cellular function with an ease never previously imagined. The tool should thus make possible a much better understanding of the simple chemical interactions that are the basis of life.

—THOMAS H. MAUGH II