# Electron Microscope Center Opens at Berkeley

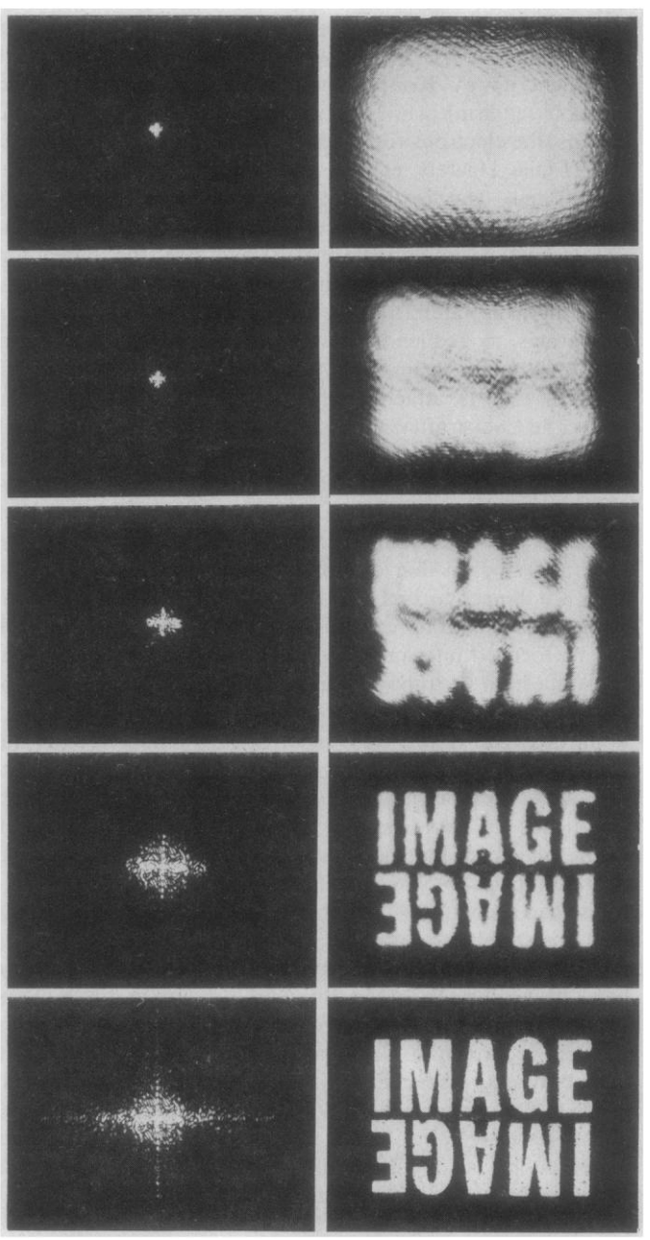
## *A 1.5 million electron volt instrument is the first of two intended to serve a broad spectrum of users*

Transmission electron microscopists would like to investigate specimens under the most realistic conditions possible and to image them with the highest achievable resolution so that individual atoms can be viewed. Both goals are easier to reach with instruments that accelerate electrons to energies of 1 million electron volts (MeV) or more than with machines of the 100 thousand electron volts (keV) or less that is typical of commercial transmission electron microscopes built for routine laboratory use. A 1.5-MeV High Voltage Electron Microscope (the HVEM) that will help materials scientists and biologists study samples in more true-to-life situations will soon open for business at the Lawrence Berkeley Laboratory (LBL). About 2 years from now, the laboratory will put into operation a 1-MeV Atomic Resolution Microscope (the ARM) that should allow microscopists to distinguish atoms even in closely packed metallic structures. Together the two instruments and the buildings to house them will cost the materials science division of the Department of Energy (DOE), which is sponsoring the facility, almost $8 million.

The DOE has designated the LBL electron microscopes a national center open to all qualified scientists having projects related to the department's energy mission. Donald Stevens, director of DOE's materials science division, notes that the relevancy criterion can be interpreted rather broadly. Since the agency does sponsor some biological research, for example, proposals for studies of biological materials are being accepted at Berkeley. A nine-person steering committee comprising four LBL researchers and five from other institutions will review requests for time on the microscopes. According to Ken Westmacott, who is facility manager for the HVEM, about 30 research proposals have already been received for that instrument, and all have been approved. About half of these came from LBL and the adjoining Berkeley campus of the University of California.

Advanced as it is, Berkeley's HVEM does not represent a marked improvement in the technology. It will fall to the ARM, which is now under construction in Japan, to break new ground. Ron Gronsky, the ARM facility manager, calls the development effort by the Japanese manufacturer JEOL "the moonshot of electron microscopy." The prime goal of the ARM will be to break the 2-angstrom barrier, which refers to the present limit on the effective resolution of transmission electron microscopes.

Actually, anything greater than 500 keV is considered a high voltage electron microscope. By this criterion, there are a dozen such instruments in the United States, but none has as high an energy as the HVEM, and none has atomic resolution. There are DOE-sponsored national facilities at Argonne National Laboratory (1.2 MeV) and the Oak Ridge National Laboratory (recently upgraded to 1 MeV) that are dedicated to investigations of radiation damage in metals and alloys. Radiation damage to structural and other materials from energetic neutrons in fast breeder and fusion reactors is a prime DOE worry. The Argonne electron microscope, for example, is specially fitted with an ion accelerator to




***The key to high resolution***

*Electrons passing through a specimen are diffracted. The electron microscope's lens system collects the diffracted beams and focuses them into an image. The diffracted beams form a pattern in so-called reciprocal space where the beams farthest from the incident electrons contain information about the most closely spaced details of the image. The figure shows the effect on image resolution of including diffracted beams farther and farther from the incident beam axis. The quality of electron microscope lenses is now so high that the principal factors limiting the ability to collect all the diffracted beams are instabilities in the electron beam current, the accelerating voltage, and the electric current that powers the magnetic lenses. [Source: Ron Gronsky, LBL]*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bombard materials as they are being viewed. On the biology side, the National Institutes of Health supports a 1.2-MeV instrument at the State University of New York at Albany and 1-MeV machines at the University of Wisconsin and the University of Colorado.

It is a matter of some concern to U.S. science officials that, with two exceptions, none of the high voltage instruments are made by American manufacturers. Several years ago, RCA built a 1.3-MeV microscope for U.S. Steel's Pittsburgh research laboratories and a 500-keV apparatus for the University of Virginia, but the company then got out of the business. Of the 50 or so high voltage electron microscopes in the world, says Westmacott, 39 are made by Japanese or European firms, and the rest are homemade. The United States once had the lead in this technology, but then abandoned it.

Berkeley's HVEM was made by the British company AEI (which was recently bought up by Kratos, Inc., a San Diego–based firm), while the accelerator to boost the electrons to 1.5 MeV comes from Emile Haefely et Cie. in Basel, Switzerland. The instrument occupies three stories in a specially constructed 60-foot high, $1-million silo at LBL. In the basement sits a 100-ton concrete block that is isolated from the silo by 12 air springs to reduce vibrations. The HVEM is at the main floor level on a platform that is attached only to the block; the Cockcroft-Walton electrostatic accelerator fills the upper story.

One advantage of high accelerating voltages is that the electron beam can penetrate particularly thick samples. In materials studies, for example, specimens are thinned to 1 micrometer or less so that the electrons can pass through and be focused into an image. There is some question as to how representative of bulk material the thinnest regions are. If the material is too thin, most of its atoms will be close enough to a surface to have their properties altered. There is a critical thickness, says Westmacott, below which surface-dominated rather than bulk behavior prevails. If the electrons can penetrate thicker sections, then it is easier to be sure one is observing structures that are not affected by being near a surface. Moreover, with the HVEM biologists do not have to resort to serial sectioning of thick samples but can study entire specimens at once because of the greater penetration.

Materials scientists and solid-state chemists are also getting increasingly interested in so-called in situ studies. Rather than studying the oxidation of a metal alloy by heating it in a furnace and later thinning it and transferring it to the microscope, for example, the oxidation can be done directly in the electron microscope's specimen chamber. High voltage instruments are helpful in two respects. First, the specimen chambers tend to be quite large, allowing what Westmacott calls "mini-labs" to be constructed within the chamber for high temperature, high pressure, or other studies. Second, the more penetrating high energy electrons again allow the use of thick samples rather than a thin alloy foil, which may oxidize differently than a bulk specimen. Alternatively, in studies of gas-solid surface interactions, the more penetrating high energy electrons allow the use of higher gas pressures.

A still unsolved problem in the imaging of biological material is radiation damage. The energetic electrons tend to ionize atoms and break chemical bonds, and there is no guarantee that the structure does not change as a result. There is considerable evidence, says Westmacott, that the damage from very high energy electrons is less than that from electrons with the typical 100-keV energies, making biological specimens easier to look at. But in metals and alloys a second type of radiation damage, displacement of atoms caused by head-on collisions between electrons and atomic nuclei, is increased by high electron energies. For this effect, which is similar to that caused by energetic neutrons in reactors, there is a threshold electron energy below which no displacement occurs. Heavier elements have higher thresholds. One can therefore use high voltage machines to study this kind of damage in metals as heavy as uranium.

The HVEM will not permit a significant improvement in resolution over existing microscopes. For this capability, LBL is counting on the ARM. But first, a word of caution. Interpreting high resolution electron micrographs requires the same sort of intuitive touch that top radiologists bring to bear in reading x-rays. The paths of electrons as they pass through a specimen and are collected by the lens system of the microscope are complicated enough that light and dark regions in the image need not correspond to features in the specimen; that is, a black dot in the picture does not always represent an atom or a group of atoms. It often becomes necessary to construct computer models of hypothetical structures and to compute the expected image. Adjustments in the hypothetical structure can then be made until the calculated image matches that actually

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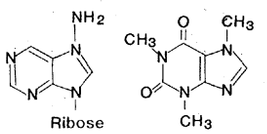
## Caffeine's Stimulatory Effects Explained

Everyone knows that the stimulatory kick delivered by a cup of coffee comes from caffeine, which Solomon Snyder calls "the most widely used psychoactive substance on Earth." Early this month at a seminar* sponsored by the Neuroscience Society, Snyder, a researcher at Johns Hopkins School of Medicine, presented new data† indicating that caffeine affects behavior by countering the effects in the brain of a naturally occurring chemical called adenosine.

Adenosine, which is a relatively simple compound consisting of a purine linked to the sugar ribose, normally depresses nerve cell firing in




Adenosine Caffeine

many areas of the brain. The chemical apparently does this by inhibiting the release of neurotransmitters, chemicals that carry nerve impulses from one neuron to the next.

Like many other agents that affect nerve firing, adenosine must first bind to specific receptors on neuronal membranes. There are at least two classes of these receptors, which have been designated $A_1$ and $A_2$. Snyder, John Daly of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD), and R. Fred Bruns, who has a joint appointment at NIAMDD and Johns Hopkins, propose that caffeine, which is structurally related to adenosine, is able to bind to both types of receptors, preventing adenosine from attaching there and allowing the neurons to fire more readily than they otherwise would.

For many years, caffeine's effects were attributed to its inhibition of phosphodiesterase, an enzyme that breaks down adenosine 3′,5′-monophosphate (cyclic AMP). Because a number of neurotransmitters exert their effects by first increasing cyclic

*Seminar for Science Writers, held at Rockefeller University, New York, on 3 March. †Paper by Solomon H. Snyder, Jefferson J. Katims, Zoltan Annau, Robert F. Bruns, and John W. Daly, to be published in the May issue of the *Proceedings of the National Academy of Sciences.*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obtained, although John Cowley of Arizona State University complained in a 1979 Nobel Symposium on direct imaging of atoms and molecules that this approach had not been terribly effective. For the most part, achievable resolution of interpretable structural detail remains at 2.5 angstroms or worse. Reducing this figure to 1.7 angstroms, as called for in the ARM design specifications, would usher in the era of single-atom resolution, since many chemical bonds are longer than this.

High electron accelerating voltages have two effects on resolution, one good and one bad. The good effect is that the mathematically achievable resolution improves as the electron energy increases, although not linearly. This is what makes cracking the 2-angstrom barrier possible. The bad effect is the inability to keep the electron accelerating voltage absolutely constant. Slight fluctuations in the voltage give rise to the equivalent of chromatic aberrations in light optics because the electron wavelength depends on its energy. In general, it is harder to keep the accelerating voltage stable as the voltage increases. Gronsky says that the choice of 1 MeV for the ARM was a kind of compromise between the two contrasting consequences of high voltages. By means of a very precise feedback system, JEOL will be able to keep its 1 million volts steady to within 0.1 volt. It will also be necessary to make the lens system to very precise specifications in order to reduce spherical aberrations.

With atomic resolution, any number of investigations in materials science, chemistry, geology, and biology would become feasible. One that is especially interesting to Gronsky and to his DOE sponsors is the study of the atomic structure of the boundary regions between crystalline regions in metals. These grain boundaries are often the weakest link in those features that determine the mechanical properties of structural materials. From the biologists' point of view, studying cell membranes might represent a problem of comparable interest.

Credit for establishment of the electron microscope center at LBL, says DOE's Stevens, should go to Gareth Thomas, who is its director. Thomas arrived at Berkeley in the early 1960's when a 200-keV microscope was the state of the art. By the end of the decade, Thomas had garnered a 650-keV machine for the laboratory. In the early 1970's, he teamed with biologist Robert Glaeser of Berkeley and physicist Cowley to propose a national center for electron optics centered around an atomic resolution instrument. An initial approach to the National Science Foundation was rebuffed. The turning point seemed to come after a 1976 workshop on high resolution electron microscopy that was held at LBL. The workshop successfully unified community support for the idea. Just as important, adds Gronsky, was that the technology had by then advanced to the point where an instrument with atomic resolution could be more seriously considered. The center later received considerable support in Congress when a budget balancing exercise initiated by former President Carter almost brought the project to a premature end.

Last year, JEOL won out in a three-way contest with another Japanese company, Hitachi, and Kratos for the right to develop the ARM. JEOL will build the instrument in Japan and test it there. Then, the ARM will be disassembled and shipped to LBL, where it will be put back together in a silo similar to that housing the HVEM. Final acceptance testing should be completed by March 1983. The LBL microscopists can hardly wait.—ARTHUR L. ROBINSON

# Data Sought on Low Cholesterol and Cancer

## *Low cholesterol levels seem associated with cancer, but it is not yet clear how strong the association is or what it means*

Accumulating evidence for a connection between low serum cholesterol concentrations and an increased risk of cancer has led researchers to pursue the question more vigorously, especially in light of other data that point to a clear relationship between high cholesterol levels and heart disease. Jeremiah Stamler of Northwestern University Medical School, who strongly supports cholesterol-lowering diets, says "there certainly seems to be something there but the relationship between low serum cholesterol and cancer is by no means clear, consistent, and unequivocal."

In an attempt to resolve the issue, the National Heart, Lung, and Blood Institute (NHLBI) contacted about 100 researchers who have been studying cholesterol and heart disease and asked them to reevaluate their data to see if they show an association between low cholesterol concentrations and cancer. Many of these investigators are expected to attend an NHLBI meeting in May.

As long as 10 years ago, evidence suggesting a low cholesterol–cancer connection was reported by M. L. Pearce and S. Dayton of Veterans Administration Hospital in Los Angeles, who noted an increased incidence of cancer in men on a cholesterol-lowering diet. But Frederick Ederer, now at the National Eye Institute, and his associates could not confirm this result when they looked at data from four similar studies.

About 5 years ago, Geoffrey Rose of the London School of Hygiene and Tropical Medicine accidentally came across a relation between low cholesterol and colon cancer. Rose hypothesized that colon cancer might be associated with high cholesterol concentrations, reasoning that the populations with high rates of colon cancer were those with high rates of heart disease and high average cholesterol concentrations. He reviewed data from a number of large, prospective studies of heart disease and found, unexpectedly, that those who got colon cancer tended to be those whose cholesterol was low—less than 190 milligrams of cholesterol per 100 milliliters of serum. The serum cholesterol concentration of the average American is 215 mg per 100 ml, and until recently it was 230 mg per 100 ml. Only 10 percent of Americans have cholesterol concentrations below 190 mg per 100 ml.

But Rose's study was retrospective. One of the first prospective studies was that of Robert Beaglehole and his associates at the University of Auckland, New Zealand, who reported last year that in their 11-year study of 630 New Zealand Maoris, men and women with serum cholesterol concentrations below 190 mg per 100 ml had a higher rate of cancer. Similar results were then reported by


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