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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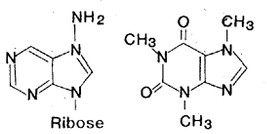
Caffeine's Stimulatory Effects Explained

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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AMP concentrations in target neurons, prolongation of the elevated concentrations, as might be brought about by a phosphodiesterase inhibitor, could lead to inappropriate nerve firing and, consequently, to behavioral stimulation.

But Snyder points out that the caffeine concentrations needed to inhibit the enzyme in brain are much higher than those that produce stimulation. Moreover, other compounds that block the enzyme's activity are not stimulants.

To buttress their case that caffeine acts instead by preventing adenosine binding, Snyder, Daly, and Bruns compared the stimulatory effects of a series of caffeine derivatives with their ability to dislodge adenosine from its receptors. "In general," Snyder reports, "the ability of the compounds to compete at the receptors correlates with their ability to stimulate locomotion in the mouse." Theophylline, a close structural relative of caffeine and the major stimulant in tea, was one of the most effective compounds in both regards.

Caffeine and theophylline also reversed the effects of a long-lived adenosine derivative that mimics the effects of the natural agent. Mice treated with the derivative, as Snyder describes them, "lie around loose, relaxed, and splayed out, but wide awake and responsive to painful stimuli." Shortly after treatment with either stimulant, however, the animals begin moving about at a higher than normal rate.

Ronald Gehrman

Laid-back mouse

Treated with adenosine mimic, this mouse has lost its get-up-and-go.

There were some apparent exceptions to the general correlation observed between adenosine receptor binding and stimulation. One of these was a compound called 3-isobutyl-1-methyl-xanthine (IBMX), which bound very well but actually depressed mouse locomotion.

Snyder, Daly, and Bruns suggest that this is not a major stumbling block to their hypothesis. The problem is that the compound has mixed effects in the brain, a not unusual occurrence with psychoactive drugs. Even caffeine, which is generally known only for its stimulatory effects, displays this property, depressing mouse locomotion at very low concentrations and stimulating it at higher ones.

In mice treated with the adenosine mimic, both IBMX and depressive doses of caffeine produce stimulation, however. The investigators conclude that IBMX does have intrinsic stimulatory capabilities, which are masked by its depressant effects but revealed by the interaction with the adenosine mimic.

Cell Defect in Mental Retardation

About 7 years ago, Dominick Purpura of the Albert Einstein College of Medicine encountered a puzzling case of mental retardation. An infant, who had appeared normal at birth, was by the age of 5 months in a profound neurological and behavioral decline for which no obvious reason could be found.

In a more or less last-ditch effort to get at the cause of the problem, Purpura took a small sample of cortical tissue from the infant's brain. Although there did not appear to be major changes in the shape and numbers of brain cells, there was a subtle abnormality in the dendrites of one of the major types of cortical neurons, the pyramidal cells. "They were almost devoid of normal spines, and the ones that were present looked like fetal spines," Purpura reports. A deficiency of normal spines would mean that the information input to the pyramidal cells was defective.

Purpura says, however, "We didn't realize at the time that we had missed something entirely." What had been missed, the investigator reported at the Neuroscience Society seminar, was the unusual beaded structure of the dendrites, which contrasts markedly with their normal tapered shape. He observed the dendritic changes in four other children whose mental retardation followed a course similar to that of the first. Further examination of the brain samples with electron microscopy revealed that the microtubules in the dendrites were also deranged.

Ordinarily, the microtubules run longitudinally through the neuronal projections and help to maintain their shape. "But in these children," Purpura says, "the microtubules were in very marked disarray, more like cooked spaghetti." He hypothesizes that the microtubular abnormality causes the beaded shape of the dendrites, which impedes the flow of nerve signals along the projections. Loss of spines and synapses would then be a consequence of poor signal transmission.

The cause of the microtubular defect is not known, but Purpura thinks that it is not necessarily genetic. There were no apparent chromosomal defects, and the parents of some of the affected children subsequently produced normal babies.

More than one cause, including factors such as environmental insults or malnutrition, might be involved. If so, Purpura maintains, "Whatever factors are operating have a common mechanism."

Just how common it might be is also unclear. In 1972, Miguel Marin-Padilla of Dartmouth Medical School found a similar abnormality in dendritic spines in brain samples taken during autopsies of infants with Down's syndrome. At present no one knows whether a microtubular defect also underlies this abnormality. Autopsy material is usually too deteriorated for electron microscopic examination, and brain biopsies are not performed on living Down's children because their condition can be diagnosed without taking this step.

The brain cell changes underlying the development of mental retardation are largely unknown, even in cases such as Down's syndrome, where a specific genetic abnormality is readily apparent. Purpura now thinks that he has a clue in the microtubules. "The spine changes originally observed are the mere tip of the iceberg. What we want to find is a common defect that could account for a variety of dendritic changes."

Jean L. Marx