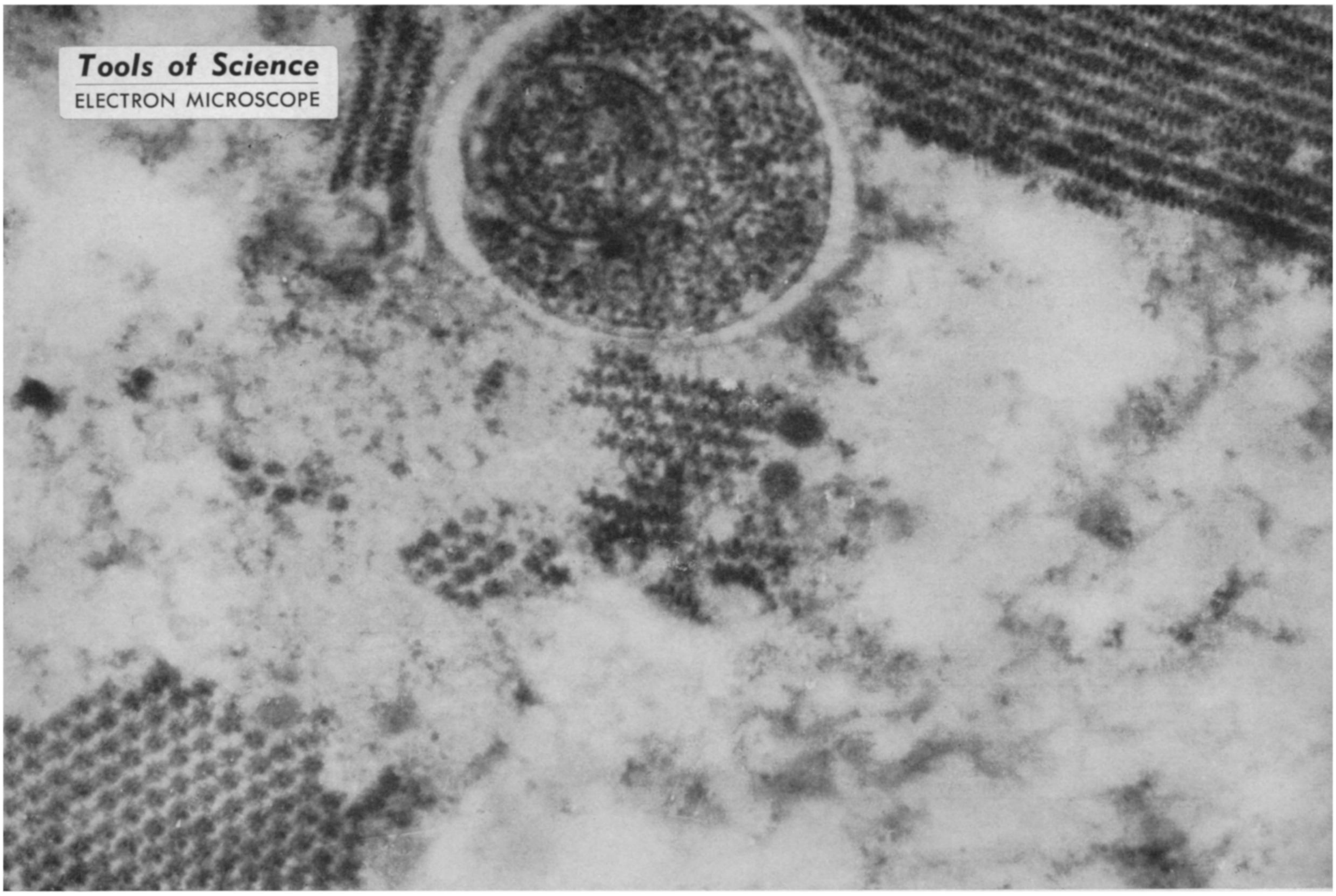
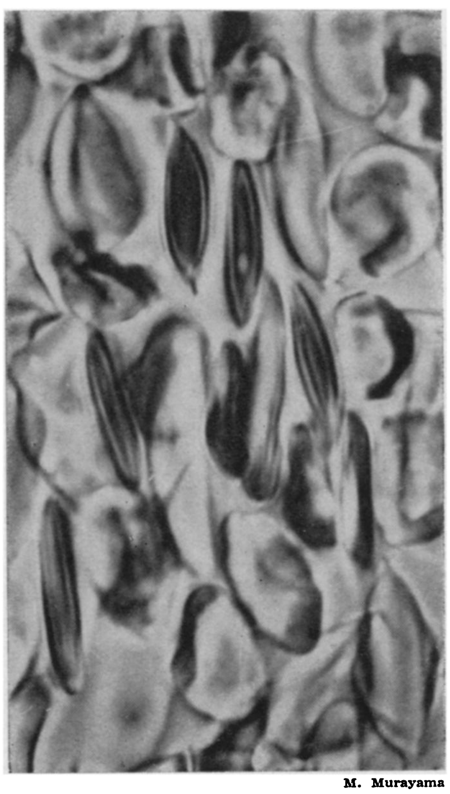


M. Murayama

Electron microphotograph shows waffle-shaped polyribosomes, left, in amoeba. Normal particles upper right.



M. Murayama

Sickle cells align in magnetic field.

by Carl Behrens

Electron's Eye View

Physicists, biologists medical researchers all find the electron microscope a daily-use tool.

• Unexpected fragility in steel exposed to radioactivity in nuclear reactors is traced to the formation of minuscule bubbles.

• An unusual form of polyribosomes—particles involved in the cellular production of proteins—is discovered in refrigerated amoebas.

• A fracture in a glass fiber-plastic material is studied to see where and how the composition failed. Aim of the research is the development of composites for airplanes and buildings; the composite should have a fifth the weight of present structural materials and all their stiffness.

None of these advances would be possible without the electron microscope, one of science's most versatile tools.

Magnification of objects by a stream of electrons is a technique developed in the past three decades. Perhaps because electrons are easier to control than light beams—and because engineers are accustomed to doing strange things with them in television and communications equipment—the number of intricate solutions to problems seem infinite.

One problem that hasn't been solved—and probably won't be—is cost. Electron microscopes cost from $50,000 up, many times the price of even the best optical instrument.

But then, an optical microscope is unable to reveal the structure of matter with anything like the detail of an electron magnifier. In cost-effectiveness terms so popular these days, the electron 'scope pays for itself.

Before the beginning of this century optical theory had been developed to show that the size of an object that could be imaged depends on the wavelength of the radiation employed. The smallest object that can be seen clearly has a diameter of half the wavelength. With visible light, this size is about two ten-thousandths of millimeter: 0.2 microns.

By 1900 microscopes were able to reveal objects that small.

With greater understanding of the nature of electricity, the possibility of using beams of electrons instead of light became apparent. Light magnification is performed by bending rays through glass lenses. Beams of electrons can be bent by applying electric or magnetic fields to them—this is how television pictures are formed—and the principle can be used in the electron microscope.

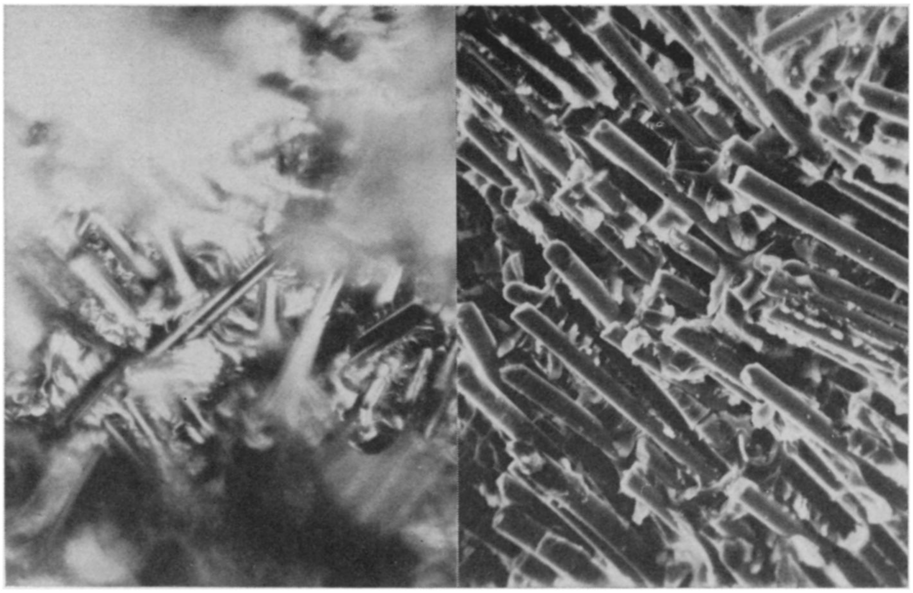
While learning to control electrons, physicists discovered that these weren't simply particles moving in straight lines. According to the theory of wave mechanics, electrons—as do all other matter—have a wave motion that depends on their mass and their energy. With ordinary objects—such as this piece of paper—the wavelength of their motion is so incredibly small, and its frequency so high, that the waves can't be detected.

Electrons have a detectable wave motion, but their wavelength is only a hundred-thousandth as large as that of visible light. This means that the fuzziness that occurs with small objects in light microscopes wouldn't become a problem unless the objects were far smaller than the diameter of an atom. Before that point is reached, problems of controlling the electromagnetic lenses intervene. The lower limit of size that can be resolved now is about five angstrom units, about one fifty-millionth of an inch. This is one four-hundredth of the smallest particle to be seen by a light microscope.

One of the most fertile applications of electron microscopes is the study of metals and inorganic materials, both in the field of basic research and for practical applications.

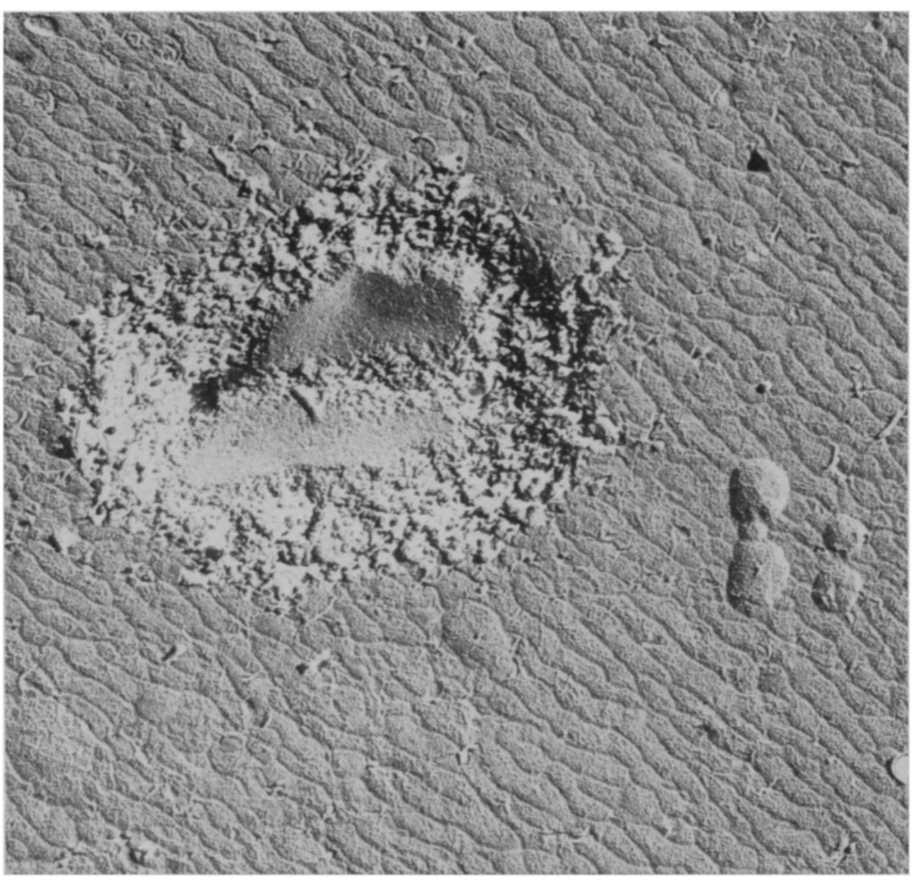
The study of steel fragility in nuclear reactors, carried out by J. R. Weir Jr. of the Oak Ridge National Laboratories, for example, has just led to a theoretical explanation of the effect of irradiation. It had been found that steel that could normally be stretched 10 to 20 percent of its length at 700 degrees C. would break when stretched only two to six percent after being exposed to neutrons in a reactor.

Since some radioactive substances give off alpha particles—the nuclei of helium atoms—researchers guessed that



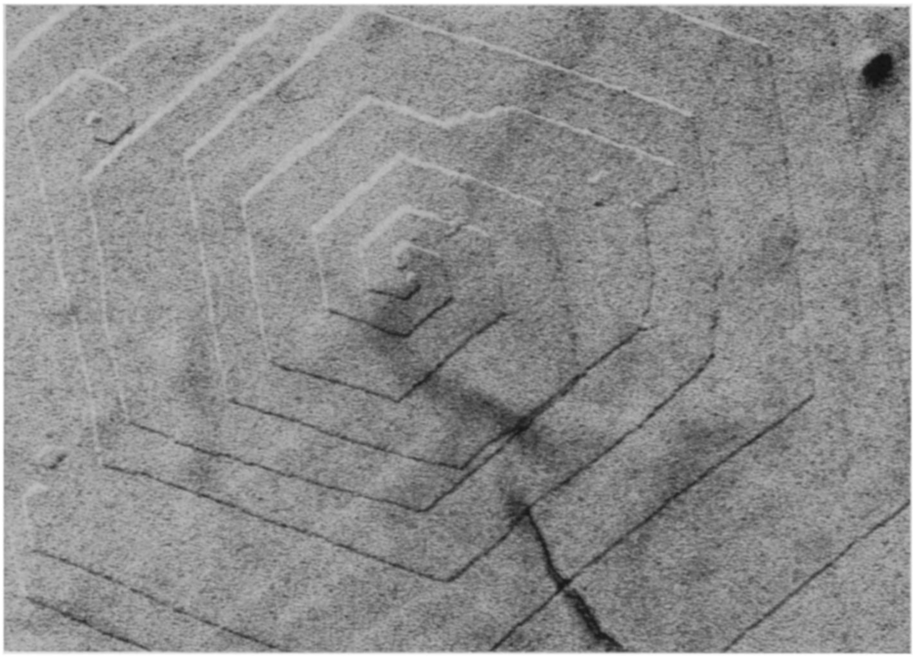
Monsanto Co.

Glass fiber fractures: Under an optical microscope, left, and electron scanner.



A. W. Ruff Jr.

Tiny spot in aluminum alloy succumbs to corrosion faster than the rest.



F. Khoury

Crystal formation in plastic shows characteristic step-wise spiral buildup.

helium might accumulate in the metal, making it more prone to fracture. Electron micrographs of irradiated steel showed bubbles along the edges of the metal crystals where they would be most likely to cause a break. These were interpreted to be helium bubbles. From knowledge of such causes for breakdown, engineers can find materials that are less likely to be affected.

Another study of metal failure, just under way at the National Bureau of Standards, is prompted by a search for a better alloy for desalination plants. Aluminum alloy tubes are needed to carry highly concentrated salt solutions, and corrosion is a major problem.

It was found that these tubes developed holes in some spots much faster than in the rest of the surface. Experimenters guessed that specific ingredients in the alloys were more susceptible than others.

So scientists at NBS's metallurgy division, under Dr. A. W. Ruff Jr., are immersing thin chips of various alloys in weak salt solutions and noting where corrosion starts. Early results are backing up experience with the tubes, leading to expectations that the particular alloy components causing the trouble can be identified and eliminated.

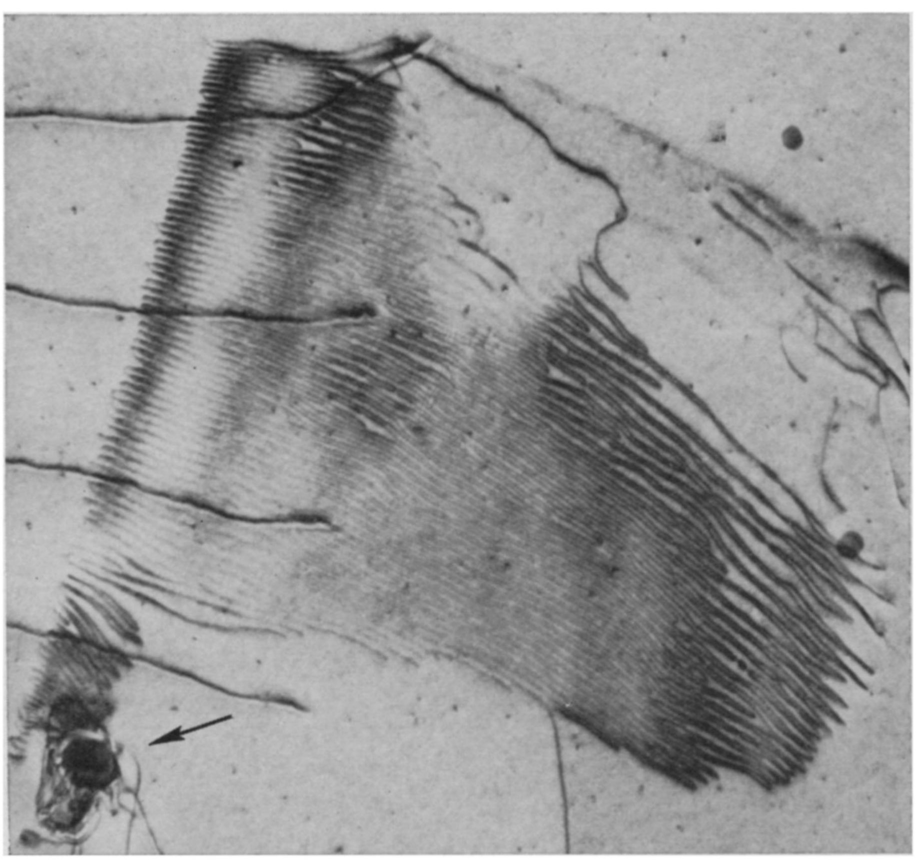
On a more basic level, Dr. Ruff is looking at individual metal crystals to see what structures are strongest and how they are formed.

Metals are made up of tiny grains or crystals. Under stress, metals often fracture when the grains shift their position and slide along the grain boundaries—this is what happened in the nuclear reactor when helium formed between boundaries. But metals can fail with the boundaries still intact—the individual crystals themselves give way.

Studies of individual crystals under the electron microscope have shown a relationship between patterns of dislocations within the crystal and the hardness of the material. In general, materials with either many or no crystal dislocations are hard, while those with just a few are weak.

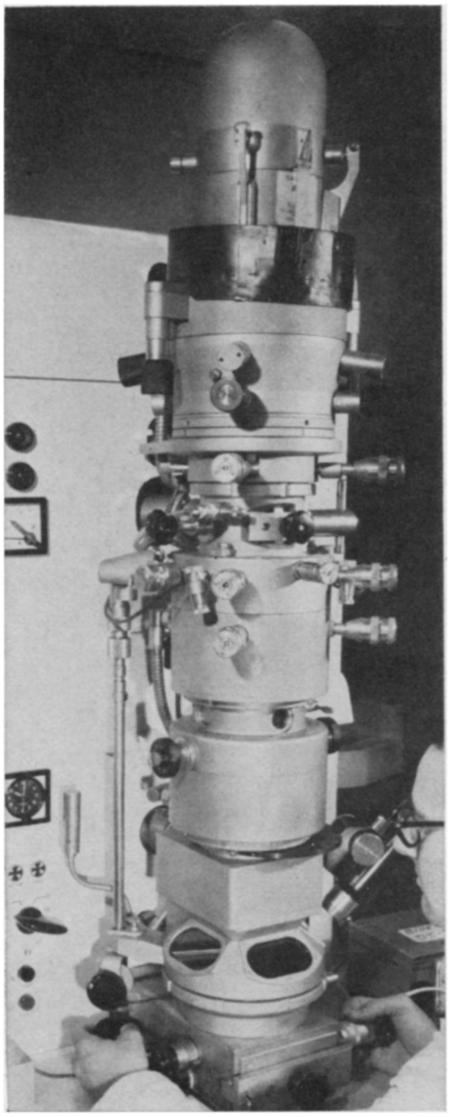
The formation of crystals itself is also being studied under the electron microscope. Although perfect crystals —regular, smooth-sided structures with no surface ridges—exist, most crystals have defects, and these defects are necessary for increasing the size of the crystal. Studies with polymer plastics show a step-wise formation of crystals which looks like a spiral when it is flattened out for viewing in the microscope.

The versatility of the electron microscope is increased by its ability to show not only surface features, as in the study of crystal formation, but also details of internal structure, which are necessary for investigation of dislocations. Another feature, useful in the studies of aluminum alloys, is that the atomic composition of the materials can be determined very easily under the microscope. The electrons passing through the material are bent or diffracted and this produces the image; but the way they are diffracted also indicates what the composition of the material is. By a simple adjustment,



A. W. Ruff Jr.

Dislocation in silver-tin alloy: arrowed particles might be to blame.



National Bureau of Standards

A typical electron microscope.

the microscopist can identify components of unknown samples by the electron diffraction pattern. In the desalination study, for example, this allows identification of the particular alloy component at the point where corrosion was severe without even taking the sample out of the microscope.

One of the most exciting advances in electron microscopes, according to Dr. Ruff, is the increased energy with which the electrons are being sent through the samples. This means that thicker samples can be used, and so more of the internal structure can be seen.

The higher energies are not so useful in the other major areas of electron microscope study: biology and medicine. With organic substances, the stream of electrons can be very damaging to the sample, and the trend is toward cutting down the energy of the electron beam and using image amplifiers to increase the contrast of the picture so that it can be seen.

In the biological field, the electron microscope has been invaluable in learning about the formation of proteins.

The discovery of polyribosomes and their relation to RNA and protein formation came from studies with the instrument.

One investigator, Dr. Makio Murayama of the National Institute of Arthritis and Metabolic Diseases recently found unusual forms of polyribosomes in amoebas. Normally, the particles are bundles of rod-like structures, but Dr. Murayama discovered that when the amoebas are cooled, a kind of lattice structure is observed.

He also found that these lattice forms were not just rod bundles viewed end-on, but were very thin chips with almost no depth.

That discovery was made by using a feature of the microscope generally ignored by researchers: stereo vision. A simple device tilts the sample slightly, allowing pictures to be taken from slightly different angles. When viewed through a stereo viewer, the two pictures give a three-dimensional view.

"Most microscopes come with this attachment," says Dr. Murayama, "but it isn't used much. You'll find it gathering dust in the accessory box, and many users don't even know what it is."

Dr. Murayama also used the microscope to verify his theory of the structure of blood's sickle cells, an abnormality in which hemoglobin molecules form stacks which sometimes jam up in blood vessels and cut off circulation. Dr. Murayama found that the sickle stack was formed by six twisted strands of molecules with a hollow space or micro-tubule in the center.

Working from that picture, he hypothesized that pressure would cause the sickles to collapse and might relieve the logjam in the blood vessel. He also found that a magnetic field around the sickles would act on the iron atoms in the hemoglobin molecules to line up all the stacks in the same direction, again loosening the jam.

Pressure therapy has been tried and gives temporary relief from sickle cell anemia (SN: 1/7), but Dr. Murayama says no one's tried the magnetic treatment yet.

Preparation of samples for the microscope is more complicated than using the instrument itself. A wide variety of methods are used.

The most straight-forward is simply slicing very thin sections of the sample with a very sharp knife. Machines for cutting thin sections of samples have been used for years for regular optical microscopes, and these have been adapted for even smaller slicing. In electron samples, the sections become so thin that moving the blade—or the table where the sample is placed for cutting—such a short distance becomes a problem. The thickness of oil lubricant on the machinery is greater than the distance—as little as 200 angstroms—the part has to move. One solution to that problem is a rod that expands a precise amount when heated. An electric heater, carefully regulated, around the rod causes it to expand, and the specimen table, attached to the rod, moves the small distance needed.

In studies of crystal structure, cutting with a knife damages the surface too much. Here chemical etching is used. Acid is sprayed on a wedge of the material until a hole shows, and the edges of the hole are mounted on the microscope viewer.

For specimens that can't take the rigor of direct viewing in the electron microscope, a number of methods have been developed. One of the most common is to coat them with a thin film of a material that can take it, such as carbon or plastic. The original sample is then dissolved away and the film, molded into the same, but reverse, shape and called a replica, is used in the microscope.

A special shadowing technique is used in making the replica to make surface features stand out. In coating the sample, the replica material is evaporated onto it from an angle, so that high points on one side pick up more deposits than those of the other giving the effect of shining a light from one side to make the shadows and thus the texture more distinct.

A new type of electron machine, called a scanner (SN: 12/24/66), is a little easier on samples, since it doesn't use such an intense beam of electrons. Instead of sending the electron beam straight through the specimen, it bounces a smaller stream off the end of the sample, scanning across its surface. As the electrons hit, they dislodge other electrons in a characteristic pattern; these are collected by another circuit and used to control a third electron beam, which forms a picture on a screen.

The great advantage to the scanning microscope, besides its gentler electron beam, is that it can show clearly much greater depths and heights on the sample surface. For the regular 'scope, some samples can be sliced thinly and viewed in sequence to give an idea of the three-dimensional make-up of the object. But a perspective view, as produced by the scanner, is much more usable. In addition, it cuts out the great cost and trouble involved in preparing thin samples.