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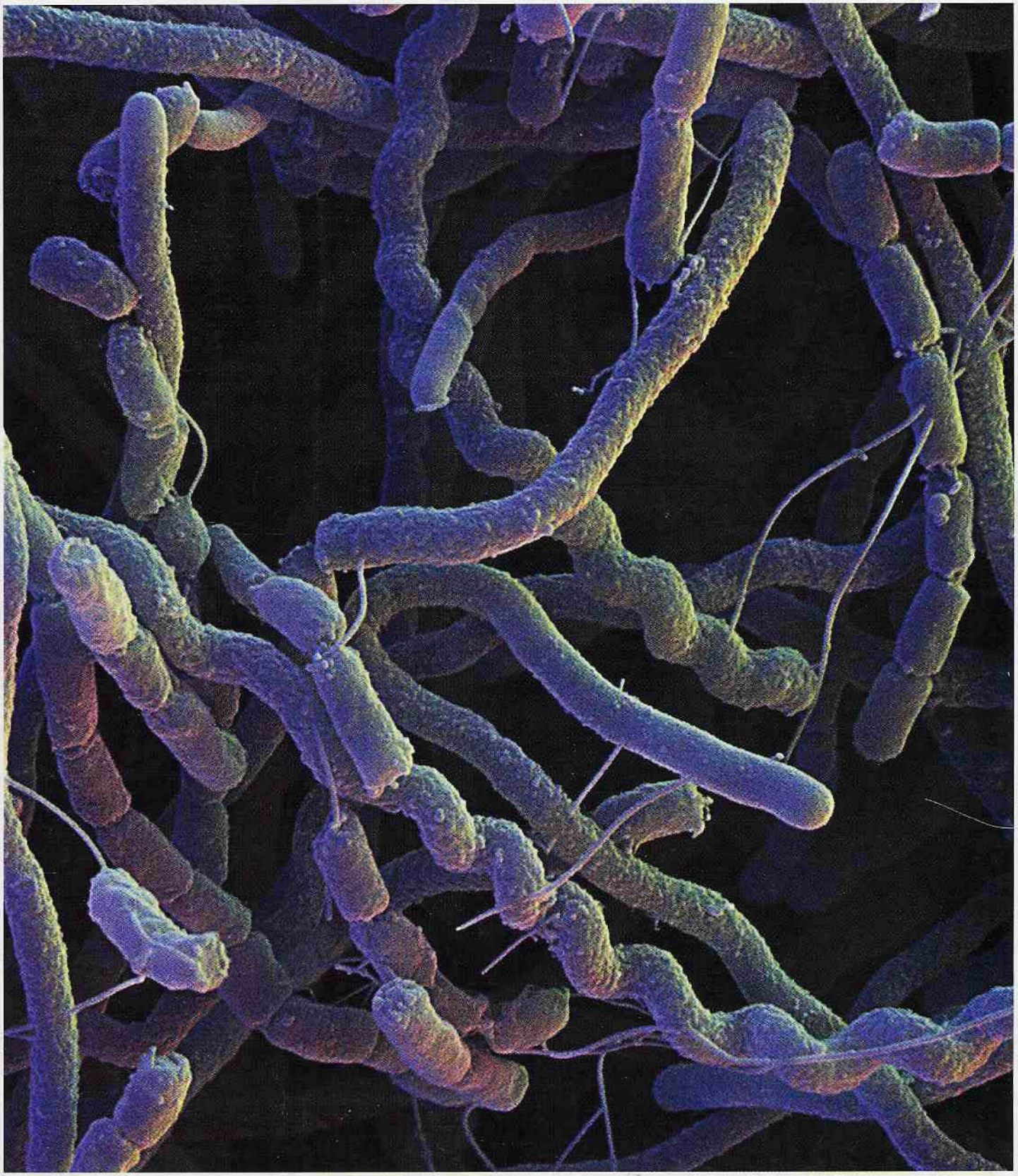

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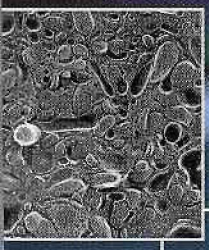

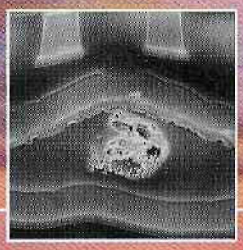

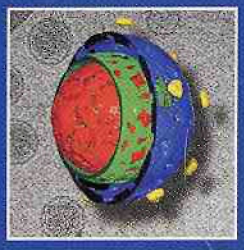

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ELECTRON TOMOGRAPHY

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The transmission electron microscope (TEM) was invented in the 1930's, and developments in specimen preparation in the 1950's led to its widespread use as a tool to study structure in biologic systems. Similar in principle to the light microscope, but utilizing a much shorter wavelength for better resolution, the TEM has the image-forming beam pass through the specimen. This results in a two-dimensional image which can be difficult to interpret because features from different depths of the three dimensional specimen are superimposed. Traditionally this was dealt with by cutting sections of plastic-embedded specimens so thin (in the 40 to 80 nanometer range) that they effectively had only two dimensions. To allow biologists to examine structures in three dimensions, serial sections are stacked and structures reconstructed. Even though computers have made reconstruction easier, the reality is that resolution in the depth dimension is limited by the section thickness. The technique of electron tomography is emerging as a way to overcome this limitation.

The development of electron tomography as an important method to examine the three-dimensional structure of organelles was impressively reviewed by Bruce McEwen and Michael Marko. As in the radiographic technique of computed axial tomography (the well known CAT scan), an intact specimen is examined from several angles and the accumulated data is computed as a three-dimensional structure. As currently used, plastic sections are cut thick enough (200 to 1000 nanometers) to include a significant portion of most cellular organelles. The section is tilted about an axis at about 1^{o} or 2^{o} increments over about $\pm 60^{o}$ to 70^{o}. At the extremes of this range the thickness that the beam penetrates is up to three times the thickness of already thick sections, so a high accelerating voltage is needed for optimal performance. To minimize certain problems from a limited tilt range (*i.e.,* less than $\pm 90^{o}$), the specimen can be rotated 90^{o} and tilted again. The physical aspects of electron tomography dictate that plastic sections up to about 2 microns in thickness can be visualized at a resolution of about 5 to 20 nanometers. This means that cell organelles are particularly well suited for examination by electron tomography.

McEwen and Marko give several examples of subcellular structures that have been examined using electron tomography. These include mitochondria, the Golgi apparatus, centrioles, membrane pores, muscle fibers, the nucleolar organizer region, and whole prokaryotic cells. As an example, it was controversial whether the folds of the inner mitochondrial membrane (cristae) enclosed a separate compartment within the mitochondria, or have unrestricted access to the intermembrane space and thereby to the cytosol. Using electron tomography, it was established that the space within the cristae communicate with the space between the inner and outer mitochondrial membranes through narrow (about 30 nanometers) tubular connections. This is a strong argument for what is called the "restricted access model" and has important consequences for the bioenergetics that occur in the mitochondrion. Another example is the Golgi apparatus where electron tomography revealed several previously unobserved features. Apparently, protein molecules do not have to go through the whole Golgi stack. Instead, vesicle transport can take place at any of the Golgi cisternae and travel through aligned fenestrations, or tubular extensions, or budding from the edges of any cistern.

The future of electron tomography looks bright. Improvements were predicted to be mainly in the area of specimen preparation, although advances in instrumentation and computing are being made. Rapid freezing and cryopreparation will more closely represent the native state and allow time-resolved studies, so improvements associated with these techniques are being developed. McEwen and Marko concluded that electron tomography has proven its worth as a method for three-dimensional reconstruction of ultrastructure. We can expect to better appreciate organelles with this technique. ■

1. The author gratefully acknowledges Dr. Bruce F. McEwen for reviewing this article.
2. McEwen, B.F., and M. Marko, The emergence of electron tomography as an important tool for investigating cellular utrastructure, J. Histochem. Cytochem. 49:563-563, 2001.

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