ciencies have not yet been evaluated, they will probably not be greatly different for fusion and fission, and it can be stated that specific afterheat power densities will be considerably less significant for niobium fusion reactors and negligible for vanadium fusion reactors, compared to fission reactors.

Possible security aspects of fusion plants. With regard to possible diversion for weapons purposes, the fact that tritium would be generated, circulated, and burned within the fusion plant means that its availability outside the plant would be minimal. Furthermore, as far as is known, there is no way to construct a nuclear weapon without using fissionable material to initiate the explosion. A fission-free nuclear weapon may, in fact, never be achieved. In the foreseeable future, the issue is therefore the diversion of fissionable material, not of tritium.

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Structure of Wet Specimens in Electron Microscopy

Improved environmental chambers make it possible to examine wet specimens easily.

D. F. Parsons

Light microscopy has the advantage of permitting one to view objects in both liquid and vapor environments. However, its resolution is limited. So far, the only practical method of overcoming the wavelength resolution limit of the light microscope has been to build a microscope with magnetic or electrostatic lenses capable of focusing charged particles, for example, electrons (*1*), lithium ions (*2*), protons (*3*), various ions in the field ion microscope (*4*), or 14-megaelectron volt nitrogen ions (*5*). The use of charged particles, with their necessarily large scattering cross sections, requires that most of the beam path of the microscope be evacuated in order to prevent diffusion of the beam by gas scattering. In nearly all work with electron and ion microscopes it has been customary to place the specimen itself directly in the microscope vacuum, and only a few attempts have been made to isolate the specimen from the microscope vacuum.

One possible solution would be to build a short-wavelength microscope that uses neutral particles, for example, neutrons (*6*), or electromagnetic radiation such as x-rays (*7*). Various point projection and curved mirror lens systems (*7, 8*) for x-rays and neutrons have been devised, but the resolution achieved so far has been no better than that of the light microscope.

In this article I consider to what degree the electron microscope allows the viewing of structures immersed in gas and liquid. Past work and recent advances will be reviewed. The main point to be made is that the routine operation of differentially pumped electron microscope environmental chambers has now been achieved, and these chambers appear to have a wide range of application in medicine, biology, chemistry, physics, atmospheric science, and other areas.

Two types of environmental chambers have been used. In one, the specimen and its environment are isolated from the microscope vacuum by two windows which are thin enough to allow penetration by the electron beam. In the second type of chamber, two small apertures are substituted for windows and the escaping gas is removed by differential pumping of one or more outer chambers surrounding the apertures. The relative advantages and disadvantages of these two approaches will be discussed.

The visualization of structures immersed in gas and liquid environments raises new problems. The presence of the liquid and gas may cause excessive background scattering with associated loss of resolution resulting from chromatic aberration and loss of contrast. Overcoming these problems involves (i) optimizing the design of the environmental chamber to reduce extraneous scattering due to gas (and film windows if present), (ii) surrounding the structure with the minimum necessary thickness of liquid, and (iii) choosing the optimum imaging mode that gives the desired contrast and resolution in rela-

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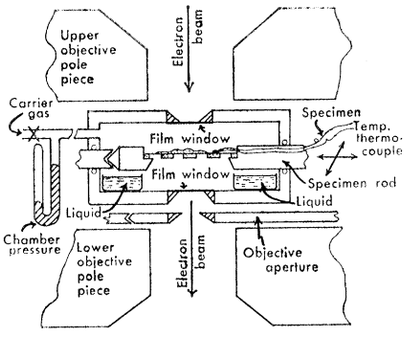


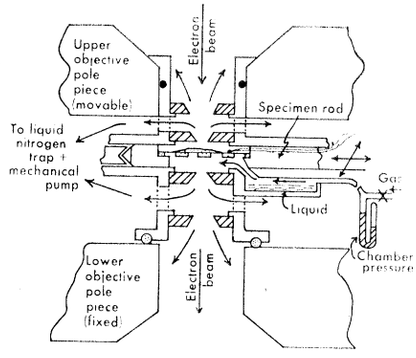


Fig. 1 (left). Generalized diagram of an electron microscope environmental chamber closed by thin film windows. The temperature of the specimen is monitored by a thermocouple and the pressure of the chamber by an external manometer. The liquid vapor pressure can be supplemented by the addition of other gases. The specimen grid can be completely scanned between the windows in the normal specimen plane in the objective pole piece gap. The objective aperture functions normally. The electron beam penetrates the upper window, the chamber vapor and gas space, the wet specimen, and the lower window in that order. Fig. 2 (right). Generalized diagram of a differentially pumped environmental chamber with inner (100-μm) and outer (300-μm) apertures. Most of the gas escaping from the inner pair of apertures passes into the pole piece gap and is pumped off by a liquid nitrogen trap and mechanical pump. Gas escaping from the outer apertures is removed by the microscope vacuum system with or without the aid of a small booster diffusion pump. The aperture assembly is aligned on the beam by a slight displacement of the upper pole piece. For clarity the objective aperture assembly and the arrangements for temperature control of the chamber are not shown.

tion to a minimum of electron beam radiation damage. The choice of the imaging mode with respect to whole cells lies between medium-energy (20- to 50-kilovolt acceleration potential) scanning transmission electron microscopy (STEM) and high-voltage (1- to 3-megavolt acceleration potential) fixed-beam transmission electron microscopy (HVEM). In both approaches, the maximum effective penetration is obtained and the effects of chromatic aberration on the resolution and contrast of thick objects are reduced. Quantitative comparisons between STEM and HVEM micrographs of whole cells (*9*) are not yet complete. Reported HVEM results on whole wet cells (*10, 11*) indicate that conversion of present-day 1- and 3-Mv HVSM's to the scanning mode is required to penetrate the nucleus and cell cytoplasm of cells. Converted scanning HVEM's will have sufficient resolution for the initial phases of the wet cell electron microscope work, but eventually the application of field emission scanning HVEM's (*12*) will be necessary to give higher resolution. Field emission requires a vacuum of 10^{-10} torr at the gun. Such a low pressure will be difficult to obtain with the use of differentially pumped environmental chambers, and chambers closed by film windows are indicated in this case.

The theory and practice of operation of the different types of environmental chambers have already been extensively reviewed (*13, 14*). Here, I will focus on applications after briefly describing the principles involved.

Principle of the Thin Film Window Chamber

The manner in which the specimen, in equilibrium with liquid and vapor, is isolated from the microscope vacuum by thin windows is illustrated in Fig. 1. The electron beam penetrates the upper window, the gas space, the wet specimen, and the lower window in that order. For ease of obtained thermodynamic equilibrium, the liquid reservoir is placed inside the chamber. One may check the equilibrium by comparing the chamber pressure indicated by the manometer with that expected for the vapor alone at the temperature indicated by the specimen thermocouple. An additional mixed gas atmosphere can then be added as required. In general, the inside height of the chamber, depending on the acceleration voltage and the amount of loss of resolution due to gas scattering that can be tolerated, will be 1 to 10 millimeters. High-resolution chambers require careful designing and exact machining if the height of the chamber and the gas path are to be reduced to less than 1 mm.

Specimens are prepared in a glove box saturated with vapor. The wet specimen mounted on the specimen rod is carried to the electron microscope in a tube containing saturated vapor. One brings the chamber to atmospheric pressure by admitting air, saturated with water vapor. The wet specimen is then quickly inserted into the chamber and the saturated air supply closed off. If the chamber operates at less than atmospheric pressure, a gas-lock is desirable to prevent the film windows from being subjected to too high a pressure difference, thus causing breakage.

Marton in 1935 was the first to use such a chamber (*15*); he attempted to use two 0.5-micrometer aluminum foils as windows. Abrams and McBain in 1944 (*16*) used plastic film windows less than 100 nanometers thick, and since then the windows have usually consisted of a plastic film coated with one or more additional layers of evaporated material (for example, silicon monoxide, silicon dioxide, silver, or gold). However, windows made of evaporated layers alone have also been used (*13*).

Other thin film window environmental chambers for the conventional (40- to 100-kv) fixed beam electron microscope include the design of Stoyanova and Mikhailovskii (*17*), Fernandez-Moran (*18*), Heide (*19*), Escaig (*20*), and Fullam (*21*). Designs for the high-voltage (fixed beam) electron microscope include those of Dupouy *et al.* (*22*), Nagata and Ishikawa (*23*), and Fukami and Katoh (*24*). Allison *et al.* (*25*) have developed a chamber for the high-voltage microscope in which ion-etched single crystal corundum windows are used. Designs for the scanning microscope include those of Swift and Brown (*26*) and Morgan *et al.* (*27*); in the chamber of Morgan *et al.* there is only one window since the transmission electron detector was placed inside the chamber, beneath the wet specimen.

Principle of the Differentially Pumped, Aperture-Limited Chamber

In differential pumping restricting apertures in the vacuum system are used to divide up the pressure drop between the extreme high- and low-vacuum regions into regions that are individually pumped (*28*). Recently, this principle was used by Schumacher to develop electron beam welders that bring the electron beam out from the vacuum, through a helium compart-

ment, into air (*29*). It is fortunate that this principle can be carried over to the electron microscope. The small size of the aperture required (about 100 μm) to maintain full gas pressure is not so small as to lead to difficulties in the alignment of apertures, blockage by dirt, or the introduction of excessive astigmatism. Experience has shown that, once a differentially pumped chamber has been set up, large numbers of specimens can be rapidly examined.

The general arrangement is shown in Fig. 2. The inner chamber in this design is closed by two 100-μm apertures instead of thin films and is connected to a liquid reservoir, gas supply, and manometer. The specimen rod has a traverse mechanism which allows the whole grid to be "viewed" by the electron beam passing through the apertures. The outer apertures have a size of 200 to 400 μm and are set exactly in line with the two inner apertures. In the simplest arrangement (*30*), the four apertures are screwed to the movable part of the objective pole piece and a slight displacement of the pole piece is used to center it onto the beam. The gas escaping from the inner apertures is pumped out of the pole piece gap by a liquid nitrogen trap and mechanical pump. The remaining gas escapes from the outer apertures and is pumped away by the microscope vacuum system with or without the aid of an additional small diffusion pump attached to the column. The pressure in the rest of the microscope column remains in the normal range. The two-compartment design, in conjunction with adequate pump speeds, ensures that there is no severe change in column pressure when air is admitted while a specimen is being changed. The wet specimen is prepared and inserted in the same way as for the thin film window environmental chamber. A detailed discussion of the gas flow rates and flow patterns through the parts of this system has already been given (*13*). Over the environmental pressure range of usual interest (a few torr to 760 torr), streaming of gas through the apertures does not occur. For 20 torr of water vapor, the mean free path of the water molecules is 1.7 μm and a water molecule near the specimen would go through about 10^7 collisions before escaping from one of the 100-μm apertures. These multiple molecular collisions (or turbulence) ensure that all parts of the specimen, even the part directly beneath the aperture, will be in equilibrium with the vapor and will not dry. A sensitive way of

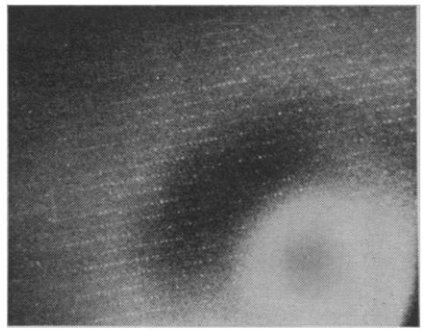


Fig. 3. Electron diffraction pattern of a wet microcrystal of ox liver catalase recorded on No-Screen medical x-ray film at 200 kv. The projection was $P2_1$-2_1 symmetry and corresponds to an orthorhombic habit of catalase.

testing whether wet material illuminated by the beam remains fully hydrated is based on the electron diffraction of wet catalase crystals (*31*) (Fig. 3), which rapidly and irreversibly lose their ability to provide a diffraction pattern when the water vapor pressure is dropped more than 10 percent from the equilibrium (saturated) value. Matricardi *et al.* (*31*) found it possible to keep the catalase crystals fully wet for more than 2 hours in the chamber. Nearly all crystals gave a diffraction pattern after this interval.

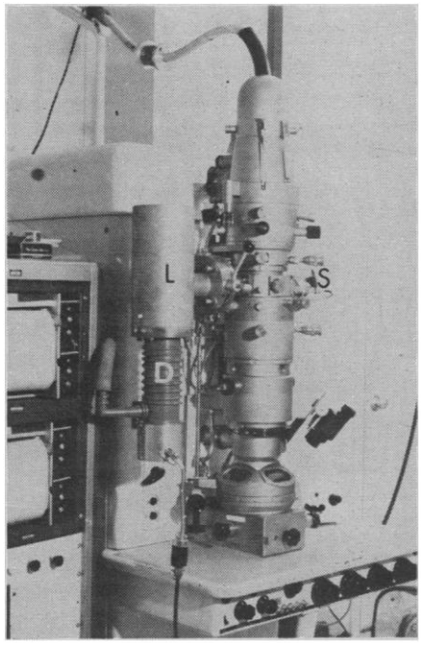


Fig. 4. Environmental chamber as fitted to the Siemens Elmiskop Ia electron microscope by S. W. Hui. The chamber is placed at the level of the upper half of the objective pole piece and is temperature-controlled (− 10° to + 45°C) by a Peltier thermoelectric cooling device. The wet specimen is inserted at *S*. The column pressure is boosted near the chamber by diffusion pump *D* and liquid nitrogen trap *L*. The mechanical pump connection to permit the pumping out of the intermediate chamber is not visible.

In a number of studies of gas reactions in the electron microscope a simple form of differential pumped chamber was used (*32*). Since 1968, investigators at the Electron Optics Laboratory of Roswell Park Memorial Institute have developed several types of differentially pumped environmental chambers in order to carry out electron microscopy of live cells and electron diffraction of wet biological crystals and wet cell membranes. Two versions were built for a Siemens Elmiskop Ia (100-kv) electron microscope in which all the apertures were placed in a single removable turret (*33, 34*). Recently, the chamber has been made temperature-controllable (−10° to +45°C) so that phase transitions in the electron diffraction patterns of cell membranes can be observed (*34*) (Fig. 4). Another version was built for the 200-kv Jeolco JEM 200 electron microscope (*35*). In both the Siemens Ia and JEM 200 versions the access ports at the level of the objective lens gap were inadequate, and thus the chamber was placed above the upper pole piece. A temperature-controlled version was also constructed in the objective lens gap of the U.S. Steel 1.0-Mev electron microscope (*13*) (Fig. 5). Swann has constructed an environmental chamber for the pole piece gap of the AEI EM7 1.0-Mev electron microscope (*30*) and used it extensively for metallurgical and gas reaction studies. A single-stage differentially pumped unit was constructed for the Cavendish Laboratory high-voltage microscope (*36*) for use in the examination of wet biological specimens.

The relative merits of the thin film window and differentially pumped types of environmental chamber have already been discussed in detail (*13*), with respect to ease of operation and resolution loss due to the gas layer and scattering from the windows. Film window chambers involve less modification of the microscope and may be particularly indicated for the high-voltage microscope where thicker and stronger windows can be tolerated. However, scattering from the windows can mar images and diffraction patterns, and the windows can become opaque as a result of the accumulation of contamination on them. Chambers that require the windows to be assembled with the specimen in its holder and do not allow internal translation of the specimen with respect to the windows are inconvenient to use.

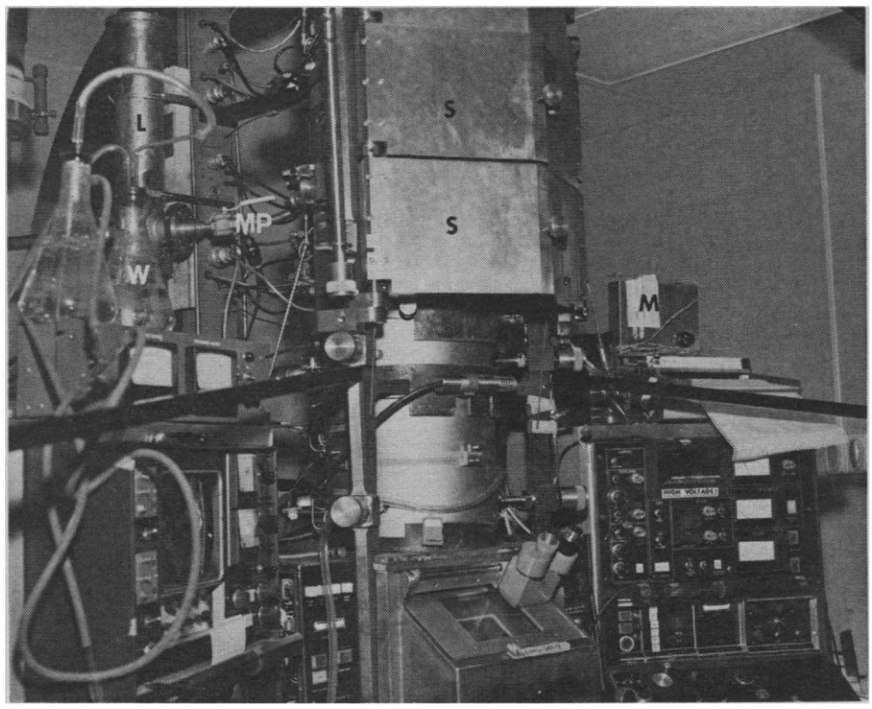


Fig. 5. Differentially pumped environmental chamber fitted to the U.S. Steel 1.0-Mev electron microscope (designed by V. R. Matricardi). Abbreviations: *MP*, mechanical pump connection; *L*, liquid nitrogen trap; *M*, manometer measuring water pressure in the chamber and thermocouple measuring the wet specimen temperature; *W*, humidified air inlet for bringing the chamber up to a pressure of 1 atm before the insertion of a specimen; *S*, x-ray shielding.

Table 1. Applications of environmental chambers.

Biological
Live cells
General biology
Improved visualization of live cells
Motility studies (cytoplasmic streaming, pinocytosis, phagocytosis, heart muscle beating)
Microchemistry: effects of reagents on organelles (for example, adenosine triphosphate on mitochondria)
Studies of the freezing of cells
Cancer research
Detection of the early stages of transformation of whole cells (types of cell processes and cell contacts, cell surface antigen movements using ferritin-labeled antibody, cytoplasmic streaming)
Sensitive detection of tumor-specific antigen with ferritin-coupled antibody
Wet replication of cancerous and normal cell surfaces
Radiation biology
Microbeam irradiation of specific organelles
Radiation sensitivity of cell functions
Ecology
Toxicity of pollution gases to cells (effect on cytoplasmic streaming)
Space biology
Cells in martian environment
Gas bubbles in cells and plasma ("bends"-decompression)
Molecular biology
Electron diffraction of crystals
Small wet protein crystals (structure determination, conformation change with oxygen, carbon monoxide, and other gases)
Wet oriented nucleic acids and chromosomes
Wet natural and artificial membranes
Oriented polysaccharides
Imaging of single wet molecules
Macromolecular interactions (for example, myosin + actin)
Surface charge distribution (for example, on myosin)
Observation of protein synthesis (Nirenberg ribosome system)
Nonbiological
Atmospheric science
Rain nucleation
Hail formation
Smog nucleation
Chemistry
Surface catalysis and polymerization
Electron diffraction of oriented water and ice
"Origin of life" experiments
Oxidation and other reactions at surfaces

The dynamically pumped chambers are more complicated to design and to construct but thereafter make it possible to rapidly view many specimens without interference by windows. The constant efflux of gas from the apertures also prevents "contamination" (carbon coating by the beam) of the specimen. Further experience will be required before the ultimate resolution of both types of chambers can be determined, but a resolution of better than 4.0 nm in the presence of 24 torr of water vapor has been observed at 200 kv for the JEM 200 differentially pumped chamber.

Electron Microscopy of Live Cells

In order to maintain cells alive in an electron microscope environmental chamber, they must be supplied with an adequate amount of nutrient medium, oxygen, carbon dioxide, and a sufficient total pressure to prevent bursting of the cells. The radiation damage of the electron beam must be kept sufficiently low to preserve the living processes under study. For useful imaging it is necessary for the cells and the water layer to be thin in relation to the electron penetration at the acceleration voltage used. The exposure time during photography must be made extremely short (possibly as short as 0.001 second) by the use of a time-controlled beam deflector in order to prevent blurring by Brownian motion of cell organelles. The optimal method of maintaining spread cells in a thin layer of medium and gas has not yet been worked out. The total pressure requirement to prevent bursting of cells or excessive gas bubble formation in the cytoplasm has to be adjusted for a gas composition that gives the least possible extraneous electron scattering; for example, the required oxygen and carbon dioxide pressures are kept at a minimum and helium is substituted for nitrogen. Problems associated with the accumulation of cell excretion products in a thin layer of liquid also need to

be worked out (*37*). In the absence of such data, it must be recognized that all earlier work has been of a "look and see" nature, and negative results with respect to motile functions of cells such as growth and division may be the result of inadequate environmental conditions rather than of excessive radiation damage. At the medium level of resolution required to see cell movements it is possible to considerably reduce the usual level of radiation damage in electron microscopy either by the use of extrasensitive photographic emulsions (*13*, *38*) or of image intensifiers (*13*). Radiation damage caused by high-voltage (1-Mev) microscopes may be up to seven times that of a 100-kv microscope as a result of the decreased efficiency of the phosphor screen and photographic emulsion at 1 Mev (*39*). Although the penetrating power of the high-voltage microscope is attractive for whole wet cell microscopy, lower-voltage scanning microscopy may give the same degree of penetration with less radiation damage.

Several investigators have claimed that they had observed the growth and division of cells that had been examined in an electron microscope hydration chamber. Stoyanova and her co-workers claimed that *Bacillus mycoides* had been observed to divide (*17*) and increase in size (*40*) in a thin film window chamber in a 75- to 80-kv microscope. Dupouy and his co-workers found that *Corynebacterium diphtheriae* and *Bacillus subtilis* could be specifically removed from the illuminated area of the grid and made to reproduce in culture after examination in a 1-Mev microscope fitted with a thin film chamber (*41*). The electron beam radiation given to the cells in these experiments was not stated. However, Nagata and Ishikawa (*42*) found that the dose for arrest of development and growth of 50 percent of the spores of *Bacillus megaterium* was small (about 1.5×10^{-6} coulomb per square centimeter at 100 kv, 7×10^{-6} coulomb/cm^2 at 800 kv). The spores were examined in the electron microscope without a hydration chamber since the spores are not affected by a vacuum. Additional confirmation of this reported viability, together with quantitative measurements of the electron beam radiation doses needed to arrest growth must be determined to establish whether any cells can be viewed in the electron microscope without loss of growth and multiplicative capacity. It appears possible that low-magnification pictures with resolution significantly better than that of the light microscope should be obtainable for some wet organisms since an electron micrograph taken on x-ray film at $\times 1000$ magnification requires only 1.12×10^{-6} coulomb/cm^2 of electron exposure at 1000 kv (*38*). This is significantly less than the dose for arrest of development of *Bacillus megaterium* spores. Motile processes of cells are, in general, more radiation-resistant than development or reproductive capacity and are being investigated quantitatively in our laboratory with the use of an environmental chamber. Swift and Brown (*26*), using a 20-kv scanning microscope fitted with a hydration chamber, observed an amoeba-like organism constantly changing shape and moving across the field of view. However, Morgan *et al.* (*27*), using similar equipment, were unable to observe motility in sperm. In our current work, we are adjusting the motility conditions using an environmental chamber fitted to a light microscope. Only when this modification is completed will it be possible to distinguish adverse environmental conditions from radiation damage as causes for stoppage of cell motility.

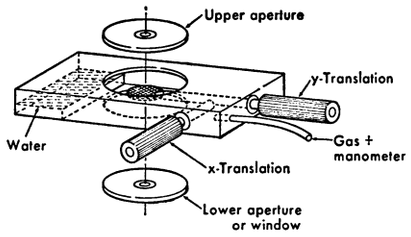
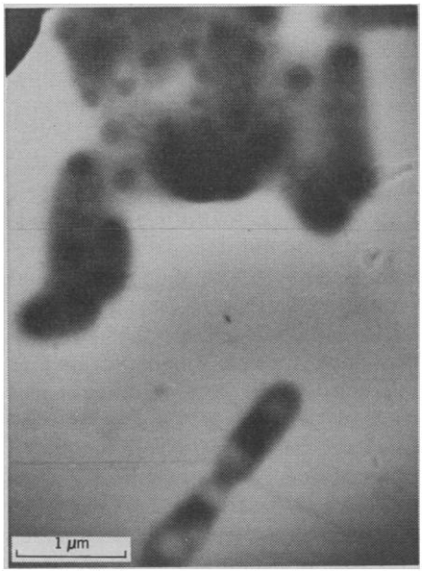


Fig. 6. Slide hydration chamber which permits microculture of cells on an electron microscope grid to be examined in either the light microscope or the electron microscope. The drop of medium on the grid can be changed by removing the upper aperture and the drop thinned down prior to electron microscopy.

Many interesting biological applications can be foreseen if it turns out that some motile functions of cells can be observed in the electron microscope (see Table 1). The slide type chamber shown in Fig. 6 facilitates the viewing of microcultures both in light and electron microscopes. With the use of such chambers it is possible that more information about the relation of cytoplasmic streaming to bundles of microtubules, and about the mechanisms of pinocytosis and muscle contraction, can be obtained. By allowing a small drop of reagent to diffuse across the grid, microchemical reactions, for example, the morphological effects of adenosine triphosphate or uncoupling reagents on mitochondria, can be studied directly. In cancer research a micro model of metastasis or malignant infiltration can be studied in the hydration chamber (*43*). This could be prepared by placing a small piece of tumor tissue adjacent to a piece of normal tissue and allowing the cells to grow toward each other and



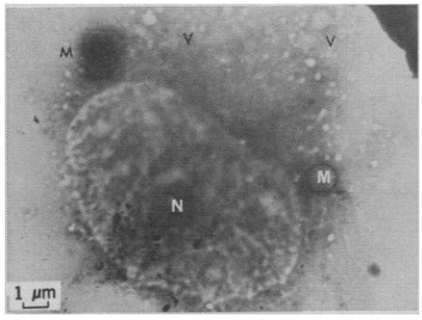


Fig. 7 (left). Coliform bacteria immersed in a thick layer of water. Dense inclusions and vacuoles are visible (800-kv acceleration voltage on the U.S. Steel high-voltage electron microscope). The chamber was at room temperature with a water vapor pressure of 22 torr (sample 25456). Fig. 8 (right). A portion of the cytoplasm and nucleus of a wet, unfixed, and unstained thinly spread 3T3 cell cultured on a carbon-Formvar film-covered gold grid. After having been removed from the medium the grid was washed in phosphate-buffered saline and transferred to the environmental chamber without drying. The dense bodies (*M*) in the cytoplasm are probably mitochondria, but other more transparent vesicles (*V*) are present. The nucleus (*N*) in this case is sufficiently thin to show chromatin structure (800 kv on the U.S. Steel high-voltage microscope). The chamber was at room temperature with a water vapor pressure of 22 torr (sample 34391).

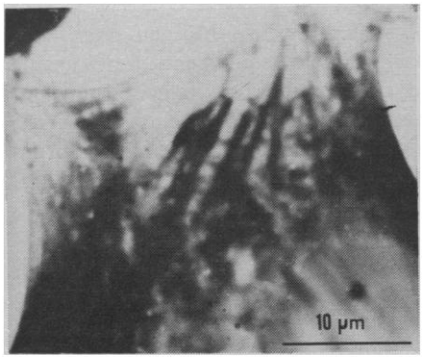


Fig. 9. Thin portions of the cytoplasm of a 3T3 cell in the wet, unfixed, and unstained state grown on a carbon-coated gold grid and washed in saline. The nucleus is not adequately penetrated by the 800-kv beam. Long cylindrical mitochondria are seen in the cytoplasm. This micrograph was obtained under the same conditions as Fig. 6.

spread out on the grid. The types of cell processes and cell-cell contacts can be studied with control double explants consisting of either two pieces of normal tissue or two pieces of tumor tissue. It has yet to be proved that such a model is a valid one for in vivo metastasis. Tumor-specific surface antigens can be detected on the wet living cells with the use of ferritin-labeled antibody. In addition, the movement of the antigen sites can be studied as a function of temperature (*44*).

So far, in our laboratory, we have been able to visualize several types of structures in whole wet, unstained, and unfixed cells (*10*). It has been possible to see inclusions in coliform bacteria, even though the cells were immersed in a thick layer of water (Fig. 7). In preparations where both the cells and the water are thinly spread, individual organelles such as mitochondria, other vesicles, and chromatin in the nucleus can be seen (Figs. 8 and 9). In other micrographs we have seen several kinds of structures not visible in the light microscope; these include fine peripheral processes less than 1000 angstroms in diameter and filamentous structures in the cytoplasm (possibly bundles of microtubules). The contrast and resolution of the wet cell electron micrographs so far obtained can undoubtedly be improved by refinement of the wet cell preparation methods, better application of dark field, and the use of techniques that reduce chromatic aberration (high-voltage, scanning transmission image mode, and the addition of an energy filter to remove inelastic scatter). Such refinements are in progress in the Roswell Park Memorial Institute Electron Optics Laboratory. Dark field, for example, shows the fine peripheral cell processes much better than bright field (Fig. 10) but shows less detail and contrast in thick areas of the cytoplasm and nucleus. With respect to wet cell conditions, Lyon and Parsons have recently shown (*45*) that the mixed gas atmosphere requirements for maintaining motility are practical and that motility can be observed at total pressures of less than 200 torr.

Other Biological Applications

The electron beam can be used to irradiate specific organelles and new light thrown on the radiation sensitivity of different target sites in the cells. Establishing the radiation sensitivity of different motile functions should lead to a clarification of the ultimate possibilities for the examination of live cells in the electron microscope. The effect of toxic and polluting gases on cell movements can be studied, and there is a possibility of studying the morphological changes that may occur when different cell types are subjected to unusual (for example, Mars-like) environments.

In examining several types of cells (human white cells, 3T3 mouse fibroblasts), what appear to be gas bubbles forming in the cytoplasm were observed (Fig. 11). Presumably these arise when the pressure inside the hydration chamber is lowered from 760 torr to between 25 and 47 torr. The hydration chamber, therefore, appears to be useful in studying the earliest changes of formation of gas bubbles in both cells and thin plasma layers in relation to problems of decompression (diver's "bends"). Vacuolar structures have been observed with the light microscope on the decompression of *Tetrahymena pyriformis* (*46*) and amoeba (*47*), but the delay of some minutes in their formation led to the assumption that they were liquid-filled vesicles rather than gas bubbles. Light microscope studies of bubble formation in the small vessels of the hamster cheek pouch (*48*) did not have the resolution or contrast necessary to permit visualization of possible gas bubbles forming in the cells of the tissue. These negative indications about gas bubbles forming inside cells are contradicted by the fact that the decompression of cell suspensions with nitrogen gas has now become a widely used method of breaking cells. Hunter and Commerford (*49*) used rapid decompression from 1300 pounds per square inch of nitrogen (14.6 pounds per square inch = 1 atmosphere) to disrupt rat cells (< 700 pounds per square inch was inadequate). Gas bubbles appear to preferentially form in the cytoplasm, since in the range of 800 to 1000 pounds per square inch the cytoplasm is disrupted but the nucleus is left intact. Even cells that are difficult to break mechanically (yeast or bacteria) can be disrupted by nitrogen decompression. Fraser (*50*) used a pressure of 900 pounds per square inch to burst 75 percent of *Escherichia coli* cells.

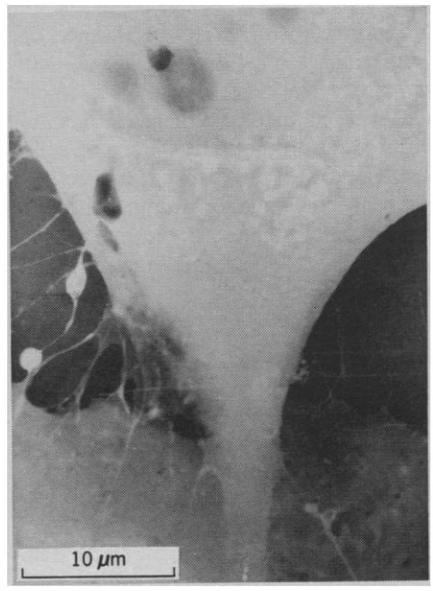


Fig. 10. Spread unstained 3T3 cell (in this case, glutaraldehyde-fixed since this caused excess water to retract away from the cells); dark-field observation. The fine peripheral processes are well visualized whereas the thicker portions of the cell are not. Displaced 30-μm aperture; 800 kv; water vapor pressure, 22 torr.

In recent work (*51*), Basu and Parsons have successfully developed the differentially pumped hydration chamber to permit the replication of wet cell surfaces inside a vacuum evaporator. Silicon monoxide or carbon can be evaporated through the apertures and water vapor layer to give a replica of the wet surface of whole cells and of the surrounding liquid. Dorset and Parsons (*52*) have quantitated the reflections from large numbers of photographs of wet catalase electron diffraction patterns. The results indicate that the data for large unit cell crystals of proteins are essentially kinematic, and a unique set of intensities can be obtained for crystallographic analysis and image computation. Other proteins (particularly myoglobin) are also being studied.

Hui and Parsons have recently shown

(*53*) that electron diffraction with the use of a fine beam of wet natural [retinal rod disks (*54*), red blood cell membranes (*53*), and liver cell plasma membranes (*55*)] and synthetic pure phospholipid membranes gives valuable information about the structure and lipid phase transitions of individual small domains of membranes. Also, electron diffraction patterns can be obtained from a single membrane layer. In x-ray diffraction a much larger beam size is used, the domain structure is averaged, and ordered stacks of many membrane layers are required. The electron diffraction patterns from dry membranes are quite different; the difference is apparently attributable to artifactual recrystallization of membrane lipids.

Finally, the use of the environmental chamber to study the structure of thin layers of water by electron diffraction should be stressed since water films of controlled thicknesses can be maintained on various polar and nonpolar substrates. Attempts are also being made to determine the structure of the water inside the cytoplasm of whole wet cells and to compare it with the structure of bulk water.

Conclusions

Several recent technological advances have increased the practicality and usefulness of the technique of electron microscopy of wet objects. (i) There have been gains in the effective penetration of high-voltage microscopes, scanning transmission microscopes, and high-voltage scanning microscopes. The extra effective penetration gives more scope for obtaining good images through film windows, gas, and liquid layers. (ii) Improved methods of obtaining contrast are available (especially dark field and inelastic filtering) that often make it possible to obtain sufficient contrast with wet unstained objects. (iii) Improved environmental chamber design makes it possible to insert and examine wet specimens as easily as dry specimens.

The ultimate achievable resolution for wet objects in an environmental chamber will gradually become clear experimentally. Resolution is mainly a function of gas path, liquid and wet specimen thickness, specimen stage stability, acceleration voltage, and image mode (fixed or scanning beam) (*13*).

Much depends on the development of the technique for controlling the thickness of extraneous water film around wet objects or the technique for depositing wet objects onto dry, hydrophobic support films. Although some loss of resolution due to water or gas scattering will always occur, an effective gain is anticipated in preserving the shape of individual molecules and preventing the partial collapse that usually occurs on drying or negative staining.

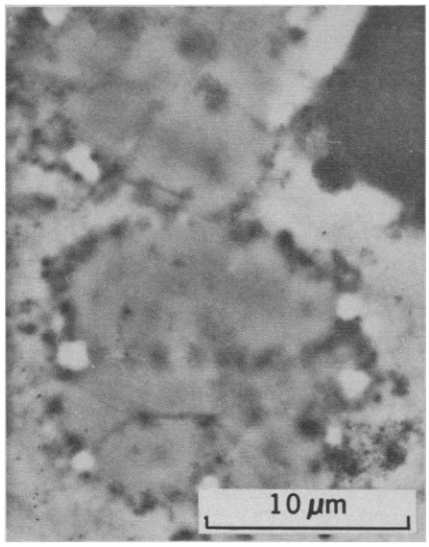


Fig. 11. Human white blood cell in a thick layer of medium in an environmental chamber after having been decompressed from 1 atm to 27 torr of water vapor. Markedly electron-transparent vesicles (assumed to be gas bubbles) are visible (sample 33354).

The most basic question for biological electron microscopy is probably whether any living functions of cells can be observed so that the capabilities of the phase contrast and interference light microscopes can be extended. Investigators are now rapidly approaching a final answer to this question. The two limiting factors are (i) maintaining cell motility in spread cells immersed in thin layers of media and (ii) reducing beam radiation damage to an acceptable level. The use of sensitive emulsions and image intensifiers can bring the observation dose below that required to stop cell motility. Use of a timed, pulsed deflector system enables sufficiently short exposures to be obtained to eliminate blurring due to Brownian motion.

Environmental chambers have enhanced the possibilities of electron diffraction analysis of minute crystals and ordered biological structures. High-resolution electron diffraction patterns (especially kinematic) of protein crystals can only be obtained in a wet environment. Hence, it may now be possible to obtain undistorted images of protein molecules. Moreover, by subjecting diffraction patterns to image-iterative techniques (*56*), it will be possible to phase the electron diffraction patterns to give a calculated image with a higher resolution than that which can be produced by electron microscope objective lenses.

Environmental chambers offer exciting prospects for the determination of water structure and water and ice nucleation (atmospheric science). Nucleation data near the molecular level have been badly needed for some time.

The application of environmental chambers in industrial chemistry, for example, in studies of polymerization, catalysis, and corrosion, are awaiting exploration. They offer an unusual approach to measurements of reaction kinetics through images that should be both sensitive and rapid.

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Computer Networks: Making the Decision to Join One

By joining a network, an academic computing center gains economies on scale yet can specialize in a chosen field.

William F. Massy

Today it is not only possible, but routine, for researchers at the University of Illinois to do serious computing on machines in San Diego or Los Angeles, or to see a time-sharing user in Paris be indifferent to the fact that the computer on the other end of the line is in Cleveland, Ohio. Computer networks are commonplace, and they occur in many different forms.

Discussion of computer networking has grown enormously during the last 18 months. Three working seminars, sponsored by the National Science Foundation and held by the Interuniversity Communications Council (EDUCOM) at Airlie House, Virginia, in the winter of 1972 to 1973, served to focus interest on networking (*1*), and a Planning Council on Computing in Education and Research has been formed. This council consists of senior academic officers and computer specialists from a number of institutions and will operate in conjunction with EDUCOM. Many of the issues I consider in this article will be addressed by the council as it attempts to chart a course for large-scale academic computer networking.

I shall first discuss some of the pressures put on university computing centers and their directors. Because I am a faculty member and university officer, rather than part of a computing organization, I can express my views freely on the subject. I shall then distinguish between two types of networks, the computer utility concept and the distributive network, and comment on how each type of network is likely to affect the decisions about computing on university campuses—which is the main subject of this article. I fear that some may find this treatment unduly speculative: my defense is that informed and hopefully insightful speculation is about all we have to go on at the present time when we assess the broad-scale questions of computer networking.

Pressure on College and University Computer Centers

The pressures put on computer centers and their directors are already well known; I will give only a few examples to illustrate them.

1) There are increasingly broad demands for computer services. New fields for computer use, new applications, and new approaches to computer systems are multiplying rapidly, both because of the velocity of technological change in computer hardware and software and because of the continued diffusion of understanding of computers and interest among potential user groups. New uses are to be found in both the academic (teaching and re-

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