limited and is confined to the monocytes, the principal event being that the red cells are bound tightly to the monocyte surface. This binding of red cells coated with either antibodies or aggregates containing γG does not ordinarily represent merely a preliminary phase of erythrophagocytosis in the usual sense, but it is nevertheless rapidly injurious as indicated by the sphering, deformation, and apparent fragmentation of the bound cells. Although differing in several distinct ways from the cytophilic antibodies described by others (*4*) in animals, these 7*S* γG antibodies of man may represent a more specialized or evolved form of cytophilic antibody in which the function of binding to antigens has been divorced from complement-promoted processes such as phagocytosis and lysis. This would allow an orderly transport of particles to specific tissue sites, and trapping or containment therein, without immediately or necessarily causing phagocytosis, or even lysis, and without the abrupt or dangerous effects of activating complement. The functional adequacy of antibodies that can induce removal of antigenic cells from the blood stream but cannot lyse them or induce extensive phagocytosis is well documented in isoimmune hemolytic processes (*2, 15*). The specific reaction of γG with monocyte and macrophage receptors provides a logical explanation for the mechanism of action of incomplete antibodies in man.

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1. Immunoglobulins G, M, and A are identified as γG, γM, and γA, respectively. In the antiserums used, isoantibodies reacting with the D (Rh_o) antigen of human red cells were entirely of the γG class and are denoted as anti-D; isoantibodies for the A, B, and I antigens were all of the γM class and are denoted as anti-A, anti-B, and anti-I, respectively; EDTA, ethylenediaminetetraacetic acid.
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21 August 1967

Electron Microscopy: Enhancement of Specimen Contrast by Injection of Atoms

Abstract. *Injections of biological specimens and substrates with cesium were made with a small accelerator in the energy range of 20 to 1000 electron volts. Subsequent electron microscopic examination demonstrated that the contrast and appearance of the specimen depend on its structure and on the energy of injection. Substrate noise is decreased over conventional contrasting techniques. The same accelerator provides controlled etching of the specimen.*

It is well known that the utility of conventional electron microscopy for investigation of biological specimens, indeed of any material of low atomic number, is very dependent on techniques that enhance contrast. The most common of these are heavy-metal shadowing, negative staining, and positive staining. The first technique outlines the specimen by a surface deposit; the second depends primarily on the drying of a solution of the heavy element around the specimen or in regions of it that are penetrated by the solution; and the third generally involves a more selective chemical interaction between specimen and solution. As instrumental resolution improves, these techniques themselves impose a limit to observable detail. This occurs particularly in the first two because of inherent aggregation of the contrasting atoms. The result is a granularity of at least 5 Å, usually more, providing a noise background in which the specimen must be observed.

Injection of atoms holds promise of improving the signal-to-noise ratio for high-resolution work and of adding a new feature to the examination of specimens in the whole range of electron microscopic observation. In essence, it is an extension of the heavy-metal-shadowing technique. The extension is that of an increase in the energy of the atoms impinging on the specimen by several orders of magnitude.

Consider the appearance of a specimen to an atom speeding toward it with an energy controlled by the experimenter. Even a distant view is that of extremely rough terrain. On closer approach the complicated arrangement of complex molecules with voids and hills appears; still closer, as the atoms of the molecules appear, they are discerned in groups of varied geometrical array as dictated by the chemical bonds between them. Surface and interior voids from one to ten or more bond lengths (a few to tens of angstroms) are seen. There are regions of softness and hardness—weak and strong bonds. Finally there is the inevitable collision of the impinging atom somewhere in this structure—early, if the path encounters a promontory; later, if it coincides with a surface void. If the energy of the approaching atom is low, as in shadowing ($\sim$ 0.1 ev), weak surface forces will be adequate to hold the atom in a surface layer, although not necessarily at the place it strikes. At energies of the order of the bond strength ($\sim$ 1 ev) at the particular point of impact, the atom may be elastically scattered and escape, or it may be captured. Capture can depend on several factors. A sufficiently strong chemical bond, relative to the incoming kinetic energy, may form between the incoming atom and atoms of the specimen. The latter can be displaced, thus

admitting the "foreigner," and the rapid dissipation of its energy among the many degrees of freedom in the neighborhood will then prevent its escape (interstitial formation). The "foreigner" may relieve a preexisting strain and be caught by such a minimum-energy process. (This is a well-known process in solid-state phenomena in which thousands of atoms can be trapped in a dislocation.) At still higher energies of the incident atom, penetration of the specimen increases, some bonds are broken, and atoms of the specimen are permanently displaced in the interior or removed from the surface. The first would be called "radiation damage," the second "ion etching." It is known that 20 to 30 ev are required to displace an atom in simple inorganic materials. In a complex structure of many degrees of freedom for energy dissipation, several or many times this energy may only rarely result in enough concentration on one atom to displace it and significantly damage the specimen.

The attractiveness of atomic injection resides in the fact that all of these processes are dependent on the atomic and molecular details of the specimen, relative to the controlled energy and to the type of injected atom. At will, the experimenter can cover a wide range of phenomena. At low energy, conventional shadowing is approached but modest penetration would inhibit surface migration on both specimen and substrate, thus decreasing background noise from granularity; at high energy, a carefully controllable etching is available. In between, selective features of the specimen can be revealed by both variation of energy and selection of the electron-dense atom for its chemical bonding properties. Specimens may, of course, be etched at one energy and "stained" at another.

In order to test whether these concepts provide meaningful contrast by atomic injection, a source of energetic atoms of relatively large electron scattering power is required. Accordingly, a small accelerator has been built to operate at energies from a few volts to a few kilovolts. For reasons of technical simplicity, cesium has been chosen as the contrasting agent. Since it is a very reactive element, chemical effects of its capture in the specimen can be expected. Appropriate choice of source design would permit the use of almost any element, for example, xenon, if chemical effects are unwanted. A schematic drawing of the accelerator

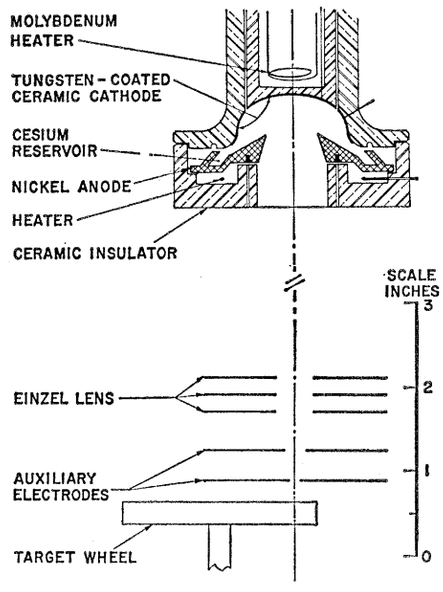

Fig. 1. Schematic drawing of cesium accelerator.

is shown in Fig. 1. The source, mounted on sylphon bellows for alignment, consists of a specially shaped nickel anode with an annular slot in which a fraction of a gram of cesium azide is placed. A heater maintains a decomposition temperature. Cesium atoms strike the tungsten-coated, heated cathode where they convert to ions that accelerate through the anode opening in a beam formed by the electric field between the surfaces of the specially shaped cathode and the anode. This beam is concentrated by the einzel lens and auxiliary electrodes through a hole [⅛ inch (0.3 cm) in diameter] in the lowest electrode. This hole has a fine screen so that the region between it and the target wheel can be made one of zero field. Standard 3-mm specimen grids are held in the 18-position wheel. External control permits successive injection of this maximum number of samples at one loading. All electrodes are equipped with adjustable voltages from 0 to 3 kv and with current meters. Two thermocouples are strategically located on the source and another measures temperature of the target. The impinging energy is determined by the net voltage difference between cathode and target and may be chosen as desired. The target current is independently controllable up to a few microamperes. At 1 μa, a 10-minute exposure results in an injection of 4.3 atoms per square angstrom, about the density of heavy-metal shadowing. The word atom is used advisedly, for although ions are accelerated it is likely that a low-velocity ion will be neutralized close to the surface and penetrate as an atom.

The design of the accelerator is such that some neutral cesium atoms and thermal radiation from the cathode can strike the specimen. The latter can be computed as, at the most, 30 mwatt over a 3-mm grid, only a minute portion of which is absorbed by the specimen. However, no effects have been observed on controls exposed under identical conditions, but without the accelerated beam. A geometric change in the accelerator will eliminate any effect from neutrals or thermal radiation if it ever proves troublesome. An additional control is customarily obtained by placing a 100- or 200-mesh grid over the specimen. Subsequent examination in the electron microscope can then be made of adjacent areas—injected areas of the opening and areas masked from the cesium by grid bars.

Contrast of any specimen supported by a substrate depends on a differential acceptance of injected atoms by specimen and substrate. Furthermore, temperature-dependent surface migration can change the distribution of density during or after the injection. Accordingly, a number of experiments on substrates have been done. From these it can be concluded that there is significant variation in acceptability of injected atoms between carbon, formvar, and parlodion films and thin sections of embedded specimens. By observations of identical areas after injection at 20°C and again after holding the specimens at 37°C overnight, direct evidence for release, subsequent migration, and nucleation of cesium compounds has been obtained. Thus the expectation of decreased mobility of injected atoms has been confirmed. Evidence has also been obtained that some films and biological specimens begin to show characteristics of having been etched at energies as low as 400 ev.

An example of a cesium-injected araldite section including a cell of *Neurospora*, fixed in osmium, is shown in Fig. 2. This was a fortunate case in which the mask divided the cell almost equally between the darker injected area and the lighter area protected by the mask. The plastic itself is not a good example of what can be obtained with care in decreasing granularity, for there has been obvious migration. On the other hand, the biologi-

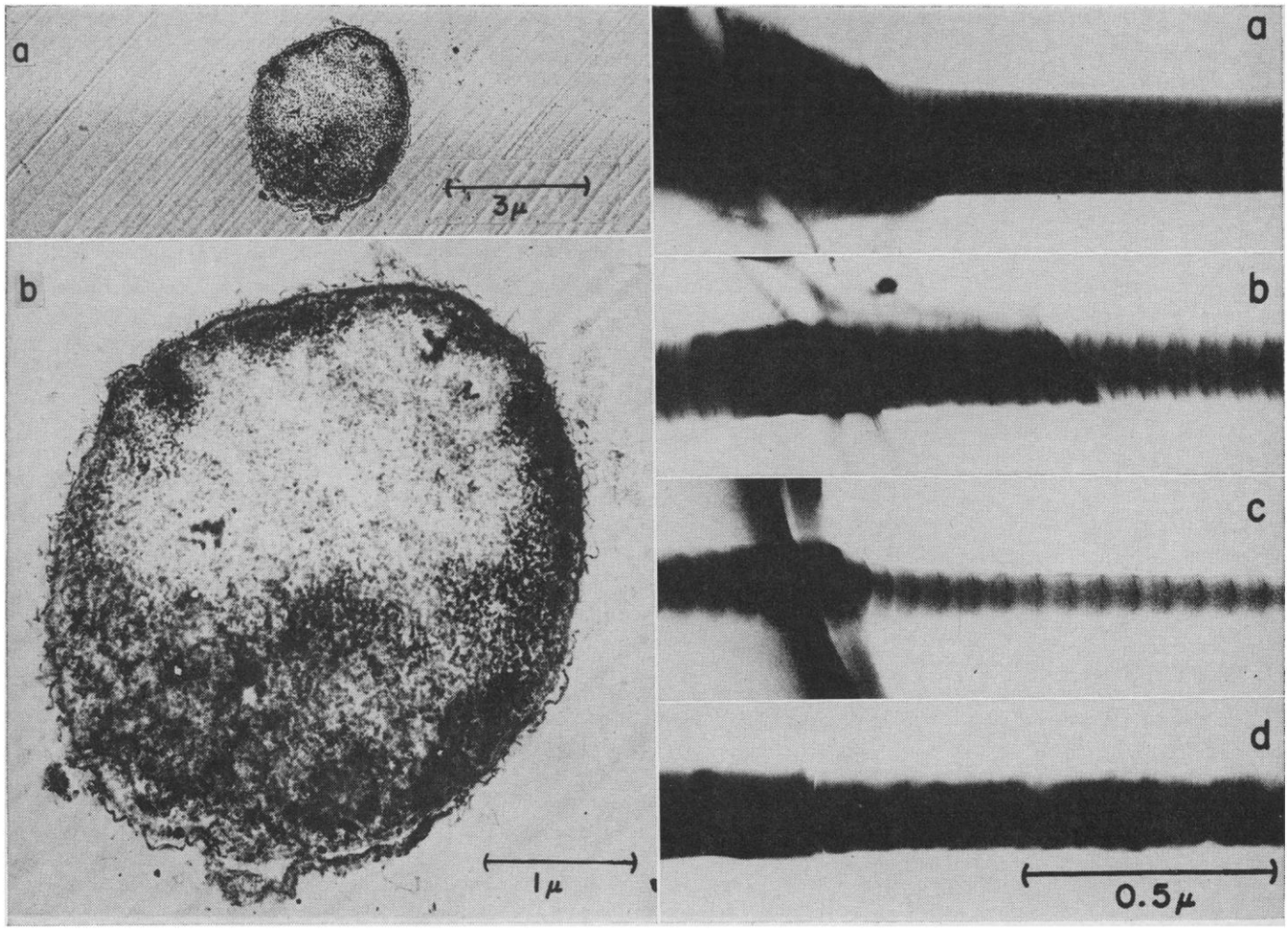

Fig. 2 (left). (a) Araldite section including *Neurospora*. Upper portion masked from 800-ev cesium beam to illustrate contrast with lower portion receiving 1.4 atoms per square angstrom. Electron microscopy: Elmiskop I, 80 kv, 100-μ condenser, 30-μ objective apertures ($\times$ 4000). (b) Same, but electron microscopy $\times$ 12,000 to illustrate specimen contrast in and out of cesium area. Fig. 3 (right). A series of collagen fibrils on films having many holes with different cesium injection conditions. (a) Shadowed by thermal decomposition of CsN_3; injected (b) at 50 ev, 3 atoms per square angstrom; (c) at 200 ev, 1.5 atoms per square angstrom; and (d) at 800 ev, 3 atoms per square angstrom.

cal specimen shows considerably more detail, internally and in the cell wall, in the injected half.

A rather extensive series of experiments with collagen fibrils has been carried out, partly because their fibrous nature permits observation on films with many holes and, therefore, both with and without substrate effects. All preparations of this protein have been checked with conventional phosphotungstic acid positive and negative stains for control of fibril quality. Figure 3 shows a series of reconstituted collagen fibrils prepared from an acetic acid solution injected at several energies, but to approximately the same cesium density. Differences in acceptability, as a function of location on the fibril and of injection energy, are immediately obvious. Variations have been observed with injection of cesium even when not detectable with conventional contrasting agents. This is not surprising in view of the rather different mechanisms operating. Ultrahigh resolution of various samples has not yet been attempted, but details of the order of 20 Å or less have been observed.

Experiments to date confirm essentially all the concepts that led to this investigation. Specifically the most important are: (i) Surface migration is inhibited if atoms are injected into the material rather than deposited by vapor condensation or from neutral solutions. This decreases the aggregation responsible for background noise and improves signal-to-noise ratio. (ii) The acceptability of injected atoms depends on the structure of the specimen and the energy of injection. The contrast provided by such atoms therefore provides a new approach to investigations of ultrastructure in which features of the specimen are revealed by mechanisms different from those operating in conventional contrasting techniques. (iii) Ions of 400 ev or more, with the carefully controllable energy derived from an accelerator of this type, provide selective etching of sample surfaces.

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References and Notes

1. Work supported by the U.S. Atomic Energy Commission. Portions of this report have been presented at the silver anniversary meeting, Electron Microscope Society of America [*Proceedings EMSA* (1967), p. 369] and at the autumn meeting, National Academy of Sciences [*Science* **154**, 424 (1966)].
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21 September 1967