ELECTRON MICROSCOPIC OBSERVATION OF SPECIMENS UNDER CONTROLLED GAS PRESSURE

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ABSTRACT

A technique for encasing specimens in a thin gas layer during their observation in the Siemens Elmiskop I is described. All gases can be employed at pressures up to one atmosphere. Destruction of specimens can occur in the beam; all organic specimens are particularly liable to decompose. The conditions under which this can be avoided are given. A useful application of the technique allows one to prevent specimens from drying out, as they normally do in vacuum. A further application uses the controlled removal of carbon for thinning organic layers and for selective etching of organic materials.

TECHNIQUE

In many instances during observation with the electron microscope, it would be interesting to expose a specimen to a controllable gas environment rather than to vacuum. In order not to reduce the quality of the image, the thickness of the gas layer must not exceed a certain limit, depending on the nature of the gas and its pressure. This limiting thickness for air at atmospheric pressure is 5 to 10μ if the highest obtainable resolution is required; otherwise it can be greater. The generation of such a gas layer around the specimen is technically not very difficult, and has already been described by several authors (1 to 8). Figs. 1 to 3 show a device for this purpose which is designed for use with the Elmiskop I. The specimen chamber is formed by two specimen grids of the Siemens type with the flat surfaces facing each other and kept apart at the desired distance by pieces of thin metal foil (Fig. 1 *a*). Both specimen grids are covered with a supporting film of low contrast, which has to withstand the gas pressure over the central openings (diameter 50 μ). One of them serves at the same time as a supporting film for the specimen. The specimen holder can be inserted into the microscope in the usual way and will automatically seal the gas inlet against the vacuum in the column. The gas is injected through a tube placed in the opening of the column provided for the stereo drive (Fig. 2). The necessary valves, etc., outside the microscope, should be clearly arranged and easy to use (Fig. 3). With this device it is relatively easy to maintain a sufficiently thin layer of gas around the specimen, so that scattering of electrons by the gas molecules does not disturb the image to any perceptible extent. Good pictures are obtained without difficulty at air or gas pressures up to 760 Torr (mm Hg). The lowering of contrast due to the second supporting film sealing the chamber is usually compensated for by the complete absence of specimen contamination.

RADIATION DAMAGE

More important difficulties are those which arise from interactions between the electron beam, the specimen, and the gas molecules, and which result in damage to the specimen. Of greatest importance is the interaction between the carbon atoms and the gas molecules due to irradiation. Volatile compounds are formed and the carbon is carried off. Therefore, with all organic materials, radiation damage which would not occur in a vacuum has to be taken into account.

Any removal of carbon will, of course, reduce the well known specimen contamination in the electron microscope, which is caused by the hydrocarbons of the residual gas (4). To examine the effect of different gases, the rate of contamination was measured at increasing gas pressures (Fig. 4). The double condenser was used, and the diameter of the illuminated area was 1.5 μ. This causes rapid contamination because the temperature of the specimen under these conditions is relatively low (*e.g.* 9). The gases introduced were sufficiently clean and dry, but because of the rubber seals, etc., they contained the same partial pressure of hydrocarbons (~ 10^{-6} Torr) as the residual gas in the microscope. All gases investigated caused a more or less rapid decrease in the rate of contamination as compared to the contamination rate *in vacuo*. The initial increase shown by H_2 and He can be explained as being due to a cooling of the specimen by the gas. It can be seen from the curves that the rate of contamination, which is extremely high under the conditions used, is just compensated for by the removal of carbon if air at a pressure of 12 Torr is used in the chamber. For some other gases the corresponding pressure under otherwise identical conditions is found to be much higher: for N_2 and Ar, ~ 220 Torr; for H_2, 380 Torr. At higher pressures, carbon removal prevails and all carbon-containing specimens are destroyed. The numerical values given here are, of course, not of great significance, since the critical pressures are highly dependent on the conditions of illumination and also on the thermal conductivity of the specimen, among other factors. When only one condenser lens of the Elmiskop I is used to focus the source on the specimen (illuminated area 50 μ diameter and 0.4 A cm^{-2} in the specimen), the "zero contamination rate" for a SiO-film 400 A thick on a Pt grid of 70 μ diameter was found at the following pressures: air, 0.1 Torr; N_2, 20 Torr; Ar, 25 Torr; H_2, 50 Torr.

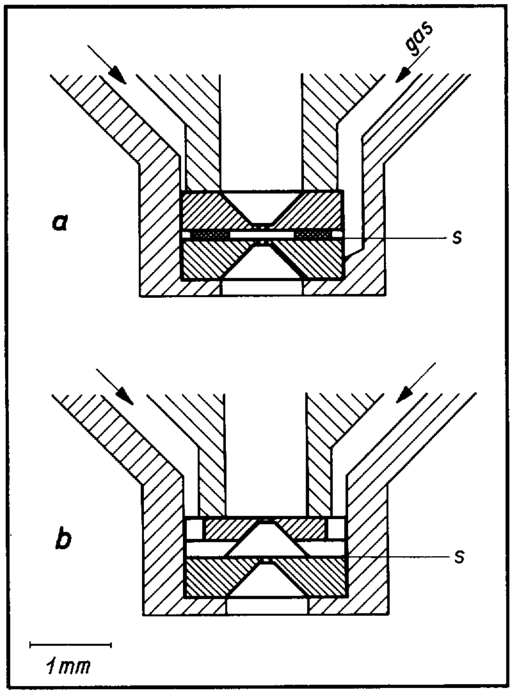

FIGURE 1

Specimen chamber for Elmiskop I.

a. For thin layers of gas (about 5 to 50 μ) at pressures up to 760 Torr (1 Torr ≈ 1 mm Hg). The holes of both grids (diameter 50 μ) are covered with supporting films; the lower one carries the specimen, *s*. The upper aperture has small grooves on the sides and lower face to permit the introduction of gases.

b. For pressures up to 20 Torr (gas layer thickness between 0.4 and 0.6 mm). The lower grid (diameter of hole 70 μ) carries the specimen on a supporting film. The upper grid (diameter of hole 50 μ) has no film and serves to reduce the escape of gas into the vacuum.

Of interest for an interpretation of the process is the increase of the rate of carbon removal for different gases at constant pressure but increasing specimen temperature (Fig. 5). The increase in temperature is obtained by increasing the illuminated area and keeping the current density constant, which, of course, causes a decrease in the contamination rate. Assuming that contamination and removal of carbon are independent of each other and occur simultaneously, the actual removal is given by the difference (on the ordinate) between the heavy curve showing the contamination *in vacuo* and the respective curves for the contamination at a given gas pressure. The characteristic difference between air and N_2 or Ar holds also for pressures other than those shown in Fig. 5. In the case of air, carbon removal increases rapidly with increasing temperature; in the case of N_2 or Ar, it does not. Evidently, in air and O_2 the elementary process that leads to carbon removal is mainly the conventional type of chemical reaction activated by thermal energy. In N_2, H_2, and the noble gases, the processes are mainly radiation-induced. For several reasons, one can conclude that the effective ionization process involves mainly molecules already adsorbed on the surface, whereas free molecules

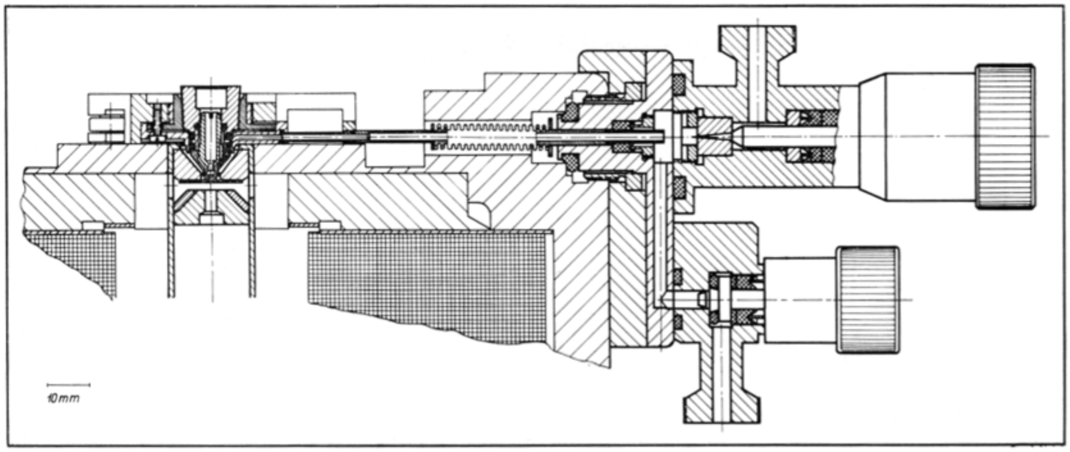

FIGURE 2

Device for admitting gas to the specimen chamber in the Elmiskop I. At the left side the special specimen holder, at the right a needle valve and a plate valve in parallel arrangement. The tube through which the gas is introduced can be moved inside the surrounding rubber vacuum seal, and therefore does not hinder the movement of the object.

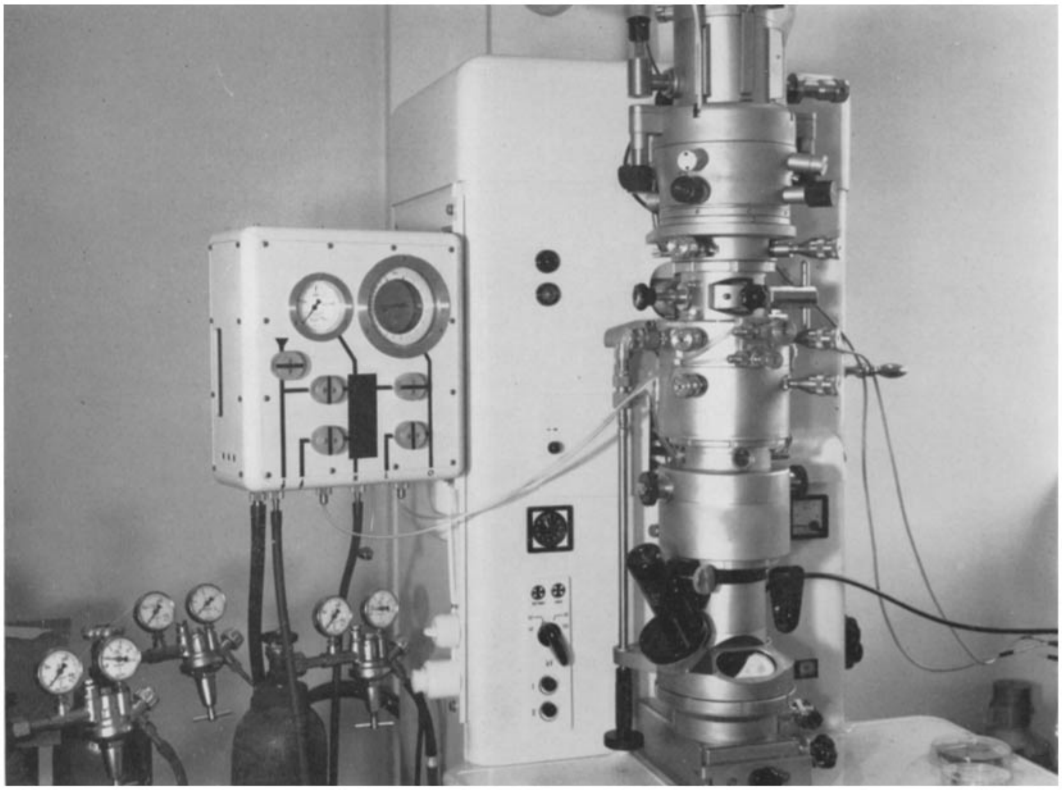

FIGURE 3

View of the installed apparatus. Two tubes connect the valves at the objective lens with the box mounted on the left side of the microscope. The box contains a membrane pressure gauge with a reference vacuum (measuring range 0.1 to 50 Torr), a gas storage tank (capacity 1 liter), five valves and the necessary connections for evacuating, filling, and flushing with gas. The indicated flow scheme facilitates the use of the apparatus.

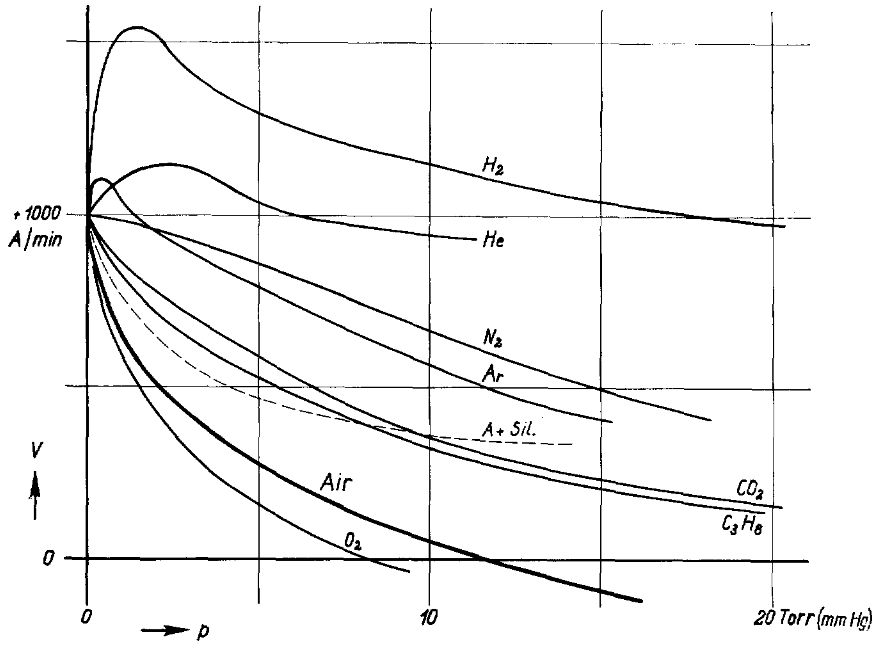

FIGURE 4

Rate of specimen contamination as a function of the pressure and the gas used in the chamber. Double condenser illumination. Negative values indicate a removal of carbon. For the *A* + *Sil* curve, air is introduced through a tube containing a silicon grease layer.

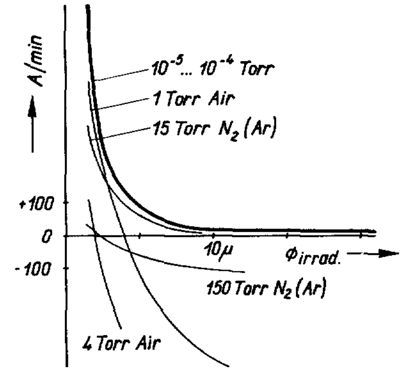

FIGURE 5

Growth and removal rates of the contaminating layer at constant beam current density (0.4 A cm^{-2}) as a function of an increase in temperature, brought about by increasing the illuminated area.

ionized before impinging on the specimen play only a minor role. But further investigation of this problem is necessary. The molecules formed in the case of the noble gases (*e.g.* CHe) can, of course, only be short-lived.

The removal of carbon is, without doubt, the most important reaction but, of course, not the only one occurring when gases are admitted to the specimen chamber. Some metals, for instance, can be oxidized in air or reduced in H_2. It may be of some interest that the reduction of copper oxide can also be brought about *in vacuo* with the aid of a layer of contamination deposited first, if the proper conditions of irradiation are given.

APPLICATIONS

Several possible applications of this technique are of interest to the biologist. We shall first discuss the avoidance of desiccation of the specimen in the microscope. With inorganic specimens there is no difficulty if air at atmospheric pressure is used in the specimen chamber and excessive heating of the specimen is avoided.[1] With organic

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[1] In this case, substrates and windows also must not be weakened by carbon removal in the beam. Good results have been obtained using films produced by evaporating silicon oxide onto normal collodion films and heating these subsequently to 200°C in air.

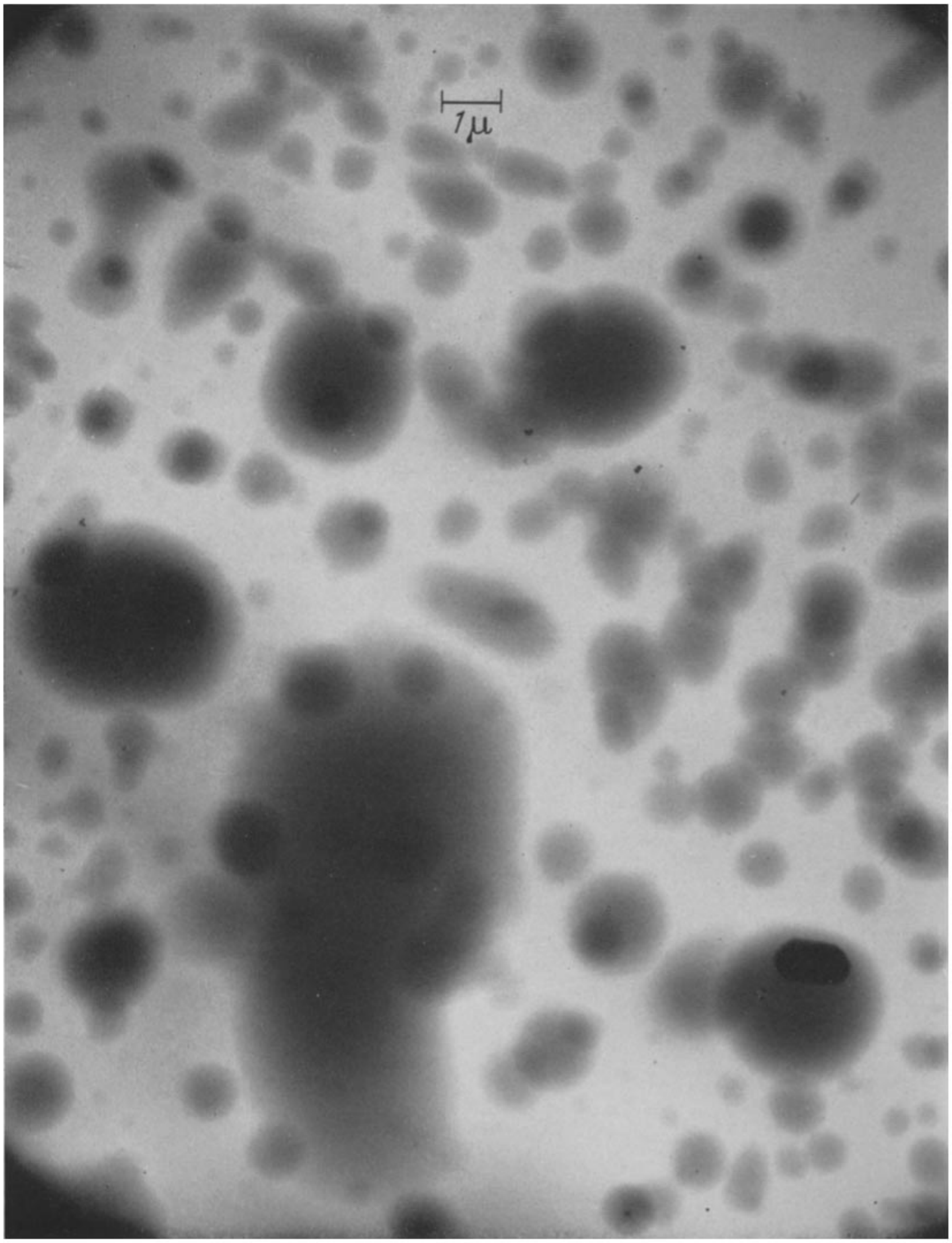

FIGURE 6

Water droplets formed by condensation on the supporting film. At a pressure of 100 Torr they are stable under the beam. Electron optical magnification × 2600.

specimens it is necessary to prevent carbon removal from increasing to a rate higher than the rate of contamination, which has been shown to occur with all gases above certain pressures, and which would result in destruction of the specimen.[2]

It is evident from the vapor pressure curve of water that, even at pressures of 100 to 200 Torr, a rapid dehydration of the specimen can be prevented if unnecessary heating is avoided. This has been proven by taking electron microscopic pictures of small water droplets in air at 100 Torr (Fig. 6). At this pressure it is possible to prevent carbon removal in the specimen if H_2, He, N_2, or Ar instead of air is used and the illuminated area is reduced to $\sim 2\ \mu$ diameter with the aid of the double condenser. If the specimen cannot be evacuated even briefly, the air in the chamber has to be flushed out with the gas. This provides some experimental difficulties. There should be still another possibility of preventing destruction of the specimen even at higher pressures and in air, *i.e.*, by increasing the partial pressure of hydrocarbons in the gas introduced into the specimen chamber, using, for instance, an oil with an appropriate vapor pressure. However, this approach has not been tested experimentally.

Further applications of interest are the direct observation of conventional and radiation-induced chemical reactions in the microscope and the controlled removal of carbon, which can be used for morphological studies of specimens. For the latter purpose the simple arrangement shown in Fig. 1 *b* can be used in combination with a needle valve to admit air. It is easy with this device to reduce relatively thick specimens of organic material to thinner, more transparent layers. The removal of material by means of gases activated by the electron beam does not occur at the same rate with all carbon compounds. Thus a differential etching of organic materials is possible which adds contrast to the image. This should be of interest in many organic specimens, especially since the process can be observed continuously, varied in rate, or stopped completely and then resumed. This is so easily accomplished, merely by opening and closing the needle valve, that the application of this technique should be very simple.

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[2] All types of supporting films could then be used.

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