BIOLOGY

The Possibility of distinguishing between Substances shown on Electron Micrographs

THE electron microscope can be used to investigate the morphological features of a specimen right down to the molecular level, but there is as yet no way of identifying the chemical nature of the substances seen, except on the rare occasions when these happen to be crystalline. In a specimen of uniform thickness, a region of high electron opacity may, in general, be said to be one containing substances of a greater weight density than those of the surrounding less opaque region[1], but this gives little help in deciding on their chemical composition. The lack of any direct way of knowing what substances one is looking at is perhaps the most serious shortcoming of electron microscopy.

It thus seems worth describing a simple way in which the image formed by an element of high atomic number differs from that of an element of low atomic number. Biological substances seldom vary significantly in the mean atomic number (calculated on a weight basis) of the elements composing them, but the method to be described can be used, for example, to identify which parts of a specimen have taken up an added 'stain' of high atomic number.

It is well known that the contrast between the different features of a biological specimen can be increased by reducing the size of the objective-lens aperture. This observation is in good accord with the theory of electron scattering deduced from considerations of quantum mechanics[2,3]. Hall and Inoue[4], for example, found that when the angular aperture of the objective was reduced from $4{\cdot}2 \times 10^{-3}$ to $2{\cdot}1 \times 10^{-3}$ radian, the scattering cross-section of polystyrene latex increased by 30 per cent. Lenz's theory[2,3] predicts an increase of about 25 per cent. But with an element of high atomic number, theory suggests that the size of the aperture will have little effect on contrast. I have made observations which in fact confirm this; for, unlike carbon, films of gold, platinum and tungsten show no detectable change in opacity when the objective aperture is changed for one of half the size.

The method of differentiating substances of high atomic number from biological or other material of low atomic number thus consists simply of taking two electron micrographs of the same field using a different-sized aperture for each. With many microscopes, changing the aperture involves only a simple manipulation that does not even require the beam to be switched off. The two plates are similarly exposed and developed. All substances with a mean atomic number (Z) less than 12 should show an image of markedly different density on the two plates. Two conditions, however, must be satisfied for this change to be seen. First, the image of any object under test must be considerably less dense than the background of supporting film (that is, the object must have a sufficient electron opacity): secondly, the plates must be exposed for long enough to give this image an optical density of about 0·5 or more (that is, distinctly darker than an unexposed part of the plate). If these conditions have been satisfied, then an object showing no change in opacity may be safely identified as one containing a large content of material with an atomic number greater than 20.

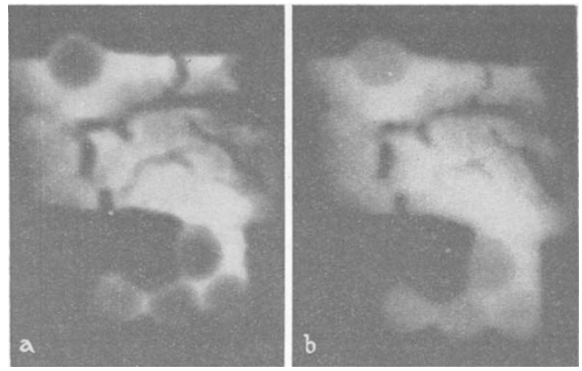

Fig. 1. Electron micrographs of polystyrene latex spheres surrounded by phosphotungstic acid (× 45,000); (*a*) using an objective aperture of 10^{-2} radian; (*b*) using an objective aperture of 5×10^{-3} radian

As an example of the method, Fig. 1*a* shows an electron micrograph of some polystyrene latex spheres surrounded by a dried-down mass of phosphotungstic acid. The latex spheres were less opaque than the phosphotungstic acid and can be clearly distinguished. A second plate was exposed of this same field but using an objective aperture of half the size. The plate was reproduced in exactly the same way and is shown in Fig. 1*b*. (Both figures are printed as negatives, that is, as the original plates.) The latex particles are now almost indistinguishable from the surrounding material, the polystyrene latex (mean $Z = 5{\cdot}6$) having gained considerably in opacity while the phosphotungstic acid (mean $Z = 58$) did not. The demonstration is made more vivid if the plate shown in Fig. 1*a* is projected in red light and the image superimposed on that of Fig. 1*b* projected in green light. The objects of low atomic number then appear green against the material of high atomic number, which looks yellow.

A full mathematical treatment of this topic will be given elsewhere, but it seems that a quantitative application of the method would allow one to differentiate calcium ($Z = 20$) or even phosphorus ($Z = 15$) from, say, protein (mean $Z = 7$). It would not, however, be possible to distinguish between the chief biological materials, proteins, carbohydrates, lipids and nucleic acids in this way, or to distinguish between any of the higher elements ($Z > 25$).

ROBIN C. VALENTINE
National Institute for Medical Research,
Mill Hill,
London, N.W.7.
Jan. 30.

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Enzymic Hydrolysis of Fucoidin by *Pseudomonas atlantica* and *Pseudomonas carrageenovora*

FUCOIDIN, a common polysaccharide sulphate in brown seaweeds, has been shown by methylation[1,2] and acetolysis[3] to consist of a chain of L-fucopyranose units joined in α-glycosidic linkages through carbon atoms 1 and 2 of adjacent units, each unit being sulphated on carbon atom 4. The results obtained