Iron as a Stain for Nucleic Acids in Electron Microscopy.*

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An electron stain that would react with nucleic acids would obviously be a valuable addition to the techniques available for the electron microscopic investigation of cell structure. Information available from light microscopy (1–3) indicates that iron has a marked affinity for acidic residues as well as an excellent capacity for the penetration of biological materials. As a heavy metal, it was expected that iron would introduce sufficient electron density into the specimen to provide for visibility in the electron microscope.

The bacteriophage T_2 of *E. coli* is known to consist of a head, containing a deoxyribonucleic acid core surrounded by a protein coat, and a tail, apparently also protein (4). In view of the known and discrete distribution of materials, this bacterial virus was chosen as a test object for investigating the use of iron as a stain for nucleic acids in electron microscopy. The observations were made on thin sections of methacrylate-embedded material, in an RCA EMU-2 electron microscope, equipped with a 25 μ objective aperture.

Samples of purified bacteriophage containing approximately 10^{12} particles per ml. were pipetted into 1 per cent OsO_4 buffered with veronal to pH 7.4. After 30 minutes at room temperature the samples were pelleted by centrifugation and washed three times in 0.9 per cent NaCl. One sample was again suspended in 0.9 per cent NaCl, another was suspended in 0.1 M $FeCl_3$. After 15 minutes both samples were washed twice in the NaCl solution, dehydrated with alcohol, and embedded in a 3:1 mixture of butyl and methyl methacrylate.

As shown in Fig. 1, when bacteriophage particles are treated with osmium tetroxide and sectioned, the tail and the protein coat of the head are stained and the nucleic acid core of the head is unstained. Bahr (5) has demonstrated that the chemical reactivity of osmium tetroxide will be largely confined to the lipide and the protein components of cell materials, and that there is no reaction of OsO_4 with nucleic acids. Preparations stained with iron as well as osmium (Fig. 2) show the protein to be stained as before, but now the nucleic acid may be seen inside the head membrane. This increase in the electron-scattering capacity of an acidic component by the addition of a heavy metallic ion may be likened to the use of stains in light microscopy to achieve comparable ends.

That the virus particles seen in Fig. 1 are whole particles in which the nucleic acid is unstained rather than "ghosts" which have lost their nucleic acid, has been demonstrated by analysis of osmium-treated samples. A treated sample and an untreated control were pelleted by high-speed centrifugation, then dissolved in warm ammoniacal detergent. Ultraviolet absorption measurements of

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the resulting optically clear solutions indicated that more than 90 per cent of the nucleic acid was retained in the osmium-treated material.

The observations presented above suggested some degree of specificity in the combination of iron with nucleic acid. To investigate this relationship further, quantitative determinations were made of the relative amounts of iron (6) and phosphorus (7) in the iron-treated virus preparations. Over the range from 0.1 to 0.001 molar, the amount of iron taken up by a fixed amount of virus was essentially constant and independent of $FeCl_3$ concentration. The ratio of iron to phosphorus was found to be approximately 4 to 1. The combining ratio and the consistency of the results are again suggestive of some degree of specificity in the coupling of iron with acidic residues, although these data are not adequate to warrant any conclusions as to the stoichiometry of the reaction.

Preliminary observations have indicated that this method may also be useful in electron microscopic investigations of acidic components of animal cells.

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Explanation of Plate 162

FIG. 1. Section through a preparation of bacteriophage, T_2, that has been treated with OsO_4. Note that only the outer membrane, the protein coat, of the virus particles is visible. × 55,000.

FIG. 2. Same as Fig. 1, but additionally treated with $FeCl_3$. As in Fig. 1, the protein is stained, but now the nucleic acid is also visible inside the head of the virus particles. × 55,000.

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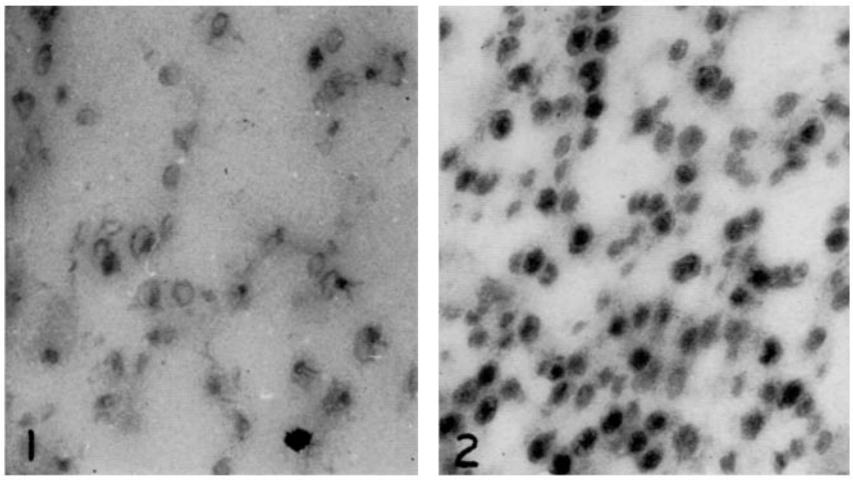

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