A METHOD OF DEVELOPMENT YIELDING FINE-GRAIN ELECTRON-MICROSCOPIC AUTORADIOGRAPHS

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Development is one of the critical operations in the preparation of electron-microscopic autoradiographs. It largely determines characteristics of the picture such as sensitivity, resolving power, background, and reproducibility of results. As a rule any development technique does not permit the highest quality results to be obtained for all possible characteristics; improvement of some is associated with worsening of others. Consequently the choice of method is always a compromise between opposing influences and choice must depend on the concrete aims of the investigation and character of the material.

A method of development widely used in electron microscopic autoradiography is by using the D-19 developer produced by Kodak. It has sufficiently high sensitivity, gives adequate reproducibility of the results and a comparatively low background, with a short development time (1-2 min) with solution diluted 1:2. Another important point is that the developer consists of the usual, readily available photographic reagents. However, despite the many advantages of the D-19 developer, it is nevertheless the most coarsely grained of all developers used in electron-microscopic autoradiography. Other conditions being the same, it therefore gives the lowest resolving power and it hides the structure of the section with grains of silver more than the rest.

This developer has been used until recently in work carried out in the Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, for high reproducibility of the results has always been essential for the solution of the problems studied at the Institute, and maximal resolution was not required. However, with the development of research into the electron-autoradiographic study of phagocytosis, the defects of D-19 developer began to create serious difficulties with the analysis of electron-microscopic autoradiographs. From the technical point of view the aim of the work was to determine the level of RNA synthesis in single bacterial cells ingested by leukocytes or lying outside the blood cells [1]. The use of the D-19 developer for this purpose enabled a labeled bacterium to be confidently distinguished from an unlabeled bacterium, even when they lay side by side. However, during intensive RNA synthesis, the concentration of large grains of silver created above a labeled bacterium was so dense that it was often almost completely covered, and the continuous overlapping of grains made it impossible to count them (Fig. 1). In order to analyze the morphology of a labeled bacterial cell and to undertake the comparative quantitative estimation of the intensity of incorporation of the label, it was necessary to obtain smaller grains of silver, in less intricate shapes. In other words, it was necessary that a large part of the bacterial cell should remain unconcealed by the grains, and the number of grains must be countable.

Several methods of development, yielding grains of silver much smaller than a bacterial cell, are used in electron-microscopic autoradiography. The one most commonly used in the West is development after preliminary latensification with solutions of gold and potassium thiocyanate [2, 7]. A method of development with phenidone also has been suggested [4, 5, 8], by means of which very small, circular silver grains are obtained. In the present experiments phenidone developer created considerable background and the reproducibility of the results was inadequate.

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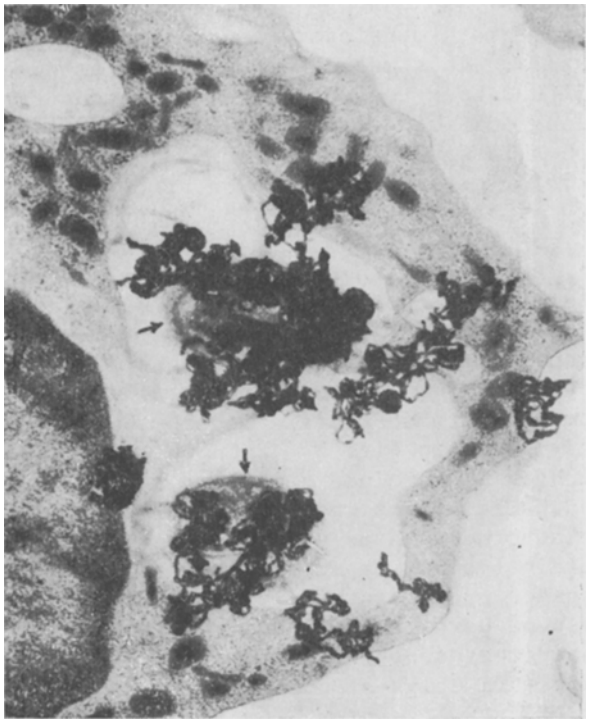

Fig. 1. Electron-microscopic autoradiograph of RNA synthesis in bacteria ingested by polymorphonuclear leukocyte. D-19 developer. Dense clumps of silver grains, interwoven with each other and covering a large part of the bacteria, are visible above them. Impossible to count the grains. 20,000 ×.

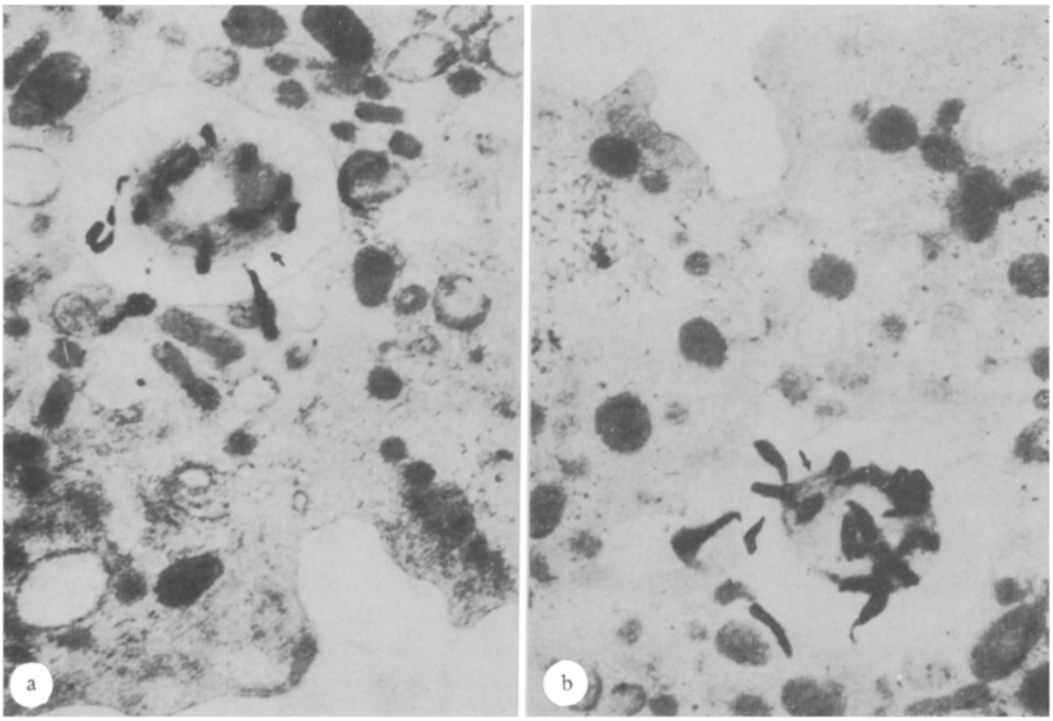

Fig. 2. Electron-microscopic autoradiographs of two sections (a and b) of a polymorphonuclear leukocyte with an ingested bacterium (RNA synthesis). Paraphenylenediamine developer. Grains of silver leave most of the bacterium uncovered (arrows). Grains can be counted. Density and distribution of grains in sections a and b do not differ in principle, indicating satisfactory reproducibility of results of development. 30,000 ×.

A third formula for a fine-grain developer was suggested by Caro in 1964, who worked with it on bacterial objects [3]. This method was not used as widely as the developers mentioned before, although it has been used occasionally for bacteriologic research [6]. Caro's developer is a solution containing 1.0 M sodium sulfite and 0.1 M paraphenylenediamine. He recommended treatment with this developer for 1 min at 20°C. However, during development of our own material, using the original conditions, a precipitate of sulfate was formed, neither the time nor quantity of precipitate could be controlled, and often it occurred in darkness during development, so that development was completed under unstandard conditions. To prevent recrystallization of sulfite, the size and density of the grains must be increased a little, and to obtain satisfactory reproducibility of the results, without altering the composition of the developer as given above, the conditions of development were somewhat modified: 5 min at 25-26°C. Preparations (human polymorphonuclear leukocytes, carrying out phagocytosis of *Staphylococcus aureus in vitro*) were obtained by the method described earlier [1]. The results of development of these preparations by Caro's developer, using the conditions which we suggested, are given in Fig. 2. Even when several grains of silver were formed above a bacterium, much of it remained uncovered, so that the structure of the bacterial cell could be observed. The grains were discretely separate from each other, so that quantitative analysis of the autoradiographs was possible. An important advantage of the method is the shape of the grain (comma-shaped), which means that they can be accurately distinguished from circular deposits precipitated during contrasting. The density of the grains in the background, in the case of development under the conditions described above, does not differ significantly from that created by the D-19 developer. Comparison of sections a and b in Fig. 2 reveals the sufficiently high standardization of the results of development.

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