ELECTRON-AUTORADIOGRAPHIC INVESTIGATION OF NUCLEIC ACID SYNTHESIS IN BACTERIA DURING PHAGOCYTOSIS

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The ordinary way of determining phagocytic activity of leukocytes, by counting the number of microbial bodies in them under the microscope, is very laborious, reflects only the ability of phagocytes to ingest bacteria, does not give a sufficiently reliable idea of the ability of phagocytes to ingest bacteria, and does not give a sufficiently reliable idea of the ability of the cells to kill the bacteria they have ingested. Attempts have been made to overcome these drawbacks by the use of radioactive isotopes. One suggested method is preliminary tagging of the bacteria by growing them on medium containing isotope. After contact between these tagged bacteria and leukocytes, the latter are washed to remove uningested bacteria and the radioactivity contained in them is determined biochemically [4]. This method does away with the long and difficult counting procedure under the microscope and gives results that do not depend on the skill of the investigator. To determine the digestive action of the phagocytes, incubation of blood cells after ingestion of microorganisms in medium with ^{3}H-thymidine followed by determination of the ability of the ingested microorganisms to synthesize DNA, by light-microscopic autoradiography, has been suggested [5].

So that the digestive action of phagocytes could be compared with the fine structure of the phagocytic cell and of the microorganism, the writers have attempted to study nucleic acid synthesis in ingested bacteria by the method of electron-microscopic autoradiography. Besides the DNA precursor (^{3}H-thymidine), which has already been used to study phagocytosis in the method of light-microscopic autoradiography, the RNA precursor ^{3}H-uridine also was used. Incorporation of ^{3}H-thymidine into the bacterial cell is, of course, evidence that the cell is preparing to divide and preserves its viability. However, absence of the label when this precursor is used does not necessarily mean loss of viability, for we know that a definite period must elapse between replication and division, during which DNA synthesis does not take place, in a perfectly normal bacterial cell [3]. No data on interruptions in the process of RNA synthesis in living microbial cells could be found in the literature, and it was therefore decided to use ^{3}H-uridine as the precursor, for it is incorporated more constantly into living cells.

EXPERIMENTAL METHOD

To obtain a leukocyte suspension 5 ml of donors' blood was transferred to a test tube containing 1 ml of 6% Trilon B solution and 2 drops of a 10% solution of gelatin, and was incubated at 37°C for 1.5 h. The layer of plasma formed in the course of this time in the upper part of the tube was collected and mixed with an equal volume of a bacterial suspension of Staphylococcus epidermidis strain No. 9198, containing 200,000 bacterial cells in 1 ml physiological saline. The mixture of leukocytes and bacteria was incubated for 30 min and washed with warm isotonic solution twice with centrifugation for 5 min at 1500 rpm to remove uningested bacteria. After washing to remove the microorganisms, ^{3}H-thymidine or ^{3}H-uridine was added to the leukocyte suspension in a dose of 100 μCi/ml and this was followed by further incubation for 30 min at 37°C. At the end of incubation the leukocytes were washed to remove unincorporated precursor with cold physiological saline by centrifugation for 5 min at 1500 rpm, and then fixed in 4% glutaraldehyde solution in cacodylate buffer. After postfixation in 1% osmium tetroxide the leukocyte suspension was dehydrated and embedded in epoxide resin

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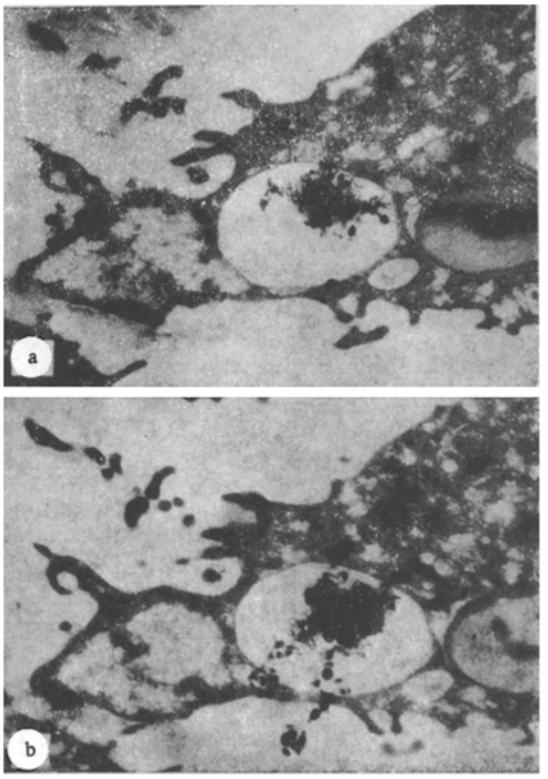

Fig. 1

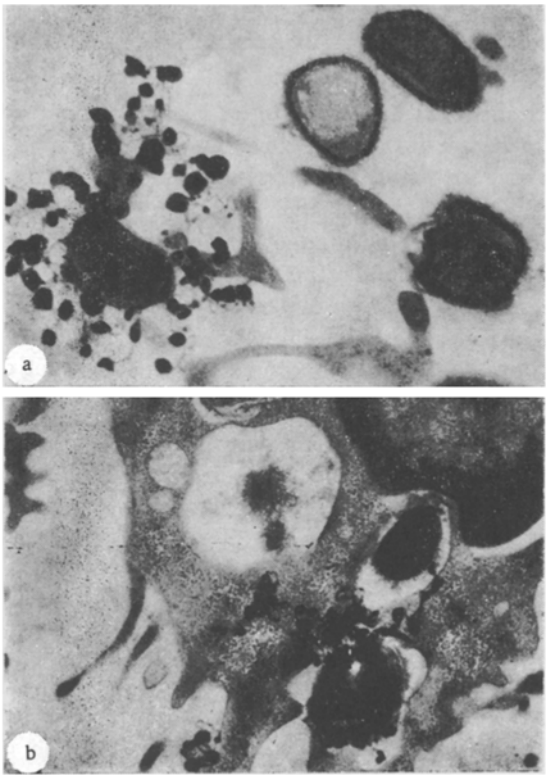

Fig. 2

Fig. 1. Two sections (a and b) through the same polymorph with phagocytosed staphylococci (incubation with ^{3}H-uridine). Intensive labeling of bacterium in both sections illustrates reproducibility of results obtained by this method; 12,000×.

Fig. 2. DNA synthesis in staphylococci in interstitial fluid (a) and in leukocyte (b). Unlabeled bacterial cells lie alongside labeled in both types of localization; a) 20,000×; b) 15,000×.

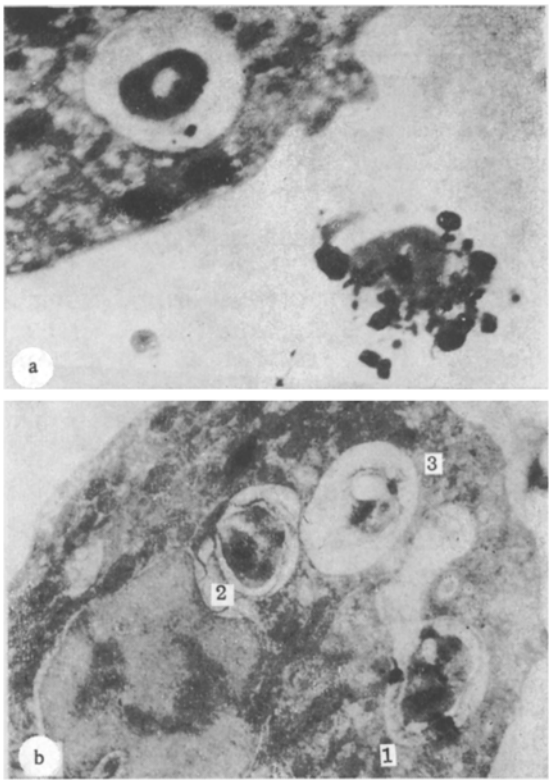

Fig. 3. DNA synthesis in staphylococci: a) intensive labeling in extracellular bacterium and absence of label in phagocytosed bacterium, 20,000×; b) different states of phagocytosed bacteria in polymorph: 1) incorporation of ^{3}H-uridine into bacterium with preserved structure, 2) uneven electron density of cytoplasm and absence of label in bacterial cell, 3) marked lysis of bacterial cell and absence of label, 16,000×.

by Spurr's method [6]. Semithin sections were coated with "M" emulsion and exposed for 1 week. The block was trimmed for ultrathin section cutting on the basis of the results of examination of autoradiographs obtained with semithin sections. Electron-microscopic autoradiographs were prepared by the method described in [1, 2].

EXPERIMENTAL RESULTS

The method used yields sufficiently reliable and reproducible results (Fig. 1), but the grains of metallic silver formed are comparatively large (0.2-0.4 μm). This fact, together with scattering of radioactive radiation, causes the grains to be located both immediately above the radiating cell and also at a short distance from it (Fig. 2a). In most cases the greatest value of this distance does not exceed the diameter of the bacterial cell, and labeled and unlabeled cells can be differentiated with sufficient assurance even when these cells are close together (Fig. 2b). Neither precursor was incorporated into the nuclei of the polymorphs, in agreement with the view that nucleic acid synthesis has ceased in these cells.

Individual labeled staphylococci could be seen in electron-microscopic autoradiographs obtained after incubation with ^{3}H-thymidine both between the leukocytes (Fig. 2a) and inside them (Fig. 2b). Unlabeled bacteria also were found between the leukocytes and in their cytoplasm. No sufficiently reliable criteria that would enable absence of label to be attributed to a pause in DNA synthesis or to death of the bacterium could be found.

The bactericidal action of the leukocytes was manifested in autographs reflecting incorporation of ^{3}H-uridine. Intensive labeling could be seen in bacteria located between leukocytes, but no label in the phago-

cytosed microorganism (Fig. 3a). Staphylococci ingested by leukocytes were at different stages of digestion. Often both viable bacterial cells incorporating ^{3}H-uridine and unlabeled, lysed cells could be seen in the same leukocyte (Fig. 3b).

Electron-autoradiographic investigation of phagocytosed bacteria with ^{3}H-thymidine and ^{3}H-uridine thus enables the level of nucleic acid synthesis in the bacteria to be recorded, ultrastructural changes developing during phagocytosis to be observed, and the digestive action of the leukocytes to be determined.

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