RNA SYNTHESIS IN POLYMORPHONUCLEAR LEUKOCYTES OF A SUPPURATIVE WOUND

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The polymorphonuclear leukocyte (PNL) of the peripheral blood is a cell which has completed the process of differentation, in the course of which it has acquired a stock of enzymes and of nonenzymic proteins necessary for performance of its function as a microphage. This view is based on morphological and biochemical features that indicate cessation of RNA synthesis in PNL: condensation of chromatin, reduction of the rough endoplasmic reticulum and ribosomes, marked inhibition of activity of the enzymes of nuclear metabolism and a decrease in the rate of incorporation of RNA precursors [3, 6]. A previous electron-autoradiographic study did not reveal incorporation of [^{3}H]uridine into inactive PNL in the blood and in uninfected tissues [5]. By contrast, in PNL taking part in phagocytosis, besides a sharp increase in the rate of energy metabolism, RNA synthesis also began to appear, although its level remained very low. RNA synthesis in PNL was first found by the biochemical method of Cline [7] during phagocytosis of latex particles by leukocytes. It was later found that if human leukocytes were bound *in vitro* not with latex particles, but with a suspension of bacteria, RNA synthesis also appeared in PNL, reflected electron-autoradiographically by the appearance of a few (2 to 5) grains of silver above certain phagocytes only [1]. RNA synthesis in PNL can be stimulated by concanavalin [8]. The authors cited used not only a biochemical method, but also a combination of light microscopy and autoradiography, by which they were able to determine the number of synthesizing cells, which they found to be 60%. All these data indicated that RNA synthesis, which ceased in the course of differentiation of PNL, can in principle be resumed, or at least, intensified. However, this intensification was discovered under artificial conditions during binding of suspensions of phagocytes *in vitro* with polymer particles or bacteria, or under the influence of a stimulator not formed in the body, and it was still not clear whether anything similar to this takes place in a focus of inflammation.

The aim of this investigation was to study RNA synthesis by electron-microscopic autoradiography, in PNL present in biopsy material taken from burns and traumatic wounds.

EXPERIMENTAL METHOD

Pieces of scab and granulation tissue removed at necrectomy on seven patients with thermal skin burns and three patients with suppurative wounds of soft tissues were investigated. Pieces measuring 1 mm^3 were cut from the material and incubated at 38°C for 1.5 h in medium 199 containing 100 μCi/ml of [^{3}H]uridine. At the end of incubation the material was washed to remove unincorporated precursor with cold medium 199 and phosphate buffer, pH 7.4, fixed with 2.5% glutaraldehyde solution and 1% OsO_4 solution, and embedded in Epon. On the day of necrectomy, the leukocyte fraction was separated from a blood sample taken from each patient. Half of the volume of the leukocytic fraction was incubated in the same way as samples from the wound. To the other half of the leukocytic fraction was added on equal volume of a suspension of *Staphylococcus aureus* or *Staph. epidermidis*, containing 2×10^8 cells in 1 ml of medium 199. A strain of staphylococcus isolated from his own blood or wound

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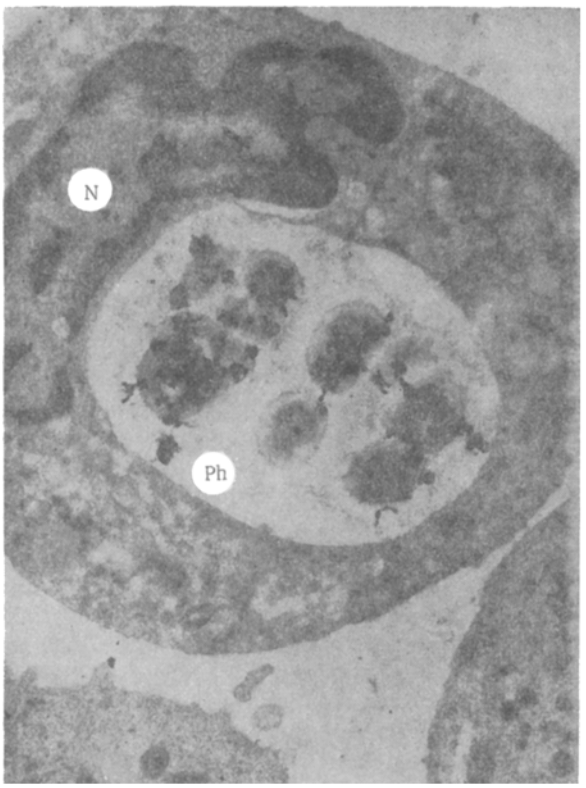

Fig. 1

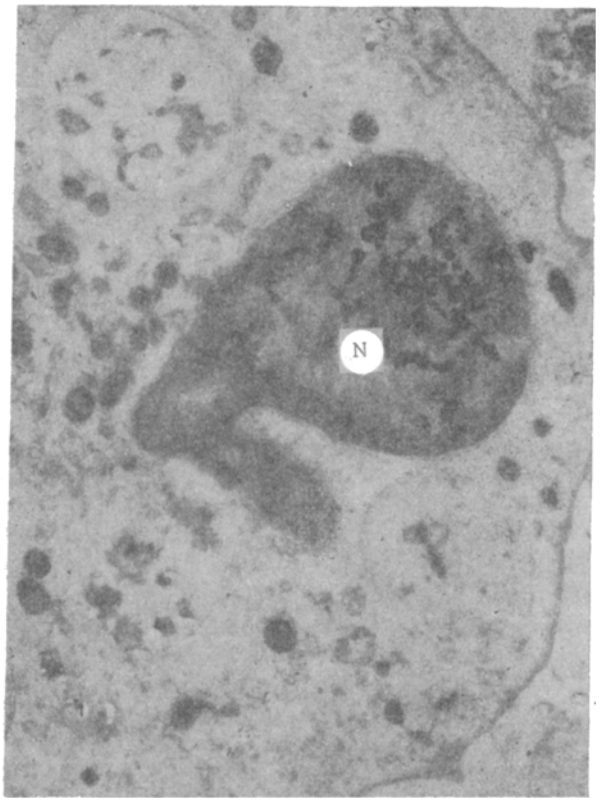

Fig. 2

Fig. 1. Single grains of silver above nucleus (N) of PNL isolated from blood and incubated with staphylococci *in vitro;* most cocci in phagosome (Ph) are labeled with [^{3}H]uridine (15,000 ×).

Fig. 2. Many grains of silver above nucleus (N) of a PNL not containing bacteria. Biopsy specimen from burn. 20,000 ×.

was bound with the leukocytes of each patient. Before binding with leukocytes the bacterial suspension was opsonized with autologous plasma. The phagocytic test was set up in tubes with film by the method described previously [2]. The material was fixed and embedded by the same technique. The incubation time, temperature, and concentration of [^{3}H]uridine were the same as for specimens from the wound. Similar investigations also were carried out with healthy human leukocytes obtained from routine blood donors. Strains of staphylococci isolated from the blood of our patients were used for phagocytosis by healthy human PNL. Autoradiographs of semithin sections (exposure 24 h) were obtained from all blocks and the number of grains of silver above the nuclei of PNL was counted in them. The labeling density in specimens from blood was counted in 200 PNL for each patient or blood donor, and in specimens from the wound, either in 200 PNL similarly or, if the number of PNL per section was less than 200, in all PNL present. To analyze the material, electron-microscopic autoradiographs prepared by the method in [4, 5] also were used. The significances of differences was determined by Wilcoxon's test.

EXPERIMENTAL RESULTS

PNL from normal subjects and subjects with wounds, incubated with [^{3}H]uridine but without bacteria, contained no label. This result was further confirmation of the view that RNA synthesis is not found in circulating PNL.

In preparations obtained after incubation of leukocytes with bacteria in the presence of [^{3}H]uridine, PNL containing label in the nucleus were found. This label consisted of a few grains of silver (Fig. 1) and it was found both in leukocytes with phagosomes containing bacteria in their cytoplasm and also in cells whose sections contained no bacteria. Many cells containing phagosomes with bacteria in their cytoplasm had no label in the nucleus. The number of PNL with labeled nuclei in this series did not exceed 18%. We found no significant difference in content of label in phagocytic PNL obtained from normal and wounded individuals.

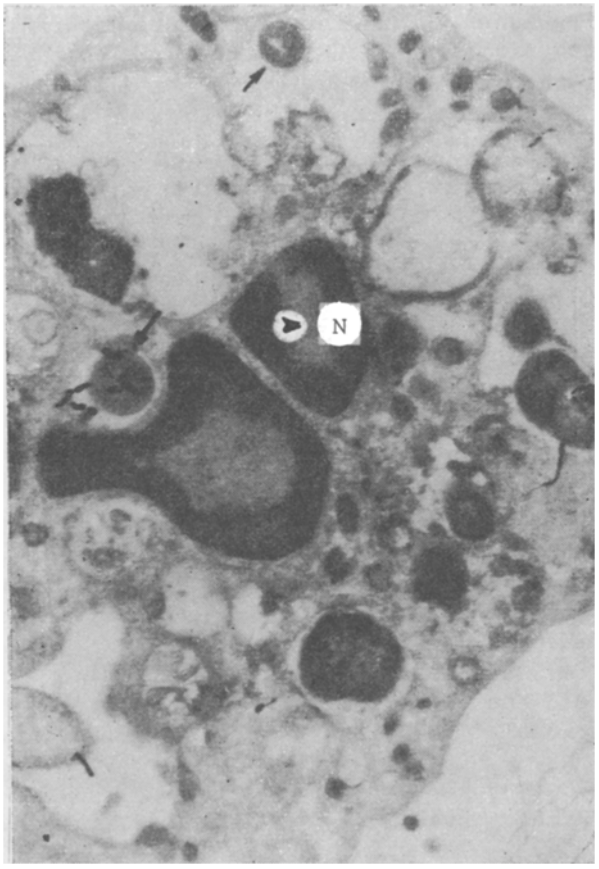

Fig. 3. Single grain of silver above nucleus (N) of PNL containing labeled (long arrow) and unlabeled (short arrow) bacteria. Biopsy specimen from burn. 15,000 ×.

PNL in the granulation tissue of burns and traumatic wounds usually were represented by single cells, most frequently with an unlabeled nucleus or one labeled with one or two grains of silver. PNL arranged in groups between the scab and granulation tissue, and among fibrin clots, differed sharply from these cells. Most (70-90%) PNL exhibited intensive incorporation of [^{3}H]uridine (Fig. 2). The density of label in them (10-30 grains) was only a little less than the density of labeling of fibroblasts and macrophages of granulation tissue in these same preparations. Intensive RNA synthesis was found in PNL of suppurative foci in all patients. The density of labeling in these PNL was significantly higher than the density of labeling in PNL obtained from the blood of these same patients and undertaking phagocytosis of staphylococci introduced into the tube ($P < 0.01$).

Most PNL in suppurative foci contain no bacteria in their cytoplasm, and it was these cells which were distinguished by the highest level of [^{3}H]uridine incorporation. Labeling was usually weaker in leukocytes ingesting bacteria (Fig. 3).

Autoradiographic experiments with phagocytosis of bacteria by leukocytes *in vitro* thus confirmed biochemical data [7] showing intensification (resumption) of RNA synthesis during phagocytosis. Autoradiography increased the accuracy of these data and showed that RNA synthesis takes place, not in all cells of the suspension, as might be supposed on the basis of biochemical analysis, but only in comparatively few of them. RNA synthesis in the case under investigation most probably was activated not by phagocytosis itself, but by metabolic changes accompanying it and the appearance of a certain activating factor in the medium, to which some cells reacted. This is indicated, first, by the fact that in most cells carrying out phagocytosis RNA synthesis is nevertheless not found, and also by data [8] showing intensification of RNA synthesis, under the influence of a stimulator, in PNL not taking part in phagocytosis.

The phenomenon discovered, namely marked activation of RNA synthesis in PNL of a suppurative wound, differs in principle, as regards both the relative percentage of cells in the wound participating in RNA synthesis and also the intensity of the process in the single cell, from the intensification of RNA synthesis discovered by Cline [7] during phagocytosis.

Another feature which distinguishes RNA synthesis in the suppurative wound from Cline's phenomenon is that this synthesis is not bound with phagocytosis, for maximal incorporation of [^{3}H]uridine was observed in cells not containing bacteria, and if bacteria are present in the cytoplasm, labeling of the nuclei is negligible.

Activation of RNA synthesis in wound leukocytes was found mainly because of the superiority of the method of electron-microscopic autoradiography, in that it can record a process taking place in only those cells that occupy an independent position and constitute only a negligible fraction of the total mass of the material chosen for study, which is beyond the scope of the biochemical method; another advantage of electron-microscopic autoradiography is ultrastructural identification of the cells and the possibility of study the distribution of label in such small objects as bacteria, which is impossible by the light-microscopic version of autoradiography.

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