RNA AND PROTEIN SYNTHESIS IN WOUND POLYMORPHS

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Maturation of polymorphonuclear leukocytes (polymorphs) is accompanied by morphological and functional changes reflecting a marked decrease in the intensity of nucleic acid and protein metabolism in these cells (condensation of chromatin, disappearance of the nucleolus reduction of the granular endoplasmic reticulum, ribosomes, and Golgi complex, and slowing of the rate of incorporation of RNA precursors). There have been few studies of macromolecular synthesis in polymorphs, when compared with the number of investigations into other manifestations of polymorph function (adhesion, chemotaxis, respiratory burst, ingestion of objects of phagocytosis, bactericidal and cytotoxic factors, etc.). The reason for this state of affairs is that these manifestations are directly connected with the protective role of polymorphs, whereas the importance of macromolecular synthesis in mature polymorphs is not clear, since the overwhelming majority of proteins necessary for polymorph function are synthesized at the preceding stages of development of the cell, and they are simply utilized in the mature forms. Nevertheless, information was obtained quite a long time ago on changes in the rate of RNA and protein synthesis developing in mature polymorphs in response to their activation. For instance, it was noted [3] that RNA synthesis in polymorphs is intensified during phagocytosis of latex spheres. The same effect was observed [5] in polymorphs stimulated by concanavalin A. The use of biochemical methods to study incorporation of precursors of lipids, glycogen, and protein into guinea pig polymorphs activated by phagocytosis of latex spheres showed [7] that the rate of synthesis of the first two types of macromolecules increases compared with that in resting polymorphs, but the rate of protein synthesis is unchanged. Human polymorphs, activated by *E. coli* endotoxin, in the presence of complement, reduce by 20% the level of incorporation of labeled amino acids into protein [4]. This seems strange, for synthesis of other macromolecules is intensified, and energy metabolism is appreciably activated. Nevertheless, data on the fall of the level of protein synthesis in activated polymorphs were evidently not mistaken, for at least they were confirmed by experiments [9] which showed, in addition, that delayed incorporation of amino acids during phagocytosis of latex takes place on account of the appearance of a low-molecular-weight inhibitor, which is neither a cyclic nucleotide nor prostaglandin E.

All these data on changes in RNA and protein synthesis relate to cases in which polymorphs were stimulated under artificial conditions by the action of a certain activating factor, introduced by the experimenter. Autoradiographic investigations of suppurating human wounds showed [2] that RNA synthesis is intensified in polymorphs of the demarcation barrier by a far greater degree than during phagocytosis of bacteria in vitro. Because of this observation it was interesting to study how natural activation factors, acting on release of polymorphs from the blood stream into a wound, affect protein synthesis in these cells, and to compare levels of RNA and protein synthesis in the same material. The investigation described below was devoted to a study of these problems.

EXPERIMENTAL METHOD

The purulent exudate (scrapings of the wound surface) obtained from 10 patients with burns was investigated. The patients' dressings contained dioxidine ointment. The exudate was transferred in medium 199 mixed with a 3% neutralized solution of Trilon B in the ratio of 9:1 and washed twice with the same medium, but containing the Trilon solution in the ratio

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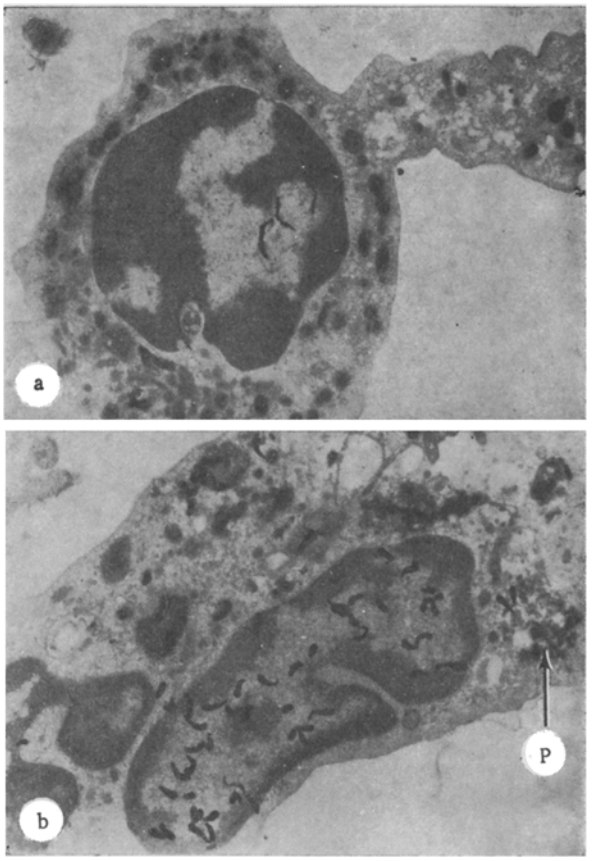
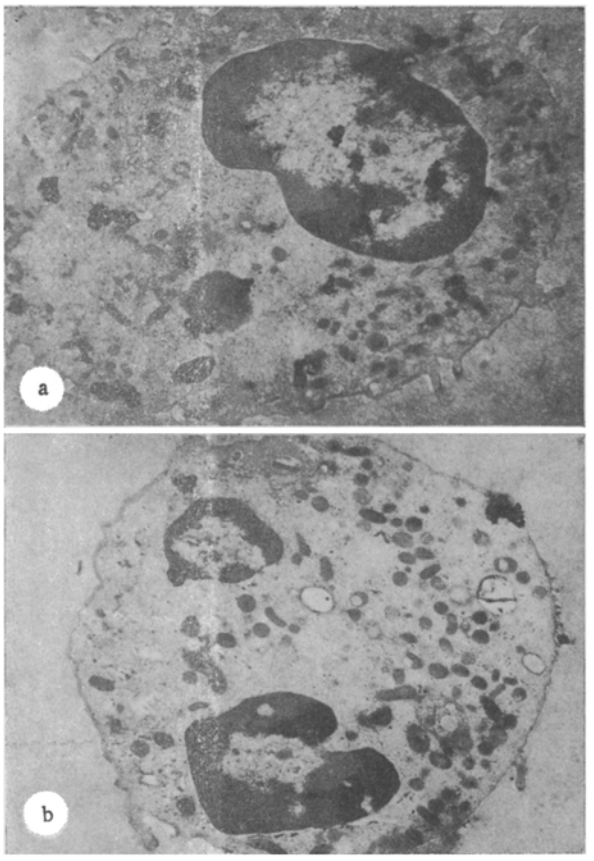


Fig. 1. RNA synthesis in blood and wound polymorphs: a) weak RNA synthesis (three grains of silver above the nucleus) in blood polymorphs containing many primary and secondary granules; b) much more intensive RNA synthesis (many grains of silver above the nucleus) in wound polymorphs containing few granules and several phagosomes (P) with detritus. Magnification 12,000×.

of 19:1. All subsequent manipulations took place in the same medium. Sedimentation of the leukocytes during washing was carried out for 5 min at 1000 rpm. A suspension of washed leukocytes containing $5 \cdot 10^6$ cells/ml was divided into two parts and incubated at 37°C for 30 min. Next, ^{3}H-uridine was added to one part in a concentration of 100 μCi/ml, after which it was incubated for a further 1.5 h. A mixture of amino acids (^{3}H-glycine, ^{3}H-leucine, ^{3}H-lysine, ^{3}H-alanine, ^{3}H-proline), each in a concentration of 100 μCi/ml, was added to the other part, which was incubated for 2 h. After incubation with the labeled precursors the leukocytes were washed 3 times and fixed with 1% glutaraldehyde solution in cacodylate buffer, pH 7.4, and were then mounted in gelatin by the method described previously [1]. The gelatin blocks were postfixed in glutaraldehyde and OsO_4 and embedded in epoxide resins [8]. Simultaneously with wound leukocytes, leukocytes isolated from the blood of the same patients were investigated by the method described above. Autoradiographs of semithin and ultrathin sections were prepared. The number of labeled cells was determined in 200 polymorphs from the blood and wound of each patient. The number of grains of silver above the nucleus and cytoplasm was counted for 60 polymorphs. The significance of the difference in levels of RNA and protein synthesis in polymorphs from the blood and wounds was determined by Wilcoxon's test.

EXPERIMENTAL RESULTS

Among wound polymorphs cells indistinguishable in morphology from blood polymorphs were found infrequently. In most cases the number of granules and the electron density of the cytoplasm were reduced in the wound polymorphs, and phagosomes of various sizes, containing tissue detritus, were frequently found in it. The number of polymorphs taking up bacteria in their phagosomes did not exceed 1% in this material. Wound polymorphs of all patients investigated were more strongly labeled with ^{3}H-uridine than blood polymorphs. This was expressed



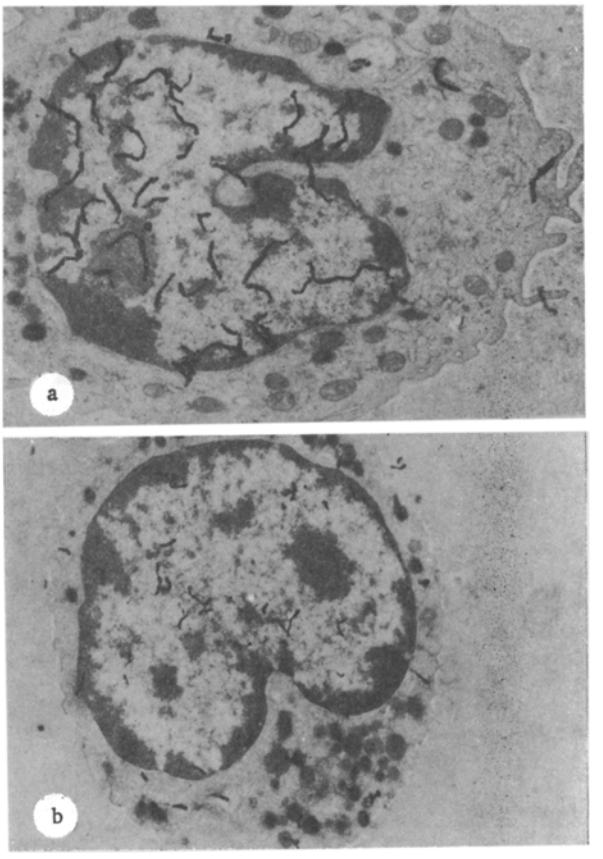


Fig. 2

Fig. 3

Fig. 2. Protein synthesis in blood and wound polymorphs: a) incorporation of amino acids into blood polymorphs reflected in a large number of grains of silver above the cell; b) the few grains of silver above a wound polymorph indicate a low level of protein synthesis. 12,000×.

Fig. 3. RNA and protein synthesis in metamyelocytes. a) Many grains of silver above nucleus, reflecting intensive incorporation of ^{3}H-uridine (12,000×); b) incorporation of amino acids reflected in the presence of many grains of silver above the nucleus and cytoplasm of a metamyelocyte (15,000×).

as a larger number of labeled cells (23-60% in the wound and 10-30% in the blood) and also as the greater average density of distribution of grains of silver above the labeled cell. The average labeling density in the wound polymorphs was 58-720% greater than in blood polymorphs from the same patient (Fig. 1a, b). The difference between wound and blood polymorphs was significant ($P < 0.01$) for both parameters compared (the number of labeled cells and the intensity of labeling). The label in the nucleus of both types of cells was located above the euchromatin, i.e., above the zone in which, in the modern view, nonribosomal RNA is synthesized [6]. We found no morphological differences between labeled and unlabeled blood polymorphs. Among the wound polymorphs, dense labeling was found both above the well preserved cells, almost indistinguishable from blood polymorphs, and above cells showing considerable injuries, with multiple and extensive phagosomes, and with a reduced number of granules.

The intensity of incorporation of amino acids by the groups of cells compared was opposite to the intensity of incorporation of uridine. Blood polymorphs, poorly labeled with uridine, incorporated amino acids faster (on average by 18-45%) than wound polymorphs (Fig. 2a, b). This result was obtained in nine of the 10 patients, and only in the one remaining patient was the labeling density in the wound polymorphs, after incubation with labeled amino acids, 5% higher than in the blood polymorphs. The higher intensity of amino-acid labeling of the blood polymorphs than of the wound polymorphs was statistically significant ($P < 0.01$) in all patients. By contrast with uridine, the more severely damaged the cells, the weaker the incorporation of amino acids into the wound polymorphs.

Cycloheximide, an inhibitor of protein synthesis, if used in a dose of 20 μg/ml, lowered by 30% the level of labeling of the blood polymorphs but did not affect that of the wound polymorphs.

During activation of the polymorph on leaving the blood to enter the wound, opposite changes were thus found in two of its functionally connected processes, namely RNA synthesis and protein synthesis: the first was intensified, the second weakened. This observation contradicts the view that RNA is an intermediary in protein synthesis, and that maximal production of it is observed in cells forming a large quantity of protein. To verify this conclusion again on an object closest to polymorphs, we compared levels of RNA and protein synthesis in precursor cells of polymorphs, which were present in large numbers in blood samples taken from our patients. The tests showed that a high level of RNA synthesis in the precursor cells corresponded to intensive protein synthesis (Fig. 3a, b). The absence of this relationship in the wound polymorphs suggests that RNA synthesis in the activated polymorph may serve some other process unconnected with protein synthesis.

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TERMINATION OF THE OVARIAN CYCLE IN A YOUNG MOUSE JOINED TO AN OLD MOUSE IN PARABIOSIS

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Weakening of the function of the reproductive system during aging is an established fact. Meanwhile it is possible by certain procedures to restore, albeit partially, the function of the sex organs in aging animals. For example, transplantation of the mediobasal region of the hypothalamus of newborn rats into the third ventricle of old females caused an increase in weight of the ovaries and uterus of the latter, with the appearance of ovarian follicles at different stages of development [8]. Transplantation of the ovaries of young mice into animals with age-dependent depression of reproductive function led to resumption of cyclic changes in the vaginal epithelium, restored the luteinizing hormone level [6], and increased the frequency of pregnancy [10]. These and other investigations have shown that changes in the reproductive system during aging are due both to changes in the central control mechanisms and to disturbances of ovarian function. To study the role of the central and peripheral control mechanisms during aging of the reproductive system, and also the possibility of restoring this function through the influence of a young animal on an old one, through the ex-

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