ELECTRON–AUTORADIOGRAPHIC INVESTIGATION OF VIABILITY OF HUMAN CELLS AFTER DEATH. POSTMORTEM ACTIVATION OF RNA SYNTHESIS IN NEUTROPHILS

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The study of the viability of cells after death can provide important information about the structural and functional organization of these cells and, in particular, their metabolism and their dependence on the blood and lymph circulation during life. The role of such investigations in resuscitation and transplantology practice is no less important. One of the leading problems which may be solved by such investigations is the determination of the length of time the cells remain viable after death, although information available on this matter at the present time is incomplete and contradictory [3].

The principal factor causing loss of viability of cells after death is considered to be hypoxia, and the most vulnerable cells are neurons of the cerebral cortex. Morphological changes in neurons visible in the light microscope, according to some workers, develop as early as after 5-10 min [1], whereas according to others such changes cannot be found, even by electron-microscopy, after 30 min [2]. However, one morphological criterion alone is insufficient for assessment of changes taking place in cells after death, and we therefore decided to supplement it with an autoradiographic investigation of RNA synthesis in cells. This paper gives the results of a study of neutrophils by the suggested method.

EXPERIMENTAL METHOD

Blood neutrophils were studied 2.5-67 h after death from burns (6 subjects), mitral valve disease (2 subjects), and gallstones (1 subject). Blood samples were taken from the jugular vein into test tubes containing 1 ml of 3% neutralized EDTA solution and 1 ml of 10% gelatin solution to 10 ml of blood. Erythrocytes were sedimented in these tubes for 30 min in an incubator. The layer of plasma with leukocytes was then decanted and washed twice with medium 199 with centrifugation for 5 min (1000 rpm). The leukocyte concentration in medium 199 was adjusted to $5 \cdot 10^7$ /ml, the suspension was incubated for 0.5 h at +37°C, after which uridine-^{3}H was added to the tubes in a dose of 100 μCi/ml, and incubation continued for a further 1 h. After the end of incubation the leukocytes were washed 3 times with medium 199 and fixed with glutaraldehyde in cacodylate buffer. Subsequent fixation, embedding in resin, and preparation of autoradiographs of semithin and ultrathin sections were carried out by methods described previously [4, 5, 7, 8]. During analysis of the autoradiographs of semithin sections the number of cells with a labeled nucleus was counted among 400 neutrophils examined in each blood sample. The labeling density was measured in 60 randomly chosen labeled neutrophils. For this purpose the intensity of fluorescence of the grains of silver in reflected light above the measured nucleus was recorded by an "Opton" cytophotometer. As the control, blood from five patients with burns was studied by the same method.

EXPERIMENTAL RESULTS

Neutrophils obtained from the blood of living persons (Fig. 1) were characterized by clear separation of heterochromatin and euchromatin, by a large number of granules in the cytoplasm, and by absence of phagosomes. In neutrophils obtained after

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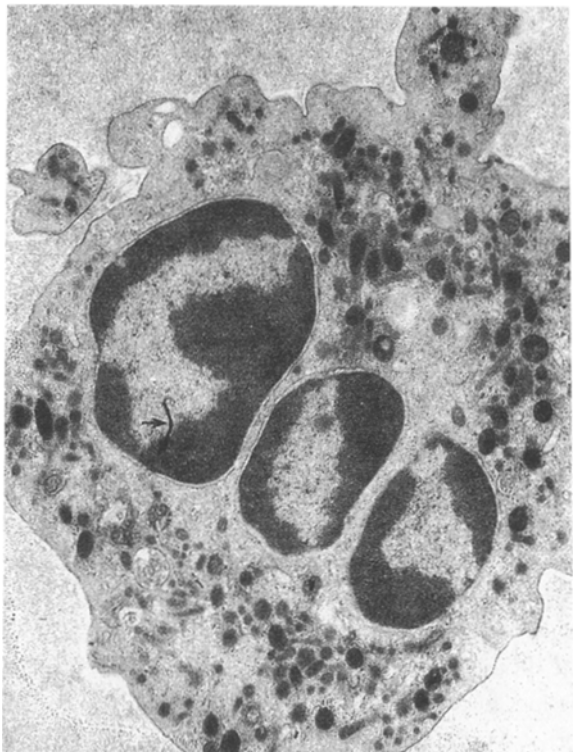

Fig. 1. Neutrophil isolated from blood of a living person and incubated with uridine-^{3}H. Weak incorporation of uridine, expressed by only one grain of silver (arrow) above one segment of nucleus. Many granules visible in cytoplasm. 15,000×.

death the number of granules in the cytoplasm was reduced (Figs. 2 and 3). Phagosomes into whose cavity granules had moved from the cytoplasm were frequently seen (Fig. 3). In semithin sections stained with toluidine blue and azure the cytoplasm of neutrophils taken from cadaveric blood stained less strongly than in cells obtained from living persons.

The difference between neutrophils obtained during life and after death, in relation to incorporation of labeled uridine, could be seen sufficiently clearly on examination of light-microscopic and electron-microscopic autoradiographs. The latter were often labeled even more intensively than the former (Figs. 2 and 3), in all cases studied, irrespective of the time elapsing after death. Quantitative analysis showed that the mean number of labeled neutrophils in the blood was 32 ± 8% during life and 70 ± 15% after death. The labeling density in the latter was 60 ± 17% higher.

Consequently, the autoradiographic investigation showed that ability to synthesize RNA not only is preserved in neutrophils, but is actually enhanced in the immediate period after death, and that this state may last for quite a long time (up to 67 h) in the cells. In our view the explanation of this phenomenon must be looked for in the specific character of function of the neutrophils.

The normal physiological activity of the body can be maintained only if the neutrophils in the blood remain functionally inert. Homeostasis maintains this state of the neutrophils. In the agony period and after death homeostasis is disturbed and the concentration of the blood gases and many chemical elements are changed. Under these conditions neutrophils, which are cells easily activated by various changes in the internal medium, react to stimuli which appear with an increase in their functional activity, and under these circumstances RNA synthesis in them may be intensified [9, 10]. Evidence of activation is given not only by the level of RNA synthesis, but also by morphological changes observed in the neutrophils after death and mentioned above: loss of granules, formation of phagosomes.

This phenomenon is of very great importance for leukocyte transplantation, which is already being carried out in practice [6]. The point is that it has been suggested that leukocytes isolated from cadaveric blood and, as was shown above, which are activated, be used for transplantation. Unlike inert neutrophils from blood of a living person, cadaveric neutrophils may secrete biologically active substances (in particular, granules and the many enzymes contained in them), they may fuse with the walls of blood vessels and with each other (i.e., facilitate thrombus formation), and may carry out phagocytosis of other cells.

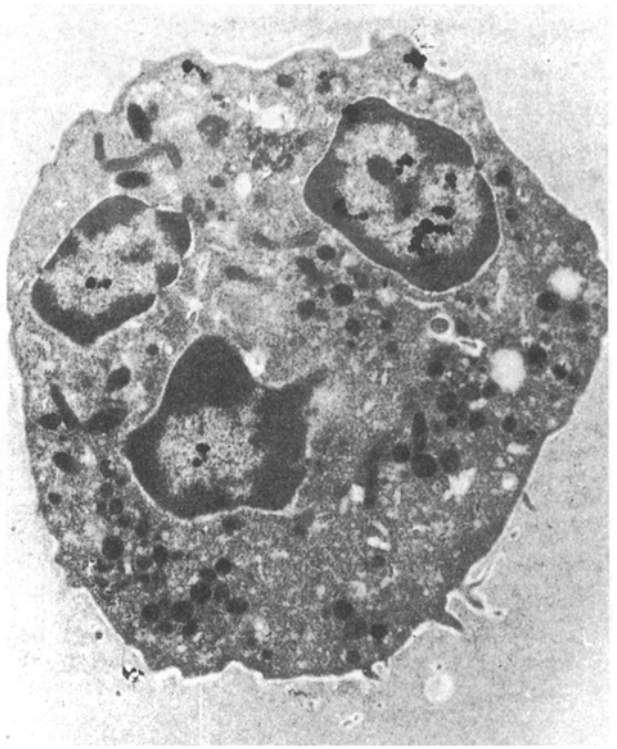

Fig. 2. Neutrophil isolated from blood 22 h after death and incubated with uridine-^{3}H. Incorporation of uridine expressed by several grains of silver, located above segments of nucleus. 12,000×.

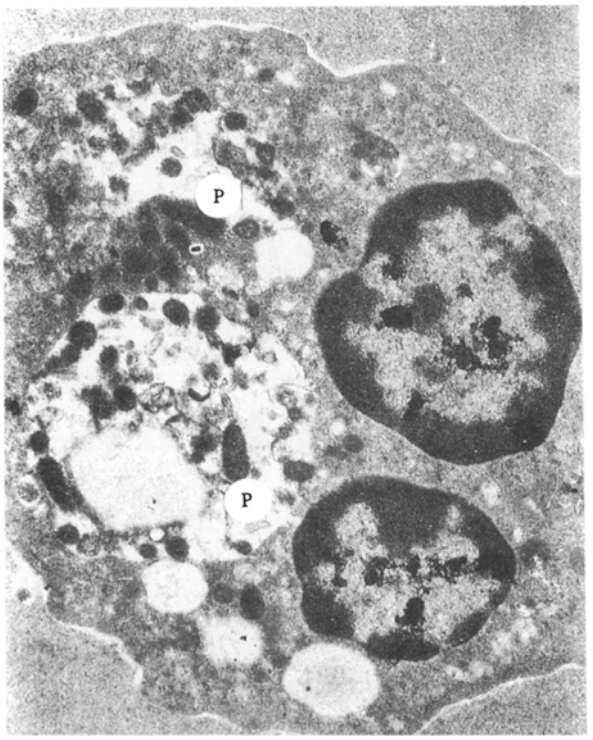

Fig. 3. Phagosome formation (P) in neutrophils isolated from blood, 22 h after death. Concentration of granules in cavity of phagosomes and decrease in their number in cell cytoplasm. Intensive incorporation of uridine-^{3}H revealed by large number of grains of silver above segments of nucleus. 17,000×.

Consequently, injection of leukocytes, isolated after death, into the blood stream must be undertaken with particular care, considering the likelihood of serious side effects. On the other hand, injection of these same cells, activated for performance of their specific function, not into the blood stream, but into an inflammatory focus, may actually be more effective than the transplantation of neutrophils obtained from living donors.

The experiments with neutrophils are evidence that autoradiography may become the most accurate test for the determination of viability of other cells also. If this same method is used, i.e., determination of the level of RNA synthesis, the assessment of viability will be carried out relative to a fundamental biological process, characteristic of all living cells and expressing the very essence of life, namely constant metabolism.

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