Physiology of Aging Leukocytes
I. Phagocytosis and Transformation (34804)

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Certain physiological activities associated with normal human leukocytes are followed and described during aging in whole blood. Phagocytosis and locomotion are characteristic of polymorphonuclear leukocytes as well as by activated lymphocytes. The time period during which incubating lymphocytes can be stimulated to transform with a mitogenic agent in the direction of macrophages is defined. The question of how long aging leukocytes are able to sustain these physiological activities is partially answered by the sequential studies described here.

Materials and Methods. Approximately 10 ml of venous blood obtained from nonmedicated human donors was drawn directly into a vacutainer (Becton, Dickinson and Co.) containing 40 units sodium heparin. There were no pH adjustments, nor were any nutrients added. Each blood sample was studied at zero time and tested again sequentially during a 30° aging period.

Sedimentation and attachment of leukocytes to glass slides. Precleaned slides were adapted to hold the sample by making a vaseline ring, and into this confined area, 0.2 ml of cell-rich plasma was pipetted and incubated in the moist chamber at 30°. After 20 min, the slides were removed, gently flooded, and whirled in Hanks' solution (Difco Lab.). This washing was repeated twice, thus, removing most of the erythrocytes, platelets, and nonadhering cells (1). The preparation was drained of excess solution and quickly overlaid with 0.2 ml of latex particles diluted 1:100 by volume with Hanks' solution. During this manipulation the monolayer was not allowed to dry out at any time. It was coverslipped, sealed with vaseline, placed in the stage warmer at 40°, and examined by phase microscopy. A minimum of 200 cells was counted and scored.

After observing the cellular events during this final period for 1 hr, the coverslip was gently removed and the adherent cells were rapidly fixed by flash drying with an air jet and stained with Wright's. Normal total white blood cell counts from healthy donors gave satisfactory cell densities on the slides.

Inert particles. Monodisperse preparations of polystyrene latex particles (Dow Chemical Co.) were used for the phagocytosis experiments. They are uniform in size having a diameter of 1.099 μ, and do not carry a charge or functional group to interact with the cell surface (2). Opsonin requirements were supplied by the plasma, and the uptake of latex particles *per se* does not seem to damage phagocytosing leukocytes (3).

Results. Sedimentation and attachment of leukocytes. There was an initial lag in cellular activity during the first hour of incubation when only a few cells remain attached to the glass slide. Differential counts on glass-adhering cells at zero time and shortly thereafter revealed an almost complete loss of normal small lymphocytes. Concomitant with this loss of typical lymphocytes was a proportionate gain in the number of large mononuclear cells resembling monocytes (Fig. 1).

After this lag period, there was a linear increase in the number of adhering large mononuclear cells reaching a maximum after 3–4 hr when virtually all cells remained attached to the slide, and the lymphocytes demonstrated their capacity to undergo a dramatic change in appearance and function. After this early burst of activity, the incu-

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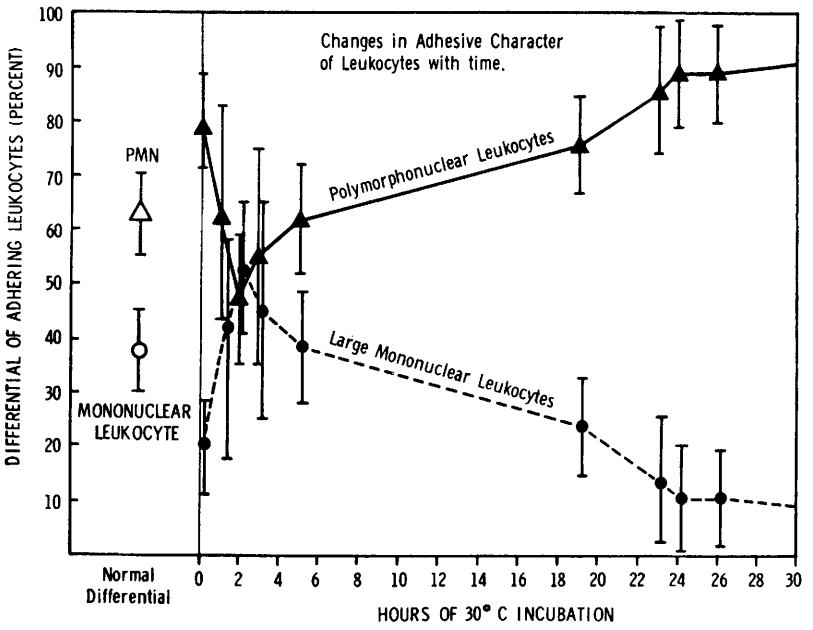


FIG. 1. *Mean values for changes in adhesive character of leukocytes with time.* Differential counts of glass-adhering leukocytes from six healthy donors were used to find the mean and standard deviation indicated by vertical lines.

bated lymphocytes became increasingly less responsive to sedimentation.

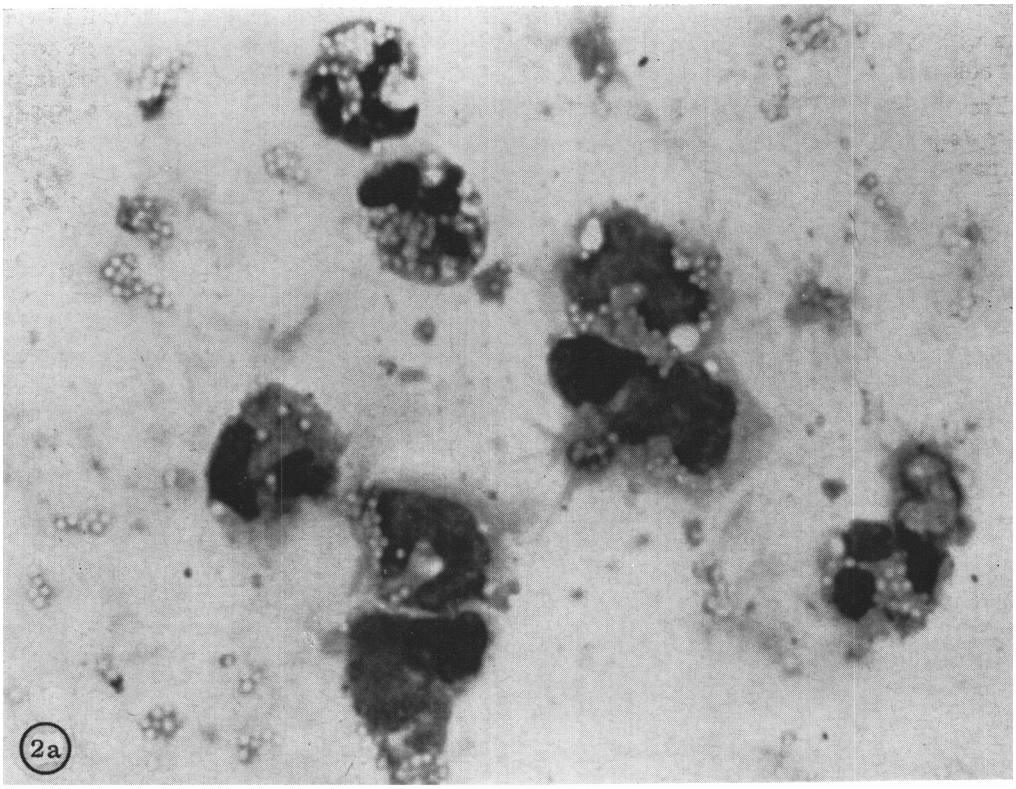
The response of lymphocytes to sedimentation onto a glass surface was not uniform. Not all lymphocytes remained attached, and those that adhered exhibited different degrees of activity ranging from very large fully developed macrophages with undulating borders to the typical intermediate-sized lymphocyte. The activated lymphocytes closely resembled monocytes.

Polymorphonuclear (PMN) leukocytes showed a reciprocal decrease in the percentage of adhering cells reflecting lymphocyte activity during the first 2–4 hr. This was followed by increasing values which reached nearly maximum levels after 24 hr of whole blood incubation (Fig. 1). PMN leukocytes continued to adhere to the glass surface even after cellular death.

Phagocytosis and locomotion of adherent leukocytes. Leukocytes sedimented on glass slides become firmly adherent, yet they were able to migrate and engulf latex beads presented to them (Fig. 2a, b). Attachment of inert particles did not necessarily trigger the events of phagocytosis (4) for latex beads attached to older or less active cells were not always interiorized. On several occasions latex beads were observed as they became attached to older cells and moved into the clear ectoplasm. These aged neutrophils seemed unable to completely interiorize the beads, and they carried them thus in the periphery during the 1-hr observation time. Particles were individually interiorized (5) by the flowing cytoplasm of amoeboid neutrophils. The attachment of particles to the plasma membrane depended on the probability of random collision, and attachment was proportional to the concentration of latex beads.

Eosinophils were much less phagocytic than were the neutrophils, and basophils gave no indication of phagocytosis at all. Both cell

FIG. 2. *Phagocytosis by neutrophils and activated lymphocytes.* Preparation showing phagocytosing neutrophils and transformed lymphocytes that was examined by phase microscopy then flash-dried and stained for further study by light microscopy. b. Note the ameboid cell with an advancing pseudopod is a transformed lymphocyte. In the same field is a typical small lymphocyte without ingested particles and a phagocytic neutrophil.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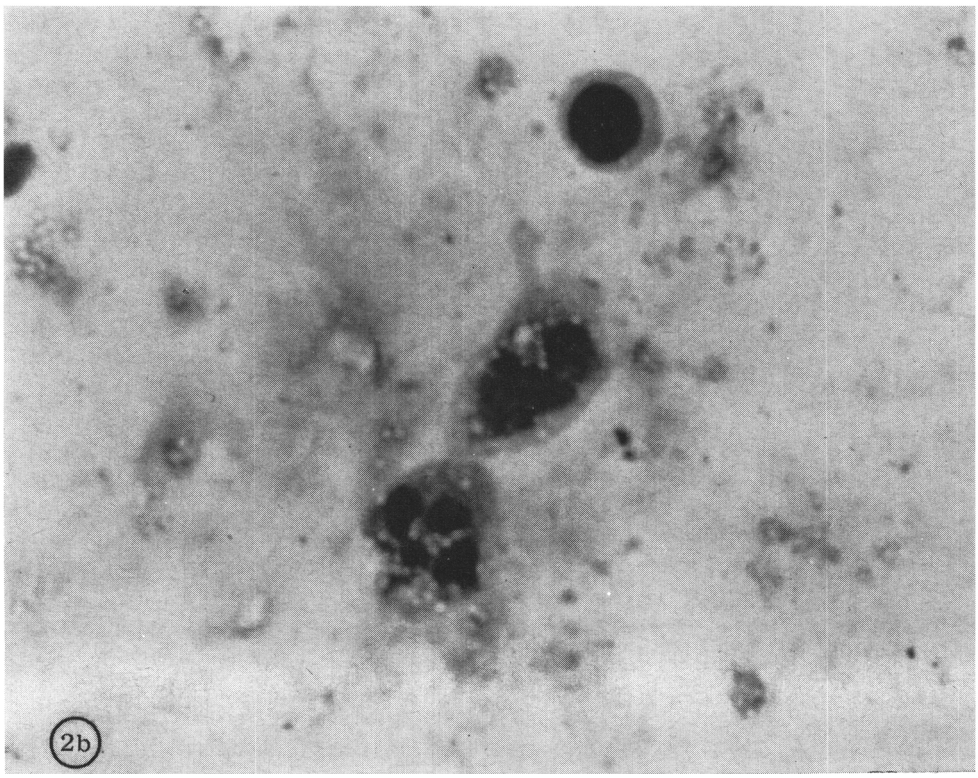
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types were observed during repeated encounters with latex beads, but the particles would not adhere to the cell.

Transformation and phagocytosis by lymphocytes. The typical small lymphocyte with its narrow rim of cytoplasm rarely contained ingested latex bead, but activated lymphocytes appeared as large ameboid phagocytes (Fig. 2b). These transformed cells were not rapidly moving, but their dynamic panorama of changing contours has been well illustrated in time-lapse cinematographic records (6). Their movements were nonpolarized with numerous small pseudopodia extending from all sides of the undulating cells. This type of activity resulted in an ineffective migratory pattern. The frequently described hand mirror appearance of small migrating lymphocytes was not so common in the early hours of incubation since most of the adhering lymphocytes had transformed into large macrophages. After 20–25 hr only a few cells remained attached and these did not become macrolymphocytes.

Early lag period. A lag period of almost 1 hr existed before near maximum activity was reached for leukocyte migration and phagocytosis. During this time the cells tended to remain rounded and inactive (7), and had no great affinity for the latex beads.

Effect of phagocytosis on the leukocyte. Neutrophils which had engulfed latex particles were found to contain fewer granules than the control cells. Degree of degranulation did not appear to be related to the aging of a cell, but it often coincided with an increased number of phagocytosed particles. Locomotion was significantly reduced in phagocytosing cells and was proportional to the number of engulfed particles (8).

Discussion. Phagocytosis of latex beads by normal human leukocytes may be divided into two separate stages. Attachment of inert particles to the surface of the cell is the initial event, and may be followed by the interiorization of the particle by invagination of the plasma membrane. Streaming cytoplasm associated with cellular motility seemed to be the main vehicle for interiorization. Particles attached to temporarily rounded cells were not ingested until the cell assumed more typical ameboid activities. There was no indication of a chemotactic attraction for the latex particle, and its attachment was the result of a chance encounter with a responsive cell.

The natural changes occurring in the plasma during aging did not seem to greatly affect phagocytosis or locomotion of neutrophils, that is, not until "starvation conditions" prevailed (9). Blood pH did not drop below 7 during the 24-hr aging period (10). No phagocytosis-promoting factor was necessary for polystyrene particles (11). Phagocytosis by PMN leukocytes requires an appropriate osmolarity (12) but during aging of whole blood there were essentially no changes in either osmotic pressure or viscosity.

Interiorization of latex particles by neutrophils decreased precipitously after 22–23 hr, and simultaneous with decreased phagocytosis was loss of motility. This period of functional failure corresponds with the onset of cellular degeneration indicated by the cells' inability to accommodate neutral red (13). Protoplasm remained a sol during this agonal period; Brownian movement temporarily increased but gave way to increased pyknosis.

Correlating biochemical changes with loss of locomotion and phagocytosis, it was found that at the time of functional failure, there was no free glucose, and that cytoplasmic glycogen had been drastically reduced (9). Apparently large stores of glycogen were metabolized as the exogenous glucose levels became inadequate. Glycogenolysis maintained the energy supply for the phagocytic process for approximately 10 hr after plasma glucose was exhausted. Supporting evidence has been provided by a number of investigators (11, 14, 15).

Transformation of lymphocytes. Small lymphocytes are not "end cells," for the observations made here reveal a potential for transformation without the stimulus of a mitogenic agent. The phagocytic nature of these activated lymphocytes was evident when they were exposed to latex particles. If cells are not examined at the appropriate time, however, it could be overlooked because of the early lag period and the subsequent loss of

responsiveness a few hours after the initial burst of activity. This loss of lymphocyte activity may be due to an inhibitory effect of the neutrophils present in the blood. Normal granulocytes markedly inhibit the uptake of ^{3}H-thymidine and ^{32}p into DNA by lymphocytes when the two cell types are present in the same incubation system (16), and it has been suggested that fragmented granulocytes as well as viable cells have an inhibitory effect on lymphocyte function (9). The behavior of the lymphocyte population in incubating whole blood was variable, and the observations made during the course of this work support the hypothesis proposed by Berman (6) that the morphological element recognized as small lymphocytes is a phase within a functionally heterogeneous population of quiescent cells.

Comments on cellular death. A precise definition of cell death under the conditions of the experiments is not practical at this time. Neutrophils appeared to be normal, functional cells by the usual criteria established for microscopy after 20 hr, but there were conspicuous signs of degeneration by electron microscopy 5 hr before this time (17). By studying the progression of changes during the aging and "death" of the cell, cytological adjustments and alterations associated with the basic pathologic process were defined within the limits of the methodology used; however, the exact time when the cell was irreversibly altered, "dead," must await further studies.

Lymphocytes do not show the same effects of aging; some showed ultrastructural damage within 24 hr while others resisted morphologic degeneration for 48 or more hours. Neutrophils, however, are differentiated cells with a functional survival time of only about 24 hr *in vitro*. Because of the high degree of specialization for phagocytosis in the neutrophil, it is unlikely that the cell could repair itself and recover. It is suggested that the neutrophil suffers damage to its respiratory mechanism because of starvation conditions. This leads to alterations in the endoplasmic reticulum, plasma membrane, and in the limiting membrane of the lysosomes causing a release of destructive enzymes into the cytoplasm. Cellular death in the neutrophil may well be synonomous with damage to the cell membranes.

Summary. Sequential changes in the human leukocyte's ability to phagocytize latex particles and lymphocyte transformation were correlated with concomitant biochemical modifications during a 30° incubation period. There were no pH adjustments nor nutritional additives during the period of sequential testing of aging blood cells.

Locomotion and phagocytosis of neutrophils continued unimpaired for 22–23 hr or until "starvation" conditions prevailed. Attachment of inert polystyrene latex particles to the plasma membrane did not trigger ingestion. Streaming cytoplasm associated with cellular motility seemed to be the main vehicle for interiorization of particles.

Small lymphocytes have a potential for transformation into large mononucelar cells after incubating 1–2 hr. There was a gradual decline in their reactivity after 2–5 hr which may have been due to an inhibitory effect of the granulocytes present in the incubating blood. Not all lymphocytes responded uniformly to the stimulation of surface contact. Their behavior was variable and they did not show the same effects of aging.

By examining the progression of changes during the aging and "death" of the cell, the cytological adjustments and alterations associated with this basic pathologic process were defined within the limits of the methodology used, but the exact time when the cell was irreversibly altered, "dead," must await further study.

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