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# AN ELECTRON MICROSCOPIC STUDY OF NUCLEAR ELIMINATION FROM THE LATE ERYTHROBLAST

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## ABSTRACT

The process of expulsion of the nucleus during the transformation of the late erythroblast to reticulocyte is described. Erythroid clones taken from the spleen of lethally irradiated mice transplanted with syngeneic bone marrow were used. 10–12-day old isolated clones were fixed in glutaraldehyde, then in osmium tetroxide. Ultra-thin sections were stained with uranyl acetate and/or lead citrate before examination. Late (orthochromatic) erythroblasts develop pseudopod-like cytoplasmic protrusions into one of which the nucleus gradually penetrates, being deformed by the extrusion through the relatively narrow passage. During the whole process, mitochondria and vesicular and membranous elements are concentrated in the cytoplasm. Once outside the cell, the nucleus reassumes its rounded form. It is surrounded by a narrow rim of cytoplasm and structurally altered plasma membrane and is connected to the rest of the cell by a bridge. Elongated vacuoles appear within this bridge, with a resulting release of the enveloped nucleus which is soon phagocytized by macrophages; this leaves behind the newly formed reticulocyte. During this process, the cytoplasmic protrusions, the agglomeration of mitochondria, and the mode of separation of the nucleus from the rest of the cell are similar to those occurring in mitotic division.



## INTRODUCTION

It has been suggested that during physiologic maturation of the late erythroblast to reticulocyte the nucleus is eliminated by karyolysis (6, 7, 16, 17). However, several investigators have stated that the nucleus is expelled (1, 8, 20, 24, 25, 27). Some authors found karyolysis to occur in primitive erythropoietic lines (3, 9, 12, 13) or in pathologic conditions (14). Microcinematography of marrow cell suspensions by Bessis and Bricka in 1952 provided evidence in favour of the expulsion theory (2).

Electron micrographs of hemopoietic centers, showing some free erythroid nuclei in proximity to the erythroid elements, or phagocytized and partly disintegrated inside macrophages, have also been taken as evidence for the expulsion mechanism (11, 19, 28). Orlic et al. (18) have presented electron micrographs in which an extruded nucleus is connected to the main cytoplasm, devoid of nucleus, by a narrow bridge. This micrograph is believed to represent a late stage of the expulsion process (18). However, an actual description of the process by which the nucleus leaves the cell has not been given on the electron microscope level.

The paucity of electron microscopic data on the expulsion process may be attributed to the rapidity of the process (2) and to the cellular distribution

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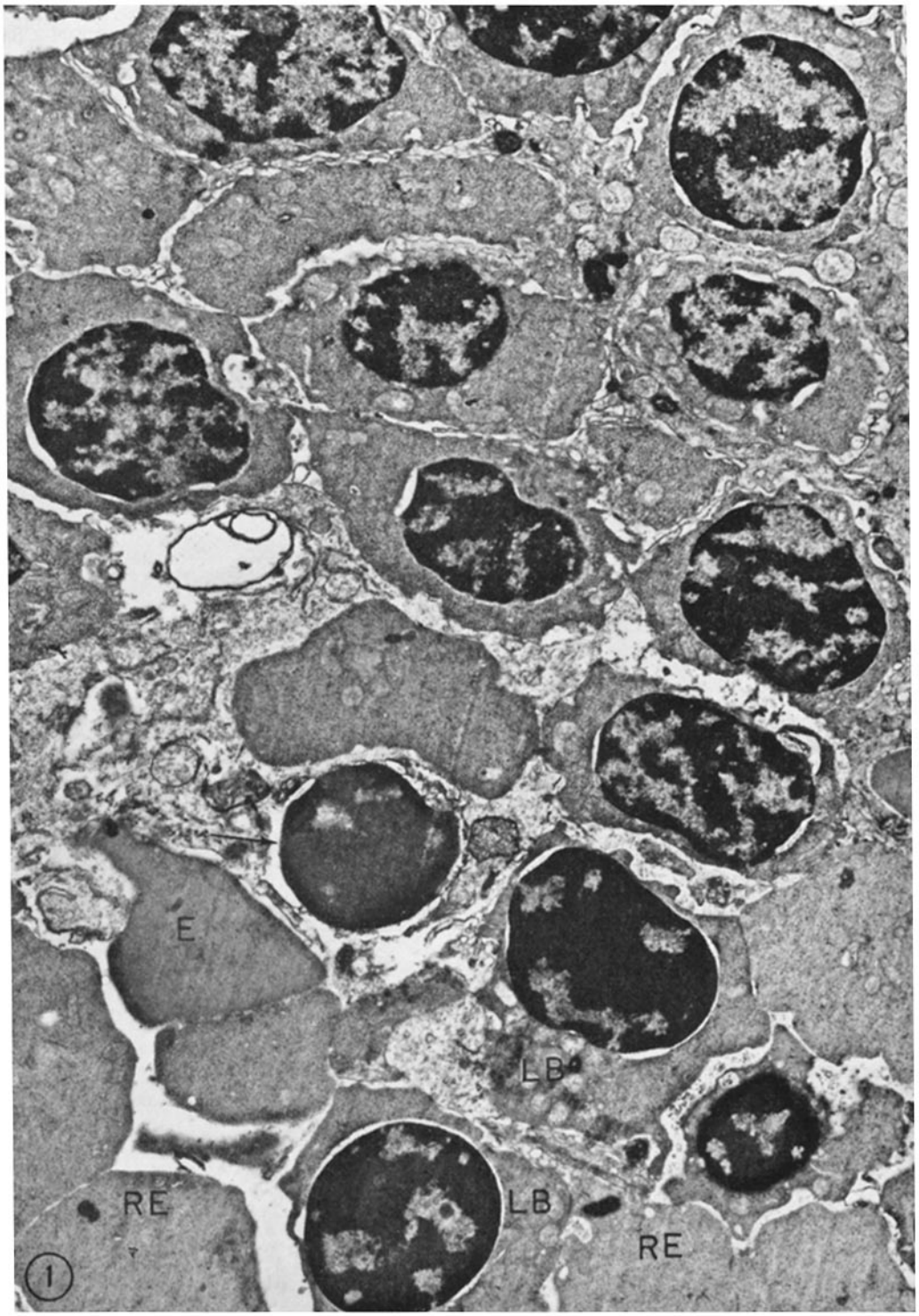


FIGURE 1. Electron micrograph of a section through an erythroid clone grown in a spleen of an irradiated mouse transplanted with syngeneic marrow cells. Mature erythrocytes (*E*) and reticulocytes (*RE*) are seen in proximity to some late (orthochromatic) erythroblasts (*LB*) with pycnotic nuclei, and to some erythroblasts at earlier stages of maturation. Thus practically the whole sequence of erythroid maturation is represented. Note the eccentric position of the nucleus of the late erythroblasts (the bottom cells). A partially digested nucleus is seen inside a macrophage (arrow). × 7,500.

in hemopoietic centers. The cells that expel their nuclei are intermingled with other cells, so that the probability is indeed small that an erythroblast expelling its nucleus will be included in an ultrathin section of about 0.1 $mm^2$ and about 600 A thick, taken in a block of 0.5 $mm^3$ in size. The probability is even smaller that the section will appear in the proper orientation to demonstrate the connection between the nucleus and the remaining reticulocyte.

Till and McCulloch (26) have described a system in which syngeneic marrow cells are injected into lethally irradiated mice; this results in the formation of pure erythroid clones in the spleen. These clones reach a diameter of 1-2 mm 10 days after injection. It was considered that this system might be usefully adapted to the electron microscopic study of the transformation of erythroblasts to reticulocytes, since cells at the same stage of maturation or in progressive stages of maturation are often found in proximity to each other within these clones. Observations of such a system are presented here, depicted in Fig. 1–12 and summarized in Fig. 13.

## MATERIALS AND METHODS

(C57BL × C3H) F 1 hybrid mice, aged 3 months, were exposed to 850 R total body X-irradiation. Then $3 \times 10^4$ syngeneic bone marrow cells (15) were injected intravenously. On the 10–12th day after injection, the animals were sacrificed, and the whole spleen was excised and placed in cooled 1% glutaraldehyde solution in 0.67 M phosphate buffer, pH 7.2.

Erythroid clones 1–2 mm in diameter were excised from the spleen and cut with a razor blade into 0.5 $mm^3$ blocks while immersed in the fixative. The blocks were fixed in fresh 1% glutaraldehyde solution in 0.67 M phosphate buffer (pH 7.2) for 2 hr at 4°C, washed six to eight times in 0.1 M phosphate buffer and postfixed in 2% osmium tetroxide in 0.1 M phosphate buffer for 2 hr. The tissue was then

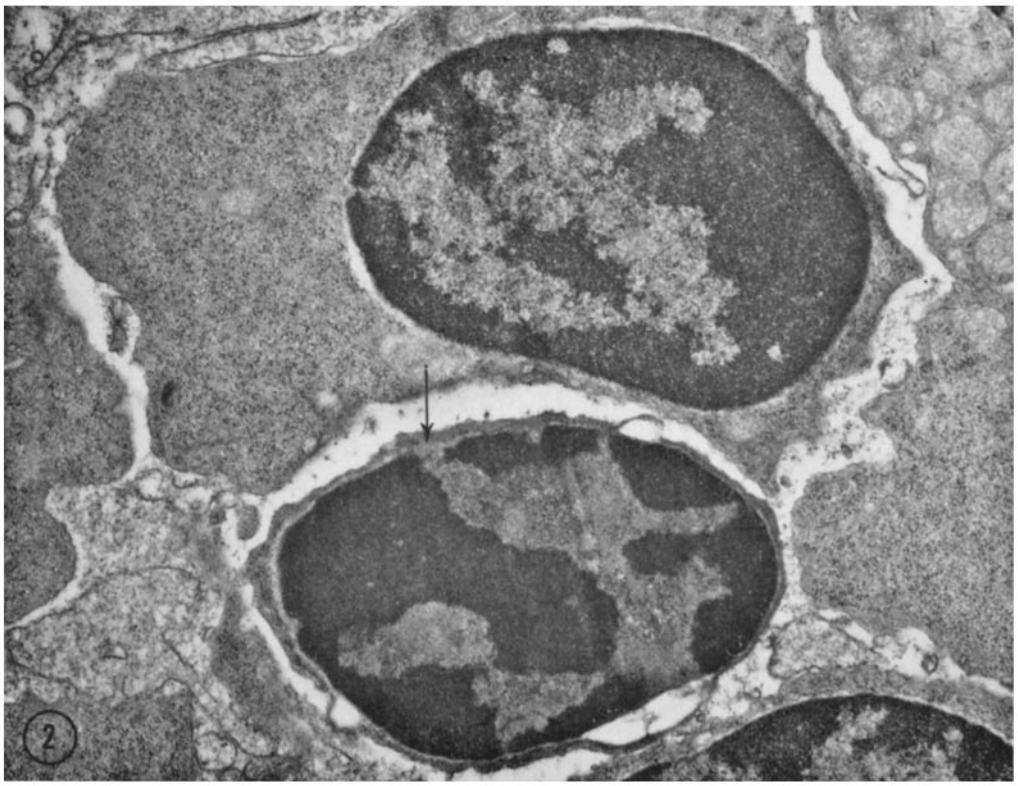


FIGURE 2. A late erythroblast (top cell) showing the eccentric position of its pycnotic nucleus. A free erythroid nucleus (bottom) is surrounded by a narrow rim of hemoglobin-rich cytoplasm containing some ribosomes. This cytoplasmic envelope is detached from the nucleus at several places. Invasion of hemoglobin-rich cytoplasm into the nucleoplasm can be seen in the extruded nucleus (arrow). × 19,000.

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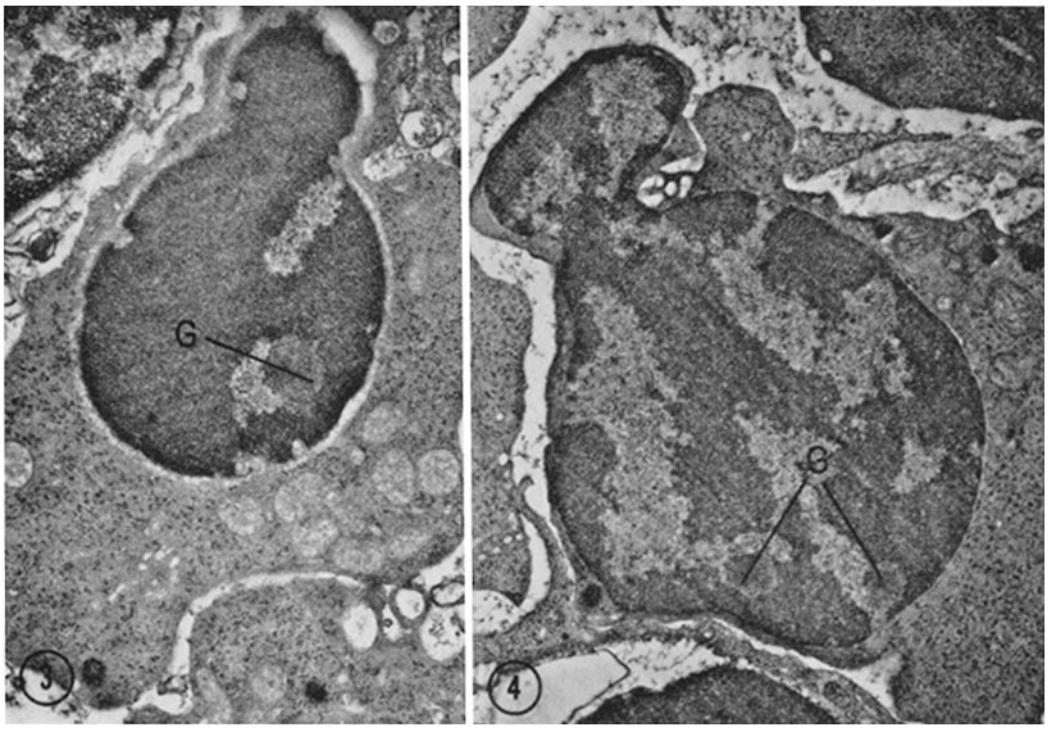


FIGURE 3. The deformed pycnotic nucleus of the late erythroblast starts to penetrate into a cytoplasmic protrusion. A nuclear granule (*G*), which has a different consistency than the chromatin and nucleoplasm, can be seen in the nucleus. It is believed to represent ribosomal aggregates (18) or remnants of the nucleolus. Similar granules can be seen in Figs. 4, 5, 8, 9, 11. Mitochondria and some membranous cytoplasmic elements are concentrated at the other pole of the nucleus. The cytoplasm is rich in polysomes and monosomes. Hemoglobin-rich cytoplasm can be seen penetrating into the nucleus. Note the irregular form of the cell. × 16,000.

FIGURE 4. As in Fig. 3, a larger proportion of the extruded nucleus is seen penetrating into a cytoplasmic protrusion. Some additional cytoplasmic protrusions are visible at the bottom pole of the nucleus (see also Fig. 8) × 18,000.

gradually dehydrated with acetone, embedded in Vestopal-W (23) and sectioned on the Danon ultramicrotome (Yeda, Research and Development Co. Ltd., Rehovoth, Israel) equipped with glass knives. Silver-to-gray sections were mounted on formvar-coated copper grids reinforced with a thin layer of carbon. Sections were stained on the grid with lead citrate (21) or saturated solutions of uranyl acetate dissolved in 50% ethanol, or were double stained with 5% uranyl acetate for 15 min followed by immersion in lead citrate (21) for 3–5 minutes. An RCA-EMU 2 electron microscope was used.

## RESULTS

The erythroid clones of the Till and McCulloch system often present large areas populated by cells at a similar stage of maturation. Cells of progressive

FIGURES 5–7. Three late erythroblasts representing sequential stages of nuclear extrusion. Progressively larger proportions of the expelled nucleus are found in the cytoplasmic protrusion. The protruded portion of each nucleus is surrounded by a narrow rim of cytoplasm and plasma membrane, which is seen damaged in one of the cells (Fig. 7). Fig. 5, × 16,000; Fig. 6, × 21,000; Fig. 7, × 23,000.

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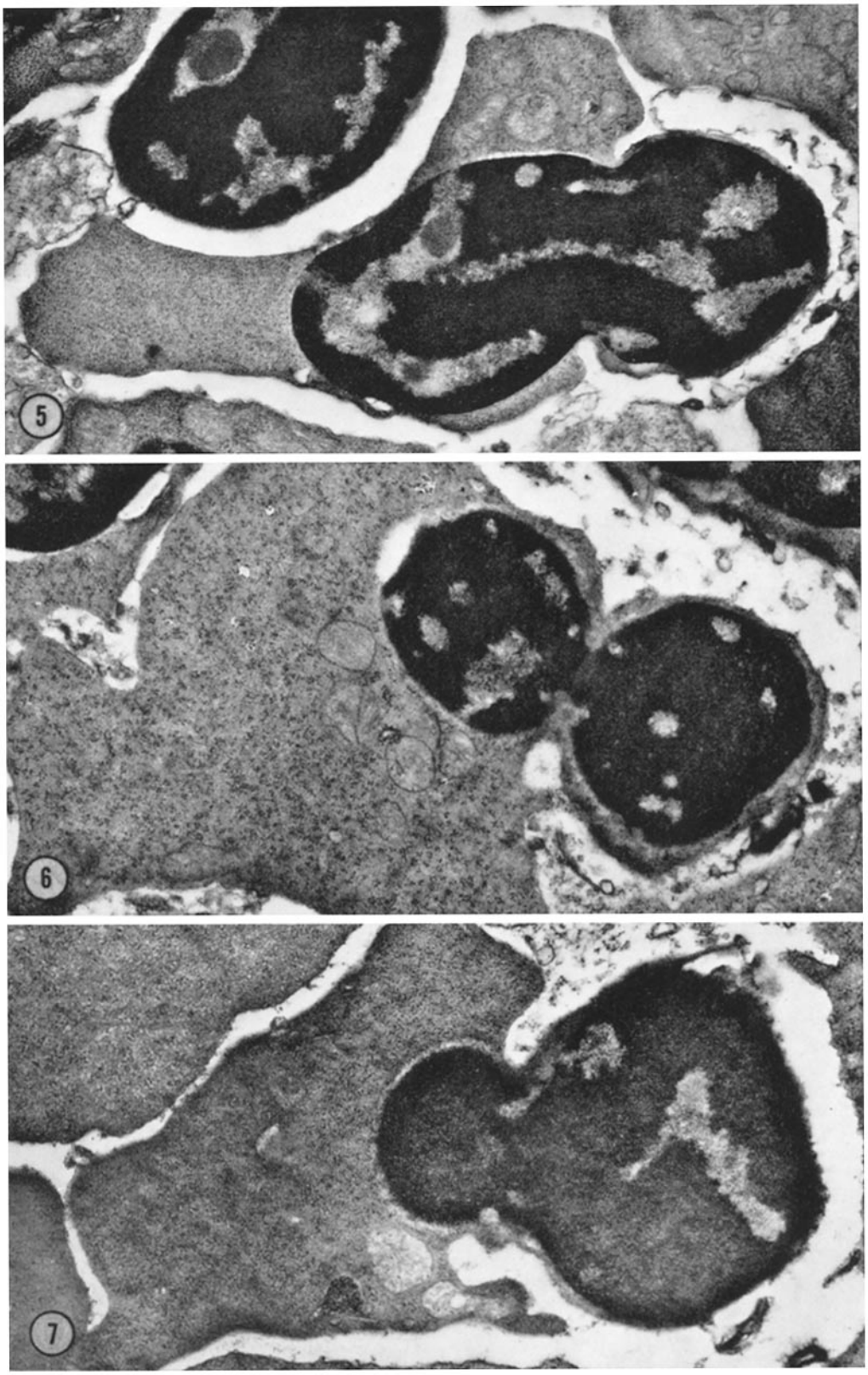

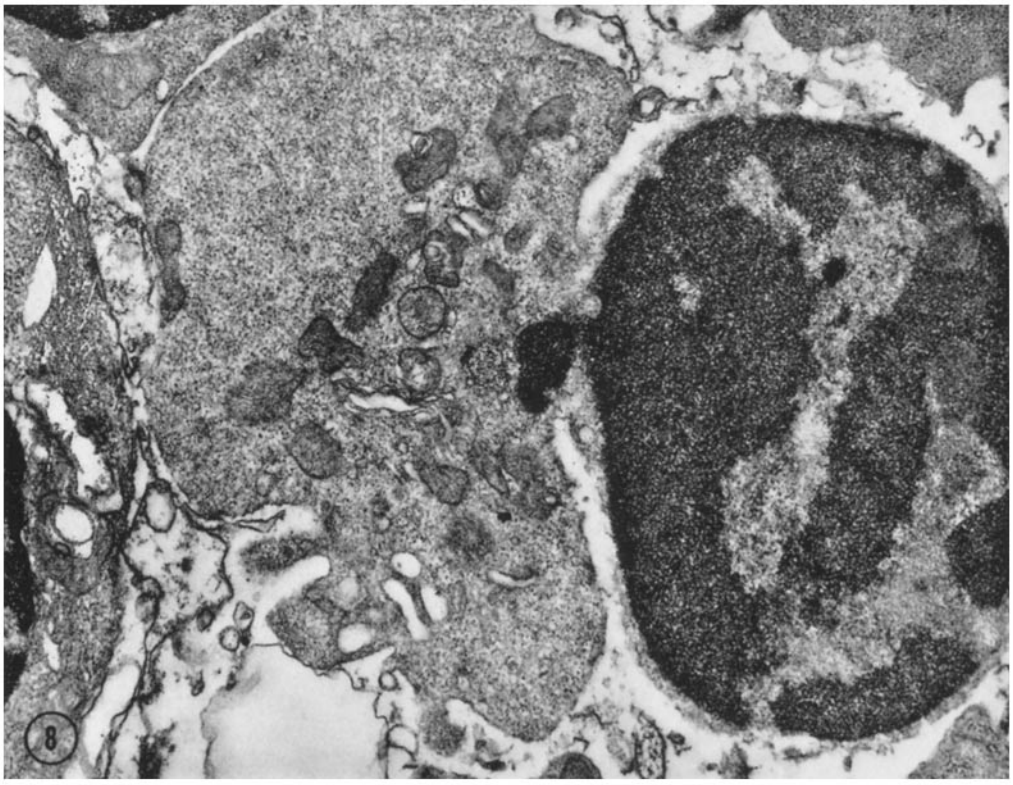

FIGURE 8. The major part of the nucleus of a late erythroblast is outside the main mass of cytoplasm. Membrane elements, probably remnants of the Golgi apparatus, are seen in proximity to some mitochondria and hemosiderin granules. Cytoplasmic invaginations and protrusions can be seen at the bottom of the erythroblast. × 19,000.

maturity can be seen in close proximity. Mature erythrocytes and reticulocytes may appear at one side of an electron micrograph and later erythroblasts and even earlier forms at the other, with intermediate stages in between; this apparently illustrates the sequence of maturation (Fig. 1).

A uniformly dark cytoplasm can be seen in the mature erythrocyte. The reticulocyte contains some free ribosomes and polysomes, remnants of mitochondria, hemosiderin granules, and ferritin particles, as well as some tubular elements and membranes, probably remnants of cytoplasmic organelles. The late erythroblast has a pycnotic nucleus with a typical radial organisation of the

FIGURES 9–11. Various stages, believed to be sequential, in the separation of the expelled nucleus and its surrounding plasma membrane from the remaining future reticulocyte. The nucleus, which has reassumed its typical rounded form, is now connected to the main bulk of cytoplasm by a wide bridge. One mitochrondrion is extruded with the nucleus (Fig. 9). Some vacuoles appear within this bridge (insert in Fig. 10). These openings become elongated (Fig. 10) thus resembling the cleavage furrow which appears at the equatorial plane at the telophase of mitotic division of erythroblasts (see reference 11). The vacuoles continue to elongate parallel to the nuclear membrane until the extruded nucleus is attached to the cytoplasm by only one or two tiny connections (Fig. 11). Fig. 9, × 25,500; Fig. 10, × 21,000, insert, × 18,500; Fig. 11, × 17,500.

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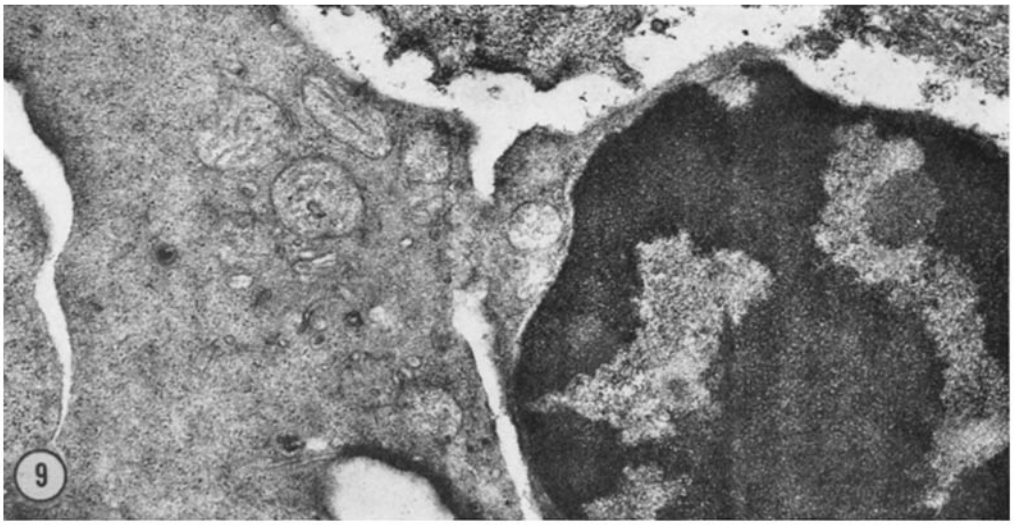


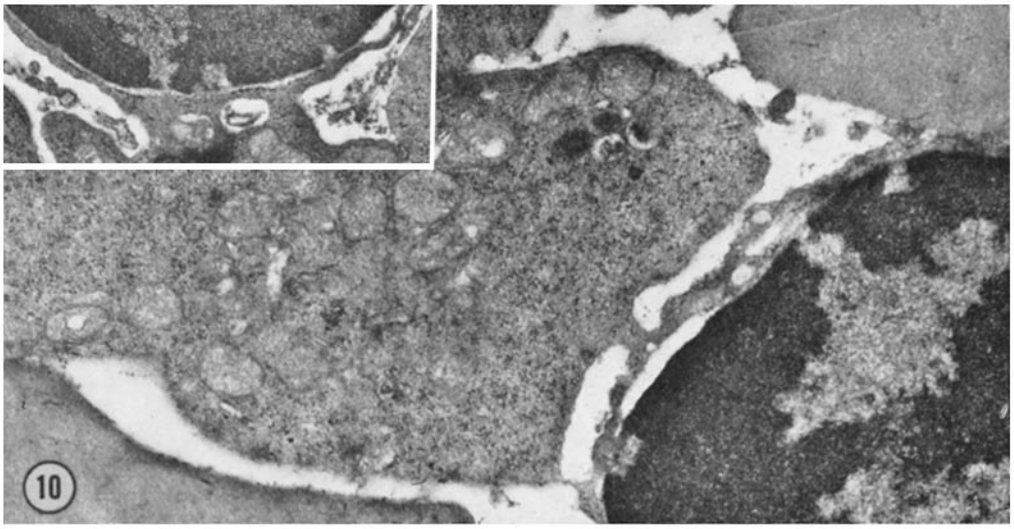


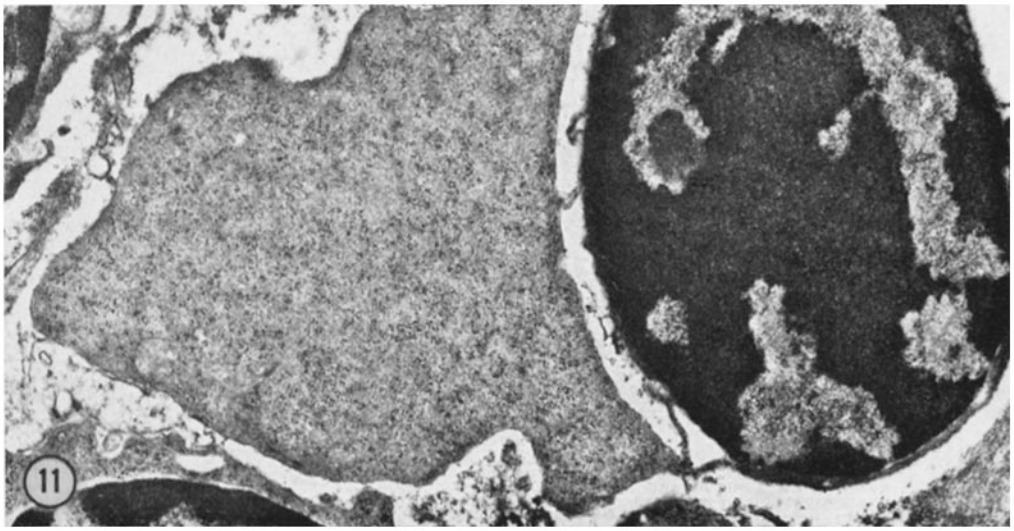

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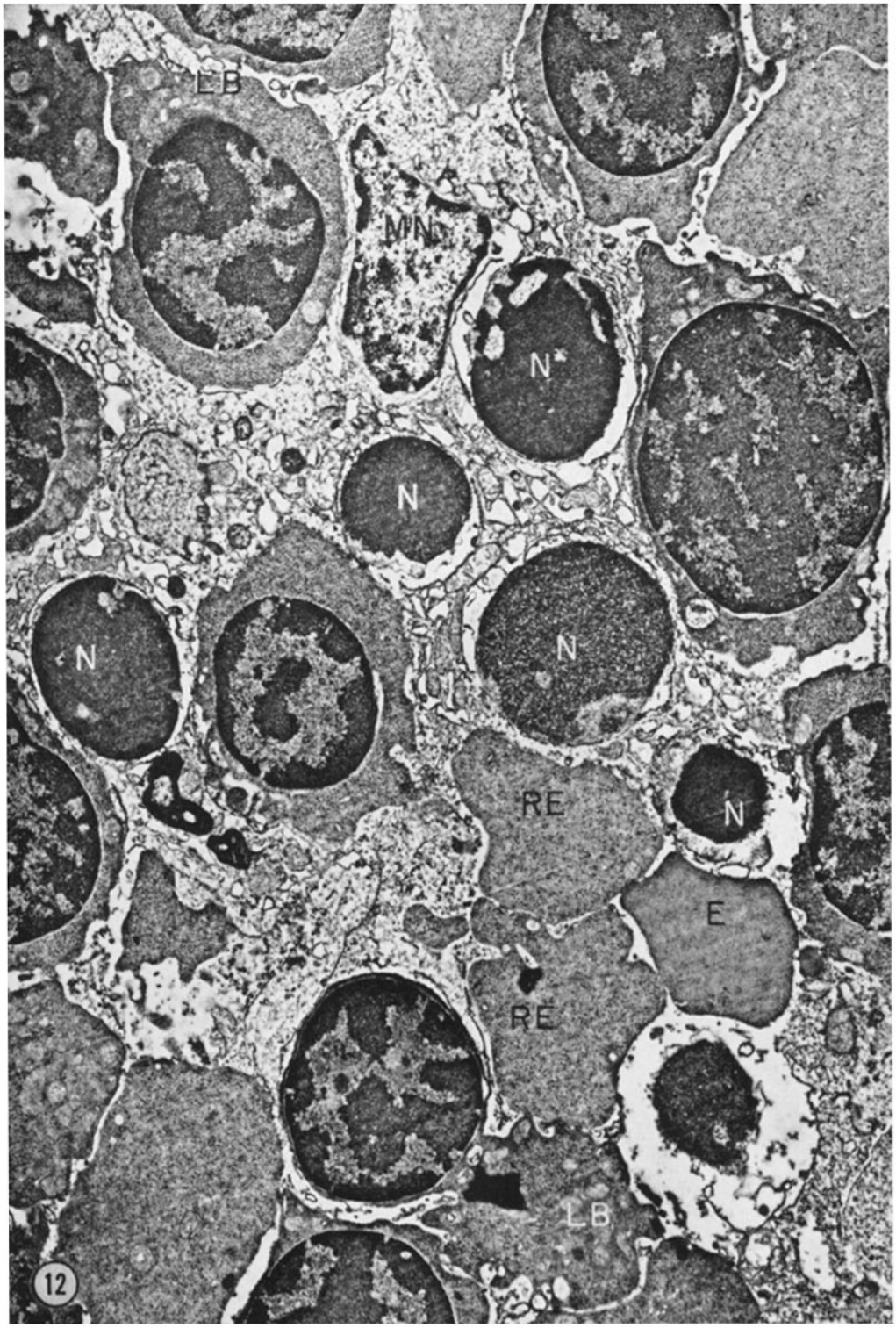


FIGURE 12. Electron micrograph of a section through an erythroid clone. A large macrophage, the nucleus of which (*MN*) is seen at the top of the micrograph, contains several extruded erythroid nuclei (*N*) at different degrees of disintegration. It is in close proximity to some late erythroblasts (*LB*), reticulocytes (*RE*), and erythrocytes (*E*). One of the erythroblasts (bottom) is at the end of the process of expelling its nucleus. Pseudopods of the macrophage have already enveloped the nucleus and its surrounding cytoplasmic rim, which is still attached to the rest of the cell. Two vacuoles can be seen between the nucleus and the future reticulocyte. × 9,000.

chromatin. The cytoplasm of this cell contains single ribosomes and polysomes, many of the latter appearing typically in pairs (5, 22). In some micrographs of cells at this stage, hemoglobin-rich cytoplasm can be seen penetrating into the nucleoplasm through pores in the nuclear membrane (Figs. 2–4). This phenomenon that can be observed in erythroblasts at various degrees of maturation (5) is seen more readily just before the expulsion of the nucleus of the late erythroblast (orthochromatic). Such an invasion of the dense cytoplasm through the nuclear membrane can also be seen in some expelled nuclei (Fig. 2). No massive invasion of the nucleoplasm by hemoglobin-rich cytoplasm or an image that could be interpreted as karyolysis has been observed in this system.

### *The Process of Nuclear Expulsion*

In the late erythroblast, the nucleus occupies an eccentric position adjacent to the cell membrane (Fig. 2, see also lower portion of Fig. 1). The mitochondria and some membranous elements of the cell concentrate near the eccentric nucleus within the bulk of the cytoplasm. A narrow rim of cytoplasm is located between the nucleus and the cell membrane. Apparently empty space between the nucleus and the cytoplasm may be observed in late erythroblasts (Fig. 1, bottom cell). These may be artifacts of fixation.

In the subsequent stage, the pycnotic nucleus can be seen penetrating into the cytoplasmic protrusion and being deformed in the process (Fig. 3). A narrow rim of cytoplasm separates the nucleus from the cell membrane. The major part of the nucleus remains in the original cell. At this stage and during the following ones, the cell is of irregular form, hence neither bidiscoidal nor spherical, and sometimes shows pseudopod-like protrusions (Figs. 4–11). As the process continues, a progressively greater part of the nucleus is extruded through an apparently narrow passage (Figs. 4–8).

In the subsequent stages, the whole nucleus is withdrawn from the main mass of cytoplasm (Figs. 9–11). The nucleus now reassumes a rounded form. It is attached to the main nucleus-free cell by a large "bridge" interrupted by "vacuoles," or only by two or three narrow links. Finally, the main cell is barely connected with the expelled nucleus, which is surrounded by a narrow rim of cytoplasm in which some free ribosomes and occasionally a remnant of a mitochondrion may still be present. The membrane surrounding this cytoplasmic rim is often undulated, detached from the cytoplasm, and discontinuous (Figs. 2, 7–11).

During the whole process, mitochondria (Figs. 4–10) and membranes of the Golgi apparatus (Figs. 8–9) can be seen agglomerated. Vacuoles containing ferritin granules are often visible, some being packed with ferritin and others containing only a few grains (Figs. 3, 4, 6–8).

Free nuclei surrounded by a narrow rim of cytoplasm and apparently damaged plasma membrane can be seen infrequently among other cells of the clone (Fig. 2). Nuclei in various stages of disintegration can be found inside the cytoplasm of the macrophages (Fig. 12).

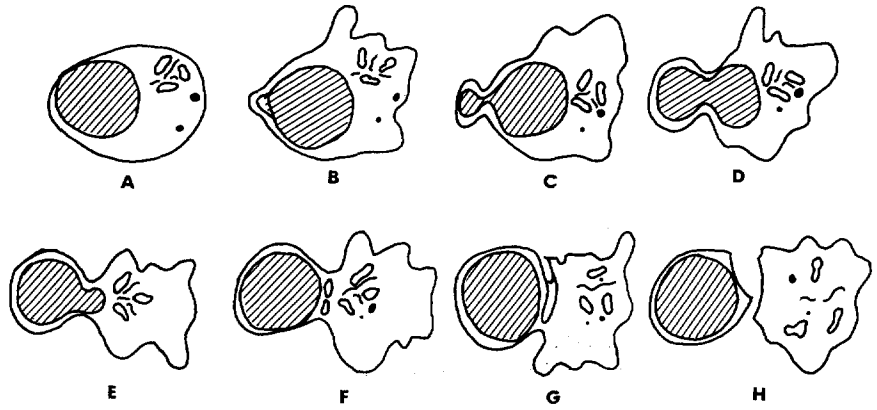


FIGURE 13. Schematic representation (*A–H*) of the sequence of events occurring in the process of extrusion of the nucleus from the late erythroblast.

## DISCUSSION

The results of this study confirm that the nucleus of the late erythroblast is expelled surrounded by a narrow rim of cytoplasm and membrane, without fragmentation, and they reveal the sequence of deformations undergone by the nucleus in the process. The sequence of events that take place when the nucleus of the late (orthochromatic) erythroblast is expelled is summarized in Fig. 13. No evidence of karyolysis could be found in the present study on 20 different erythroid clones. It has been argued that, if all of the nuclei were expelled, more free nuclei should have been found (7). That free nuclei are rare in hemopoietic centers may be due to the avidity with which macrophages phagocytize the expelled nuclei (Fig. 12).

Penetration of hemoglobin through pores in the nuclear membrane, similar to that described by Davies (4) in avian and amphibian nucleated erythrocytes and by Grasso et al. (5) in rabbit erythroblasts, was observed. However, the nucleus is usually expelled before further development of this process takes place. The observed agglomeration of mitochondria in the vicinity of the nucleus as the expulsion process begins confirms the observations of this phenomenon made by Bessis and Bricka with phase microscopy (2) and those of Orlic et al. with electron microscopy (18). It may be related to the energy requirement of the increased contractile activity (1, 2) and the synthesis of additional cell membrane which is required for the separation of the extruded nucleus. A similar concentration of mitochondria has been shown to occur in mitotic division of primitive erythroblasts (10).

The surface activity and movement of the late erythroblast with its irregular protrusions was described in the phase microcinematography and the similarity of this phenomenon to cell division was pointed out by Bessis and Bricka (2). Additional similarity between the two processes is represented by the elongated vacuoles separating two dividing cells, on the one hand, and the similar cleavage furrows separating the extruded nucleus from the remaining cytoplasm, on the other. This has been previously observed (11) and is confirmed in the present study.

The similarity between the process of cell division and the expulsion of the nucleus raises two problems for further study: (*a*) what brings about the expulsion of the nucleus rather than a division of its contents? (*b*) by what features does the structurally damaged membrane surrounding the expelled nucleus differ from the membrane enveloping the remaining future reticulocyte as to make it so readily recognizable by the macrophages?

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