THE MECHANISM OF DENUCLEATION IN CIRCULATING ERYTHROBLASTS

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ABSTRACT

Erythroblast denucleation in the peripheral blood was studied by electron microscopy. Blood was used from dogs anemic either by infection with Babesia canis or from injections of phenylhydrazine hydrochloride. One of the earliest stages of denucleation was the migration of nuclei to the plasmalemma. Mitochondria and coalesced vesicles, derived from the cell membrane of the erythroblast, congregated at the underside of the nuclear envelope unapposed by erythroblastic cell membrane. The coalesced vesicles apparently provided the limiting membrane which surrounded the deep circumference of the extruded nucleus and its associated hemoglobin rim, and also furnished a new plasma membrane for the cell in the area where the nucleus, in denucleation, had utilized the original erythroblastic plasmalemma.

It is not known whether loss of nuclei from erythroblasts occurs by extrusion, by karyolysis, or both. Most studies to date have used phase-contrast microscopy to determine the mechanism involved, and the predominant opinion seems to favor nuclear expulsion as the method of denucleation (1-11).

The present study of erythroblast denucleation was made with the electron microscope, with blood from dogs with either induced or spontaneous anemia.

MATERIALS AND METHODS

The blood studied was from dogs anemic as a result of severe parasitism with a hematozoic parasite, Babesia canis, and from dogs made anemic by treating them every other day with three subcutaneous injections of phenylhydrazine hydrochloride at a dosage of 16 mg per kg of body weight. Five to six drops of whole blood were collected in fixative when approximately 10% of the erythrocytic cells were nucleated, as determined by examination of Wright-Giemsa-stained blood smears. 1% OsO4 (12) was employed as a fixative for blood obtained from dogs parasitized with B. canis, while blood from phenylhydrazine-treated dogs was fixed in 2% glutaraldehyde and postfixed in 1% OsO4. The fixed blood was embedded in Araldite (13), and cut into thin sections with a diamond knife. Sections on grids were stained with uranyl acetate and lead citrate (14), and were examined with a Philips EM200 electron microscope.

RESULTS

Light Microscopy

Nuclei were located centrally, peripherally, or partially protruded from erythroblasts (Fig. 1). Occasionally nuclei were observed extracellularly. Basophilic, polychromatic, and orthochromatic erythroblasts were present in blood smears.

Electron Microscopy

The mechanism of denucleation was the same in both types of anemia, and thus the results were combined for this study.

Erythroblasts with centrally located nuclei contained numerous polyribosomes and a scattering of mitochondria as well as open-spaced vesicles throughout the cytoplasm. Such vesicles ap-
FIGURE 1  A nucleus (arrow) is almost extruded from an erythroblast. Wright-Giemsa stains. × 1,500.

FIGURE 2  An erythroblast with a centrally located nucleus (N) contains scattered mitochondria (m), vesicles (V), and invaginations (arrows) on the cell surface. × 15,000.
Figure 3  Intranuclear hemoglobin (arrow) may have originated from the cytoplasm through expanded nuclear pores, or possibly within the nucleus. X 15,000.

Figure 4  Extranuclear chromatin (arrows) enters the cytoplasm through expanded nuclear pores. X 15,000.
Figure 5 Mitochondria (m) aggregate on the under side of the nuclear envelope, following migration of the nucleus to the cell membrane of the erythroblast. X 15,000.

Figure 6 Vesicles (V) also accumulate in the area of mitochondrial (m) concentration. X 15,000.
Figure 7  Vesicles (V) after accumulating in the area of mitochondrial (m) concentration coalesce to form dilated open spaces. × 15,000.

Figure 8  Enlarged coalesced vesicles (V), on the under side of the nuclear envelope, open at the plasma-lemma of the erythroblast (arrow) to form irregular U-shaped channels. × 15,000.
FIGURE 9 A series of coalesced vesicles (V) almost completely undermine most of circumference of the nucleus not associated with cell membrane. Two of the coalesced vesicles have opened at the plasmalemma of the erythroblast to form irregular U-shaped channels (arrows). × 15,000.

FIGURE 10 Rows of coalesced vesicles (V) open to the plasmalemma of the erythroblast and cause its surface to be studded with many pseudopodia-like extensions (arrows). × 15,000.
Figure 11 Just prior to denucleation. A nucleus (N) is attached to the parent cell by a thin layer of membrane-limited hemoglobin (H). The membrane (arrow) on the underside of the hemoglobin-rimmed nucleus and a portion of the plasmalemma of the erythroblastic cell (double-headed arrow) were formed from coalesced vesicles. × 15,000.

Figure 12 An extracellular nucleus (N) is surrounded by a layer of hemoglobin (H), and the entire structure is enclosed by a membrane. × 15,000.
FIGURE 13  Schematic sketch of an extruded nucleus and the remainder of the erythroblast. The wavy line indicates membranes derived from cytoplasmic vesicles. Solid lines are original plasma membranes.

Apparently originated from small invaginations of the plasmalemma (micropinocytosis), became detached, and moved into the cytoplasm of the erythroblast (Fig. 2). Nuclei of erythroblasts often contained intranuclear hemoglobin which was similar in appearance to that in the surrounding cytoplasm. The occurrence of such intranuclear hemoglobin probably resulted either from the passage of hemoglobin from the cytoplasm into the nucleoplasm through expanded nuclear pores, or from formation of hemoglobin in the nucleus (Fig. 3). The existence of extranuclear chromatin apparently resulted from a similar but reverse process with migration of chromatin into the cytoplasm through expanded nuclear pores (Fig. 4).

One of the earliest stages of denucleation appeared to be migration of nuclei to the plasmalemma of the erythroblast. In this phase, approximately one-half of the superficial circumference of the nucleus was in close apposition to the erythroblastic cell membrane and was separated from it by a thin layer of cytoplasm. Concurrently, mitochondria aggregated at the underside of the nuclear envelope (Fig. 5). Vesicles also accumulated in the area of mitochondrial concentration, close to the plasmalemma (Fig. 6). Vesicles later became elongated, and adjacent ones coalesced when located in the area of mitochondrial concentration (Fig. 7). Many of these coalesced vesicles were sandwiched between the nuclear envelope and mitochondria in the area. It appeared that eventually a series of coalesced vesicles combined with another series of coalesced vesicles to form larger single vesicles. Such dilated, open spaces gradually extended to the plasmalemma of erythroblasts where they fused with the cell membrane to form "U"-shaped channels of various sizes (Fig. 8). Eventually, a series of coalesced vesicles completely undermined the deep portion of the circumference of the nucleus (Fig. 9). In this process, one membranous arm of the irregular U-shaped channel served as the lower, limiting membrane for the nucleus and layer of hemoglobin about to be extruded. The other membranous arm of the U-shaped channel became a new segment of plasmalemma for the erythroblast in the area where the nucleus was pinched off. Since series of coalesced vesicles were arranged in rows of two or three, opening of these several rows of vesicles to the plasmalemma of the erythroblast caused this cell to have many pseudopodia-like extensions on to its surface where the nucleus was to be separated from it in the terminal stage of denucleation (Fig. 10). Just before complete denucleation, a nucleus was attached to the parent cell by a thin strand of membrane-limited hemoglobin (Fig. 11).

Extracellular nuclei were surrounded by a layer of hemoglobin of varying thickness, and the entire structure was limited by a single membrane (Fig. 12). Mitochondria were sometimes entrapped in the peripheral layer of hemoglobin that surrounded free nuclei; however, most of the mitochondria in erythroblasts were retained in the cell cytoplasm subsequent to denucleation.

**DISCUSSION**

Denucleation of erythroblasts has been stated to require about 10 min, and probably occurs by extrusion rather than nucleolysis (1–11). Some of these conclusions were reached by using phase-contrast microscopy; however, many studies employed electron microscopy (4–11). The results of the present study also indicate that extrusion is the principal mechanism of denucleation of the erythroblast of the dog. However, there is some
evidence of karyolysis prior to extrusion of the main nuclear mass since extranuclear chromatin sometimes was seen escaping into the cytoplasm through expanded nuclear pores (Fig. 4). Such nuclear fragments that originate in denucleation could be the origin of Howell-Jolly bodies.

A sequence of events which may occur in denucleation is described herein. Congregation of mitochondria and vesicles in the vicinity of the deep circumference of the nuclear envelope is shown in micrographs. These are probably the cytoplasmic granules that have been described previously in the region of the nucleus prior to extrusion (5). In accord with known functions of mitochondria, it could be assumed that these organelles accumulated in this area to supply energy possibly required for nuclear expulsion; this has been suggested by others (9). Cytoplasmic vesicles which accumulated in the region of the nucleus probably originated as pinocytotic vesicles on the erythroblastic plasmalemma (Fig. 2). These vesicles did not appear to be endoplasmic reticulum or remnants of Golgi apparatus. That micropinocytotic activity occurs on cell surfaces of erythropoietic cells has been demonstrated previously (15, 16). It was concluded in the present study that coalesced cytoplasmic vesicles provided the limiting membrane which surrounded the deep portion of the extruded nucleus and its associated hemoglobin layer, and also furnished a new plasma membrane for the cell in the area where the nucleus, in its release, had carried with it the original erythroblastic plasmalemma (Fig. 13).

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