or sarcolemma and because addition of diazacholesterol to isolated sarcolemma has no effect on its adenosine triphosphatase activities. Possibly the selective increases of the (Na^+,K^+)- and Ca^{2+}-stimulated adenosine triphosphatases represent a compensatory reaction to the influx of Na^+ and efflux of K^+ associated with the repetitive action potentials that characterize myotonic muscle.

The specific membrane resistance is more than doubled in diazacholesterol-treated rats (*3*). This increase was expected to reflect a decrease in chloride conductance because chloride conductance is two- to threefold that of potassium in normal mammalian muscle (*11*). Indeed a recent study (*12*) showed that chloride conductance is only one-third of normal in diaphragm fibers of diazacholesterol-treated rats. We suggest that in diazacholesterol-treated rats the abnormal sterol composition of the sarcolemma results in decreased chloride conductance and thus accounts for the repetitive action potentials and the resultant delay in relaxation of myotonic muscle. This hypothesis is consistent with the observation that chloride conductance induced in thin lipid films by certain polyene antibiotics is dependent on the sterol composition of the film (*13*). Similar mechanisms may apply to certain forms of human myotonia, including myotonia congenita in which increased membrane resistance and decreased chloride conductance are recognized (*14*).

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20 November 1972

Fate of the Nucleus of the Marrow Erythroblast

Abstract. *Nucleated red cells lose their nuclei during passage through the endothelium of marrow sinuses. The passage occurs through cytoplasmic pores which are not gaps at the junction of two endothelial cells but perforations within the endothelium. Enucleation occurs because the pores are of relatively fixed size. Whereas the cytoplasm is flexible and squeezes through the pore, the nucleus is rigid and cannot conform to the pore size. It is, thus, caught, and the red cell becomes enucleated.*

The function of the endothelium of splenic sinuses in controlling transmural cellular passage is well known: rigid intracellular materials such as Heinz bodies are removed, whereas the rest of the red cell is allowed to pass by. Such a mechanism is commonly known as "pitting" (*1*). We now provide evidence that a similar mechanism is operative in cellular passage in the wall of bone marrow sinuses.

In bone marrow, hematopoiesis occurs outside the sinuses. At the stage of orthochromic normoblast polarization of the nucleus occurs so that the bulk of the cytoplasm lies closer than the

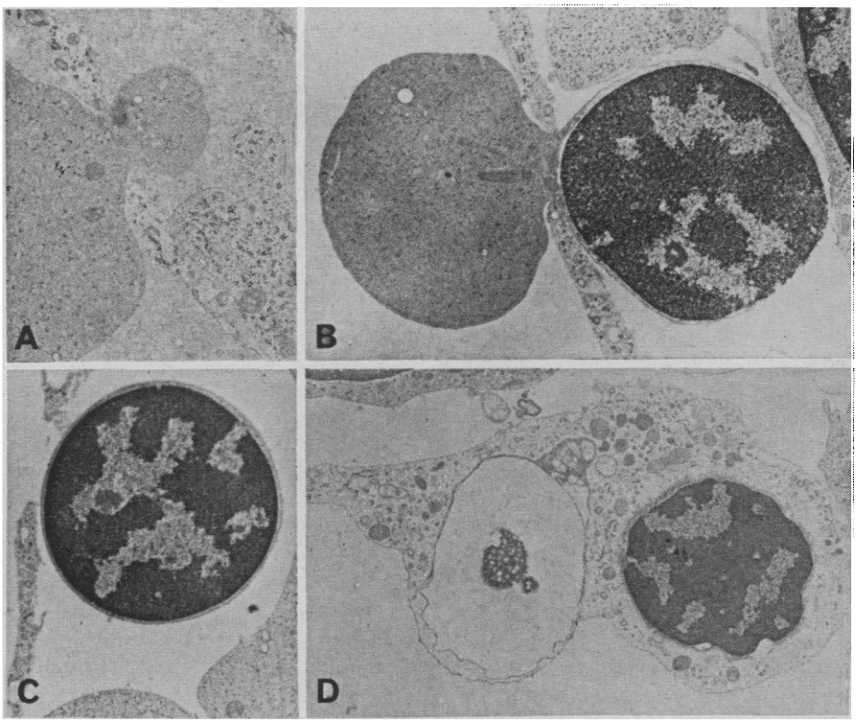

Fig. 1. Various stages of transmural passage and enucleation of red cells and mature normoblasts. (A) The cytoplasm in passage from the cord into the sinus. The size of the aperture appears to exert a control on the passing red cell, and the cell must conform to this size (× 8500). (B) Nucleated red cell in passage through the wall of a marrow sinus. The cytoplasm is completely within the lumen. The nucleus, however, cannot conform to the size of aperture and the nucleus remains behind (× 8500). (C) This nucleus, located in an area adjacent to a pore, may have been recently lost from a passing normoblast (× 8500). (D) The nucleus of a normoblast is still recognizable in the cytoplasm of this cordal macrophage. The remnant of another nucleus is seen within a phagosome (× 6800). In A, B, and C the cord is on the right and the lumen on the left.

nucleus to the sinus wall (*2*). The cell then has to pass through the sinus wall in order to enter the circulation.

Our materials were obtained from 12 New Zealand white rabbits, 1 to 8 weeks old. With the animals under general anesthesia, the marrow tissue was removed from tibia and femur after splitting the bones. The tissue was immediately immersed in Karnovsky's glutaraldehyde-formaldehyde mixture buffered in sodium cacodylate, where it was fixed for 24 hours. After it was rinsed in the buffer, the tissue was fixed in 2 percent OsO_4 similarly buffered. It was then dehydrated, embedded in Araldite, sectioned in an LKB microtome with a diamond knife, and studied in a Hitachi HU 11-A microscope.

The endothelial cell of the sinus wall had an elongated nucleus. Its cytoplasm was stretched to make up the bulk of the sinus wall, which was very thin and at times extremely attenuated. When the cytoplasms of two endothelial cells came into contact, they were held by junctional complexes, usually of the zonula adherens type. No gaps were present in these junctions, a feature distinguishing these sinuses from lymphatics (*3*). No passage of red cells through these junctions was observed. Passage occurred through pores of various sizes (1 to 4 μm) at irregular intervals in the endothelial cytoplasm of the sinus wall (Fig. 1).

The continuity of the cytoplasm in the area below the pore was shown in serial sections, which indicated that these pores are within the cytoplasm of a single cell and are not gaps at the juncture of two endothelial cells. The pore size appeared relatively fixed, exerting a control on the passing cells, which must conform to this size in order to traverse the wall. The cytoplasm of the passing cells was seen to squeeze through these pores (Fig. 1A), and the cytoplasmic organelles, such as mitochondria and ribosomes, and small nuclear fragments (Howell-Jolly bodies) were small enough to conform to the pore size. They were also seen in passage through the apertures. The rigid nucleus of the red cell, however, could not conform to the pore size. When nucleated red cells were seen in passage, the nucleus was caught and the cell became enucleated (Fig. 1B). In this manner, the red cell less its nucleus entered the circulation as a blood reticulocyte and the nucleus remained in the hematopoietic compartment of the marrow (Fig. 1C) where it was ingested by macrophages (Fig. 1D).

The morphological basis of this pit ting function in the endothelium of marrow sinuses is not immediately clear. Even in high-resolution microscopy, no specialized grommet-like structure was seen in the portion of endothelium adjacent to the pore. This is of interest because in the endothelium of splenic sinuses, bands of microfilaments determine the pore size and thereby control transmural cellular passage (*4*). In our materials, bands of microfilaments were present in the endothelium of marrow sinuses. These structures, however, were located beneath the cytoplasmic membrane in the abluminal side. We did not observe bands of microfilaments in the areas adjacent to the grommets. In the spleen, the pores are potential and the passing cell must find its way between two endothelial cells normally in juxtaposition. During the passage, the pore size is controlled by bands of microfilaments that prevent the endothelial cells from being forced too far apart. In marrow, however, the pores are actual and the passage of erythrocytes is transcellular

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Proton Beam Radiography in Tumor Detection

Abstract. *Monoenergetic protons are highly sensitive to density variations and are capable of giving radiographs of very high contrast. As an initial step in exploring their diagnostic potential, protons with energies of 160 million electron volts are used in "contact radiography" on tumor-bearing human brain specimens. The proton radiographs are compared with x-radiographs taken under identical specimen conditions.*

Unlike x-ray and neutron fluxes, which are attenuated in an exponential relationship with absorber thickness, the proton flux shows only moderate attenu ation before dropping off very steeply at the end of the particle range. This property may be used to advantage fo by placing a photographic film in the region of the steepest part of the flux-depth curve radiographs of unusually high contrast may be obtained (*1*). Furthermore, radiography based on transmission of protons is relatively insensitive to variations in chemical com-

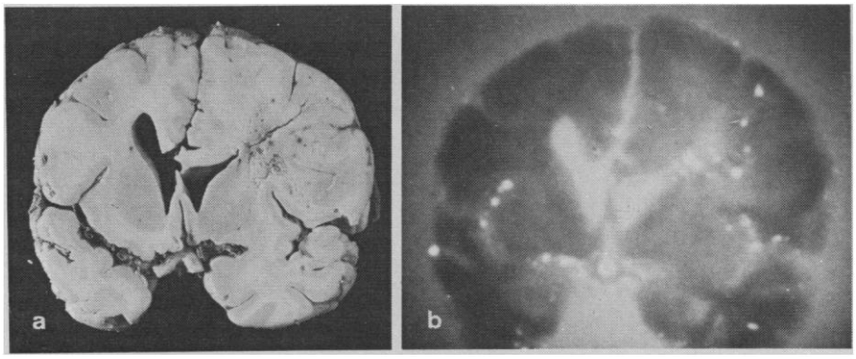

Fig. 1. (a) Front view of a coronal slice 1 cm thick of a human brain fixed in formalin. Note the glioblastoma multiforme in the white matter of the hemisphere on the right, with swelling of the hemisphere and distortion of the ventricular system. (b) Proton radiograph. Note the decreased density in the tumor area and the visualization of the basal ganglia. The white spots are air bubbles. The conditions were 115-cm focus to film, no screen, Polaroid TLX film, and a dose to the first surface of approximately 290 rad in 0.8 minute.