REDUCTION IN SURFACE CHARGE AS AN EXPLANATION OF THE RECOGNITION BY MACROPHAGES OF NUCLEI EXPELLED FROM NORMOBLASTS

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ABSTRACT

The density and distribution of electric charge on the surface of rabbit bone marrow cells was visualized by electron microscopy after the cell surfaces had been stained with charged colloidal iron particles. Expulsed erythroid nuclei are less negatively charged than any other cell in the bone marrow. They carry from about one-half to one-third of the charge density on the remaining future reticulocyte. The reduction in the surface charge density is already apparent when the nucleus is partially expulsed. Practically no positive charge was found on its surface or on the surface of any other bone marrow cell. The possibility that the reduced negative charge on the surface of expelled erythroid nuclei is one of the means by which the macrophage distinguishes it from other bone marrow cells is discussed.

INTRODUCTION

The nucleus of the late erythroblast is expelled, surrounded by a narrow rim of cytoplasm and membrane (1, 2, 12, 13, 18, 19, 21).

Similar electron micrographs were interpreted in a slightly different way, i.e. that the macrophage plays an active role in "extruding" the nucleus (16, 17). It has been pointed out, that if all the nuclei are expelled, more free nuclei should be present than are actually found (9). However, we assume that the rarity of free nuclei in the hemopoietic centers may be ascribed to the avidity with which the macrophages phagocytize the expelled nuclei (2, 12, 13, 16, 17, 19, 21). We have raised the question (19), in what features does the membrane surrounding the expelled nucleus differ from that which envelops the remaining future reticulocyte? What makes it "recognizable" by the macrophage?

Macrophages also "recognize" senescent red blood cells. It is reasonable to assume that there may be a feature common to the senescent red cell membrane and the membrane surrounding the expelled nucleus. Of all the biophysical characteristics of old red blood cells (4), the surface charge, which is markedly reduced in older cells (6, 10, 20) (Marikovsky, Y., and D. Danon. See paper in this issue), appeared to us the most likely feature to be recognized by the macrophage. Since the demonstration of this parameter by measurements of the electric mobility of free nuclei is excluded by their rarity, or inexistence as some authors claim (2, 16, 17), we have analyzed the relative differences in charge density on the membrane surfaces by a technique using positively and negatively charged colloidal ferric oxide particles, as described by Gasic et al. (7).

MATERIALS AND METHODS

Cell suspension was prepared by suspending small pieces of bone marrow from young rabbits in phos-

phate buffer solution and passing it about 10 times through a syringe needle No. 16.

The suspension was filtered through six layers of cheese cloth, and the filtrate containing the bone marrow cells was centrifuged for 7 min at 150 *g*. The sedimented cells were fixed by resuspension in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 (11) for 1 hr at room temperature, washed twice, and left overnight in the same buffer adjusted to isotonicity by glucose. Before the cells were suspended in the colloid solutions, they were washed twice with distilled water. The positively and negatively charged colloidal ferric oxide solutions were prepared according to the technique described by Gasic et al. (7). The cells were kept in the positive colloid solution, pH 1.8, dialyzed or undialyzed, for 10–30 min at room temperature, washed with 12% acetic acid and then with distilled water. The negative colloid solution was prepared from the dialyzed positive colloid and adjusted to pH 6.0. Cells were kept in the negative colloid solution for 15 min at room temperature and then were washed twice with distilled water only.

The cells were postfixed with 1% OsO_4 in 0.1 M phosphate buffer (11) for 1 hr, gradually dehydrated with acetone, embedded in Vestopal-W (15), and sectioned on a Danon ultramicrotome (Yeda, Research and Development Co. Ltd., Rehovot, Israel) equipped with glass knives. Sections were mounted on uncoated or Formvar-coated copper grids reinforced with a thin layer of carbon. They were stained on the grid with uranyl acetate or double stained with uranyl acetate followed by lead citrate (14). JEM-7 and JEM-T7 electron microscopes were used.

To compare the density of attached colloidal particles on the surfaces of different membranes, a curvimeter was used to measure the length of membrane on which the number of black dots was counted from micrographs enlarged to a 25,000 magnification.

RESULTS

All bone marrow cells treated with the positively charged colloidal ferric oxide were labeled by the colloid. Cells of the granuloid and lymphoid lines were generally more heavily marked than those of the erythroid line. The density of colloidal black dots on the cell membrane differed according to the stage of development of the erythroid line. Early basophilic erythroblasts were the most densely marked. The density of ferric oxide staining was less in the late, orthochromatic erythroblast and increased again in the reticulocyte (Fig. 1). Quantitative evaluation of the degree of labeling with colloidal iron particles on marrow cells from different stages of differentiation will be published elsewhere.

Free erythroid nuclei which have a narrow, hemoglobin-rich rim of cytoplasm surrounded by plasma membrane were less labeled with the ferric oxide particles than were any other cells in the bone marrow (Fig. 2).

Particularly striking is the difference between the particle density on the membrane of the erythroid nucleus and that on the membrane of the macrophage in the process of phagocytizing it (Fig. 3).

Late erythroblasts were found at different stages of their nuclear expulsion, a part of the nucleus being extruded and surrounded by cell membrane, while the rest was still inside the main bulk of cytoplasm. In all these cells, the part of the membrane that surrounded the extruding nucleus had a smaller number of attached colloidal particles than did the rest of the cell membrane (Fig. 4). This observation was verified by counting the positive particles per unit length on different parts of the perpendicularly sectioned membrane in 20 erythroblasts from which nuclei were in the process of being extruded. The electron micrographs were taken from sections of bone marrow of three different rabbits (Table I).

Negatively charged colloidal particles did not adsorb on the surface of bone marrow cells. Only occasional randomly distributed black dots could be observed without any preference being exhibited for any particular cell or free nuclei, or for the membrane that surrounds the extruding nuclei.

DISCUSSION

Macrophages, as well as other bone marrow cells, are negatively charged and, therefore, normally exhibit a mutual repulsion. A reduction in negative charge on the membrane of the erythroid nucleus takes place even at the early stages of the expulsion process. This may be concluded from the fact that the membrane surrounding the expelled portion of the now deformed nucleus has a reduced affinity to the positive colloid in comparison with the rest of the plasma membrane of the future reticulocyte.

The reduced electric charge on the surface of extruded mammalian erythroid nuclei was demonstrated by the reduced ability of these surfaces to adsorb positively charged colloidal ferric oxide particles. Neuraminic acid carries most of the negative charge on the red cell surface (3, 8). It was found to be the site for attachment of positively charged poly-L-lysine (5) as well as for the adsorption of positively charged ferric oxide colloidal

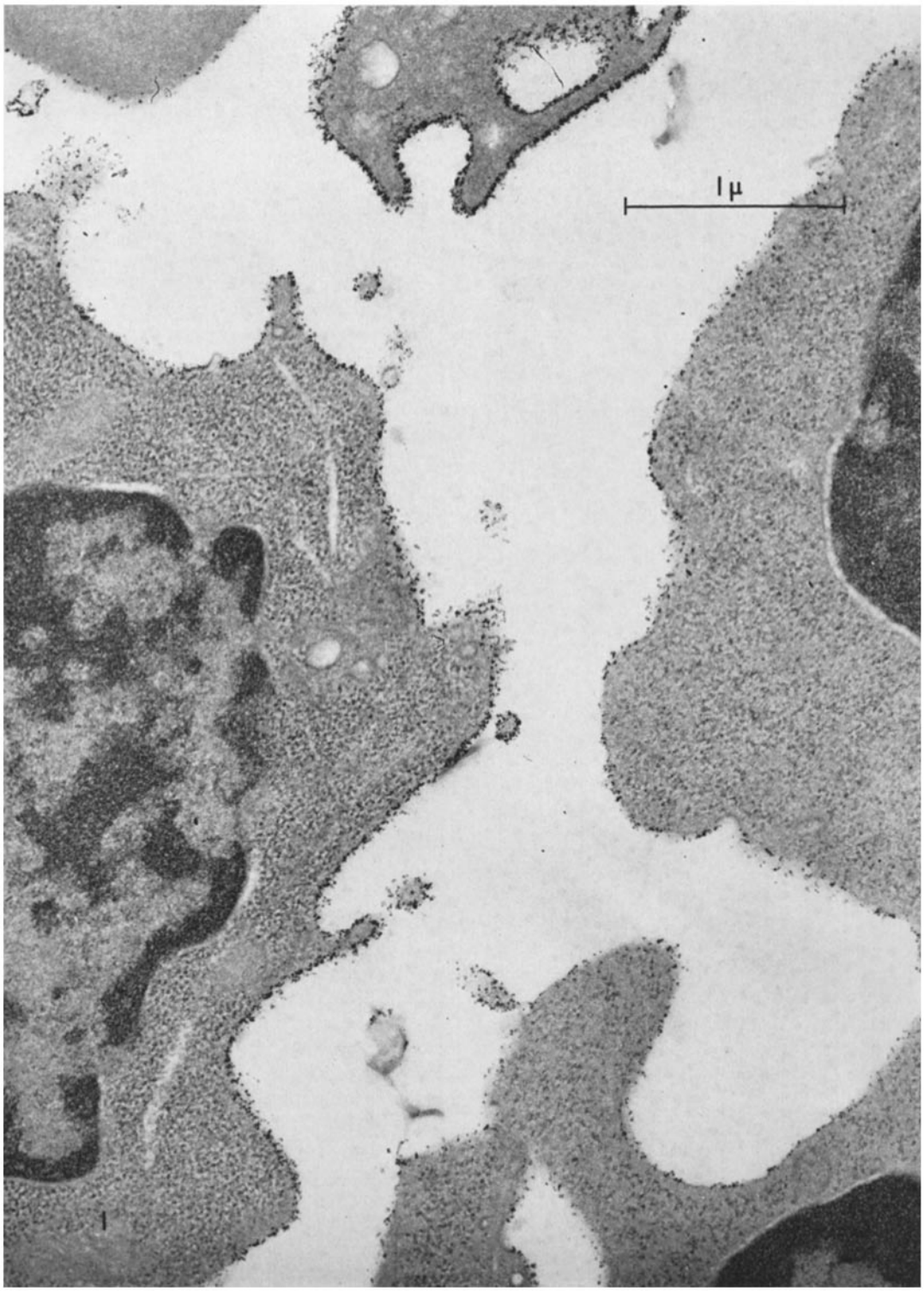

FIGURE 1 A section of rabbit bone marrow suspension stained with positive colloidal ferric oxide. Note the difference in iron particle density deposited on the various cell membranes. The leukoid cell, in the top center, is most heavily labeled. The basophilic erythroblast, on the left, is less labeled; and the two late erythroblasts, on the right, are less labeled than the basophilic erythroblast. The mature erythrocyte (top left) is the least labeled. × 35,000.

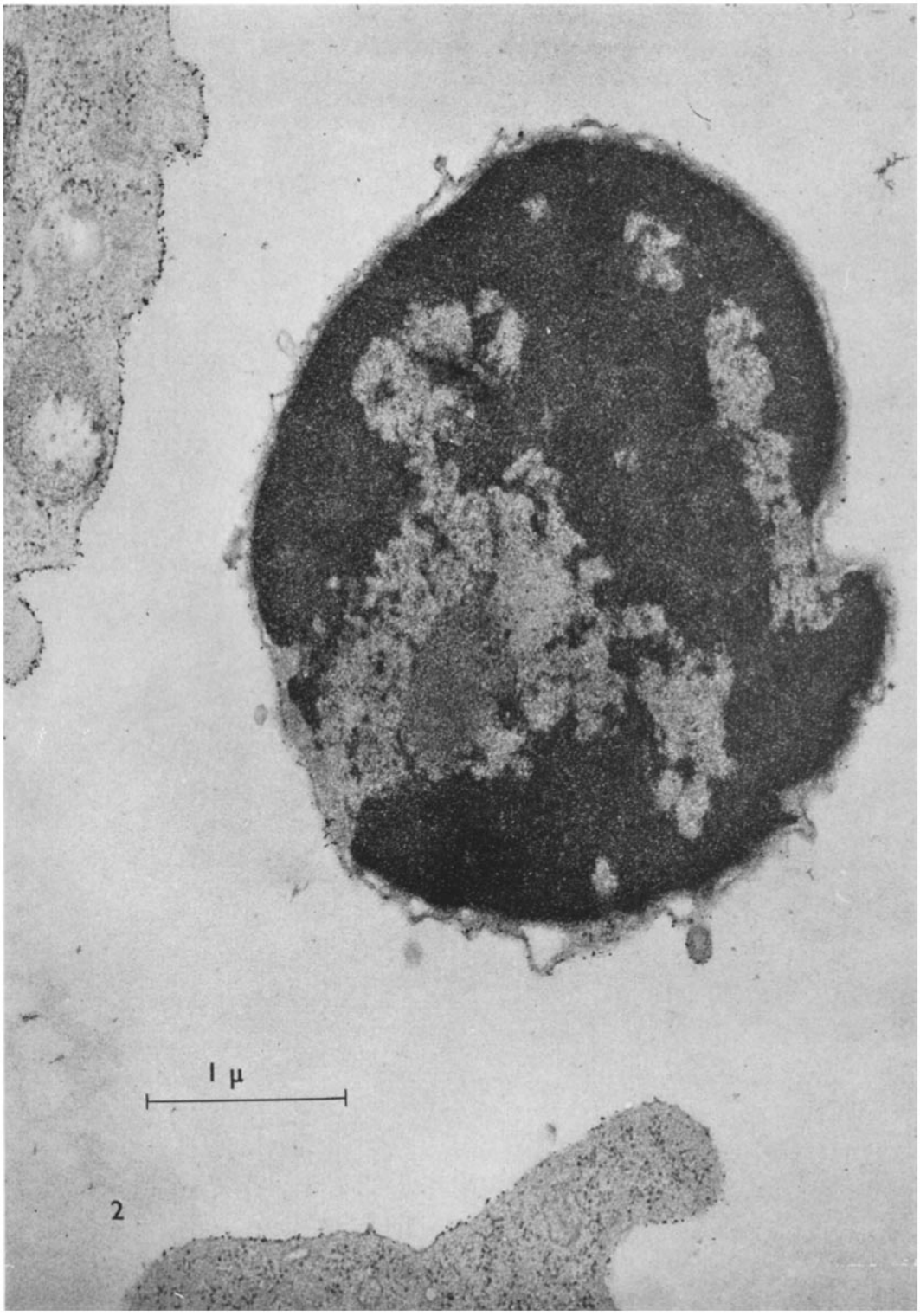

FIGURE 2 Thin section of rabbit bone marrow suspension, stained with positive colloidal ferric oxide. An extruded nucleus, surrounded by a narrow rim of cytoplasm and plasma membrane, is seen in the vicinity of an erythroid (bottom) and leukoid elements. Heavy deposits of the colloid appear on the leukoid cell membrane as compared to those on the membrane of the erythroid cell. Almost no colloidal deposits appear on the free nucleus. × 30,000.

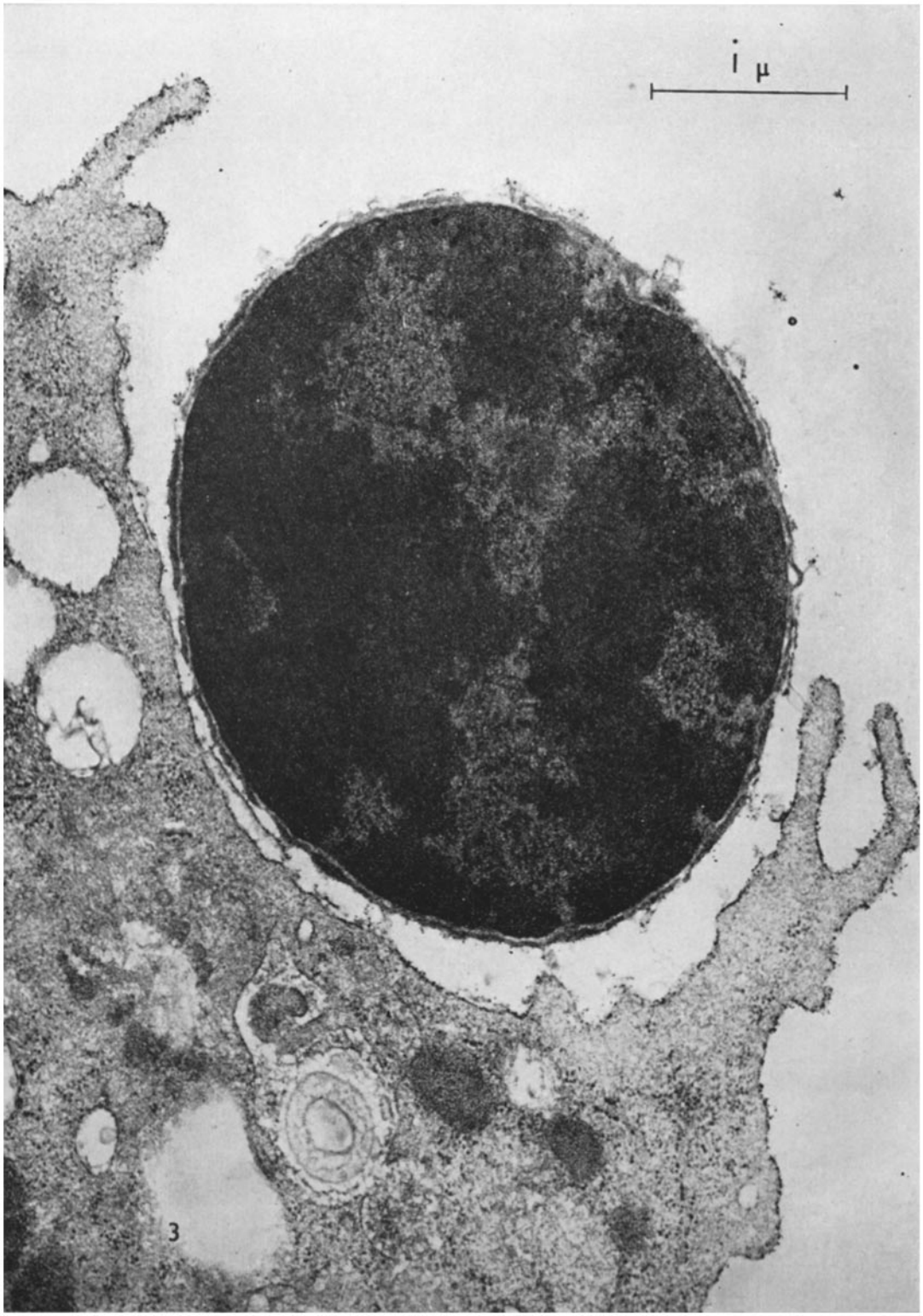

FIGURE 3 Thin section of rabbit bone marrow suspension, stained with the positive colloid. Free erythroid nucleus, situated in proximity to a macrophage, is partly surrounded by the cytoplasmic protrusions of the macrophage. The colloid black dots are abundant on the macrophage membrane and scarce on the membrane surrounding the erythroid nucleus. × 30,000.

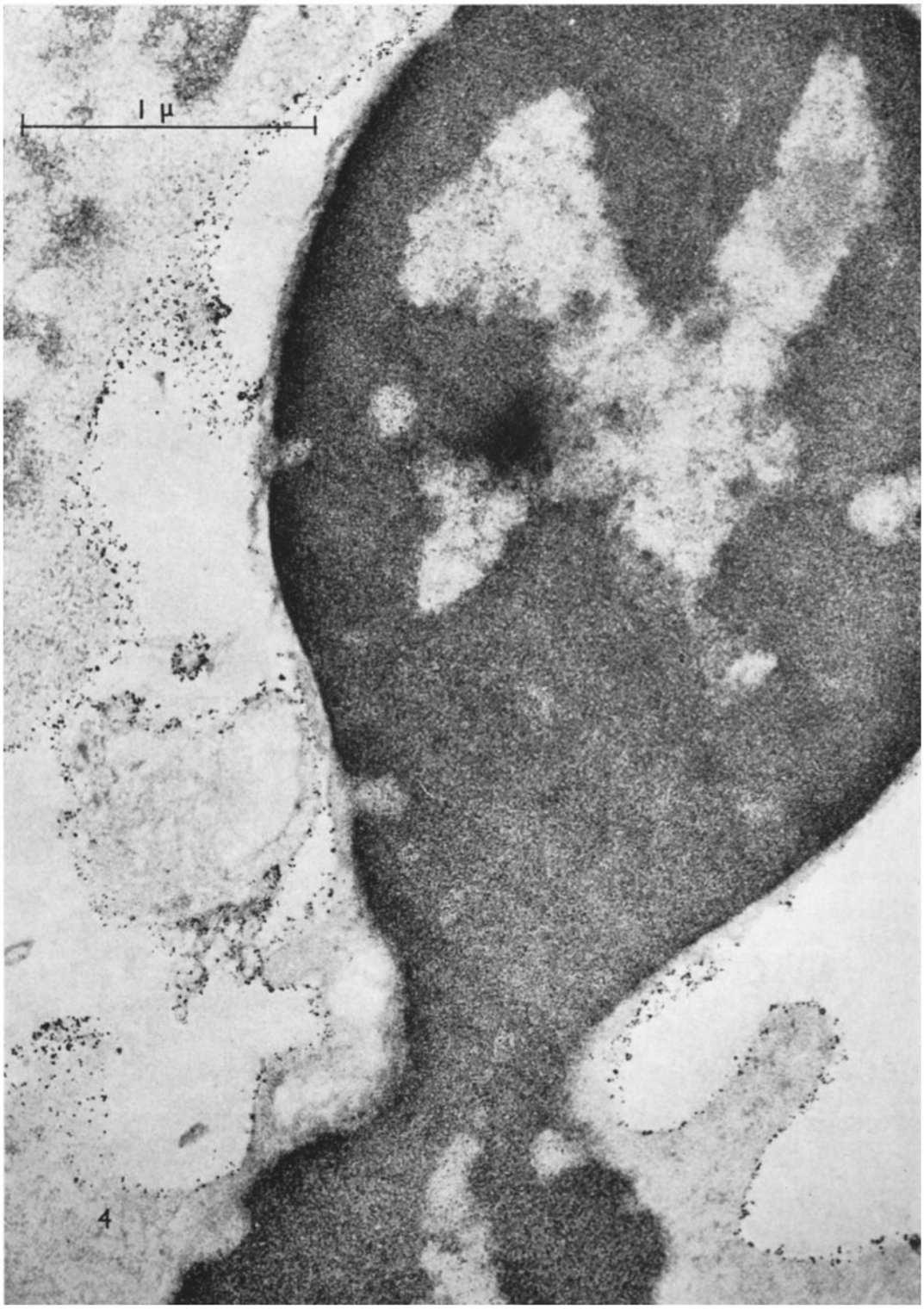

FIGURE 4 Bone marrow cells stained with the positive colloid. A late erythroblast is shown representing an early stage in the nuclear expulsion process in which a part of the nucleus is situated outside the main bulk of cytoplasm, surrounded by a rim of cytoplasm and membrane. The positive colloid on the erythroblast surface is present mainly on that part of the membrane which surrounds the remaining cytoplasm. A part of a leukoid cell (top) shows a membrane heavily coated with dense particles. × 45,000.

TABLE I

Number of Attached Positive Colloidal Particles Counted Per Micron Length of Membrane Surrounding Nuclei That Are Being Expulsed as Compared With Membrane Surrounding the Remaining Cytoplasm

Rabbit No.	Cytoplasm	Nuclei	Reduction
			%
1	55	21	68.1
1	53	20	62.2
1	42	22	47.6
1	50	18	64.0
1	49	26	46.9
1	47	28	40.4
2	42	16	61.9
2	51	20	60.8
2	53	32	39.6
2	50	25	50.0
2	40	13	67.5
2	50	20	60.0
2	41	22	46.5
2	49	20	59.2
2	46	15	67.3
3*	26	13	50.0
3	18	6	66.6
3	19	7	63.1
3	25	16	36.0

* The generally reduced number of colloidal iron particles per micron of all the membranes in rabbit No. 3 is probably due to the fact that another batch of stain was used. However, the difference between the membrane surrounding the expulsed nucleus and that of the remaining cytoplasm is practically the same as in the other two rabbits.

particles (8). We may therefore deduce that either the membrane of the expelled nucleus loses most of its neuraminic acid by deterioration of the membrane during the process of nuclear expulsion, or that the carboxylic groups are masked by structural alterations in the plasma membrane (19) during this process.

In ascites tumor cells, which had been treated with neuraminidase to "peal off" their negative charges, positive charges appeared on the cell surface to which negative colloidal iron particles were adsorbed (7). One might expect to find positive charges on the presumably neuraminic acid-free surface of the expelled nucleus. However, in our experience, practically no negatively charged colloidal iron particles attached to the surface of expelled nuclei or to the surface of circulating rabbit red cells treated with neuraminidase (Marikovsky, Y., and D. Danon. See paper in this issue.) This leads to the conclusion that positive charges do not play a role in the ability of the macrophage to "identify" and phagocytize expelled erythroid nuclei. However, the reduction of the surface charge on the expelled nucleus to less than one-half or one-third of the negative charge surrounding the remaining reticulocyte may explain the ability of the macrophage to recognize and establish contact with the extruded part of the nucleus, even before the expulsion process is completed.

This study is part of a work to be submitted by E. Skutelsky in partial fulfillment of the requirements for a Ph.D. from the Scientific Council of the Weizmann Institute of Science.

The authors are indebted to Mr. Stanley Himmelhoch for the photographic work.

Received for publication 18 November 1968, and in revised form 16 May 1969.

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