were divided into the following groups according to their snout-rump lengths (SRL): 1.5, 2.5, 3.5, 4.5, 5.5, 6.0, 7.0, 8.0, 10.0, 11.0, 13.5, 15.0, 22.0, 29.0, 34.0 and 41.0 cm. 8 adults also were used. The animals were killed by decapitation and as quickly as possible blocks of tissue were removed from the submandibular glands and fixed for 4 h at 0 °C in 3.5% glutaraldehyde buffered in 0.1 M phosphate to a pH of 7.4. The tissues were washed in buffer and osmicated in 1.0% osmium tetroxide at 0 °C for 2 h. The specimens were then passed through propylene oxide, infiltrated with and embedded in Epon 812. Thin sections of this material were cut for electron microscopy, mounted on uncoated grids, and stained with uranyl acetate and lead citrate. The sections were examined in an RCA EMU-3F electron microscope operated at 50 kV.

Results and discussion. Numerous inclusion bodies (virus-like particles) were observed in the lumina of mucous tubules and of intralobular ducts of the opossum submandibular gland. Over the 3 year period in which the study was conducted, such particles were consistently found in all adult animals and in all postnatal stages examined, with the exception of newborn (1.5 cm) animals known to be less than 12 h old. The particles were abundant in the lumina of mucous tubules and of intralobular ducts and often appeared to clump and to form irregular aggregates (figure 1). The particles also were observed in the intercellular canaliculi located between cells of the mucous tubules. Similar particles were not observed in the nuclei or in the cytoplasm of cells comprising the submandibular gland (figure 1). The particles for the most part are spherical in outline, uniform in size, and exhibit a distinct, central, electron dense core. They are limited externally by a distinct peripheral membrane (figure 2). The outer diameter of the particles ranges from 100 nm to 150 nm and the central core ranges from 50 nm to 70 nm in diameter. The particles, with regard to size, location and morphology, are quite distinct from the adjacent secretory granules of the cells that make up the mucous tubules (figure 1).

The appearance of these particles is remarkably similar to that of numerous viruses reported[8]. Unlike the majority of virus reported to date, however, the particles observed in the opossum submandibular gland were not noted in either the nucleus or the cytoplasm of component cells. The virus-like particles appear nonpathogenic and their nature and role is unknown.

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Close association of erythrocytes with surface of late mouse blastocysts: SEM analysis[1]

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Summary. SEM analysis of preimplantation mouse blastocysts reveals closely associated red blood cells on involuted surfaces of trophoblasts, an indication of the capability for initial phases of phagocytosis at an early developmental stage.

Among changes which occur in the cellular physiology of mammalian blastocysts preparing for implantation are those concerned with the development of invasiveness and phagocytic properties of trophoblasts. Evidence for the latter is based on a) histological demonstration of inclusions of cellular debris and erythrocytes (RBCs) in trophoblastic giant cells of implanted embryos[3,4], and b) light microscopic demonstration of uptake of particulate matter by guinea-pig trophoblasts in vitro[5]. Ingestion of maternal tissues and cells by phagocytosis is believed to be one of the physiological mechanisms for nutrition of implanting embryos[6]. During SEM analysis of preimplantation, zona-free mouse blastocysts, closely associated red blood cells on trophoblast surfaces were observed. This preliminary report describes this association and its possible relationship to the attachment phase of phagocytosis.

Methods and materials. Brinster's method[7] was used to obtain blastocysts from inbred, Swiss-Webster mice. The embryos were collected from the uterine flushing and washed[8] to remove contaminants. The washed embryos were cultured in vitro for 2 h[8], then fixed for scanning electron microscopy[9]. The fixed embryos were gently centrifuged (7 min at 300 × g, 5 °C) onto millipore discs which were subsequently dehydrated through increasing concentrations of ethanol (50–100%), critical point dried, then gold coated (200–500 Å) prior to examination by means of an ETEC Autoscan.

Results and discussion. Figures 1 and 2 illustrate results of the scanning electron microscopic examination. The frequency of observation of embryos with associated erythrocytes represent approximately 30% of several groups observed at this developmental stage. The associated erythrocytes illustrated in figure 1 and 2 do not have the fragile, topical attachment with trophoblast surface described by others[10] when antigenic sites of late mouse blastocysts are labeled with sheep RBCs. The involuted surface membranes and attachment of the erythrocytes to the surface by microvillous processes (figures 1 and 2) is in accord with reports describing phagocytic craters[11–13] and attachment of particles to macrophage surfaces during the attachment phase of

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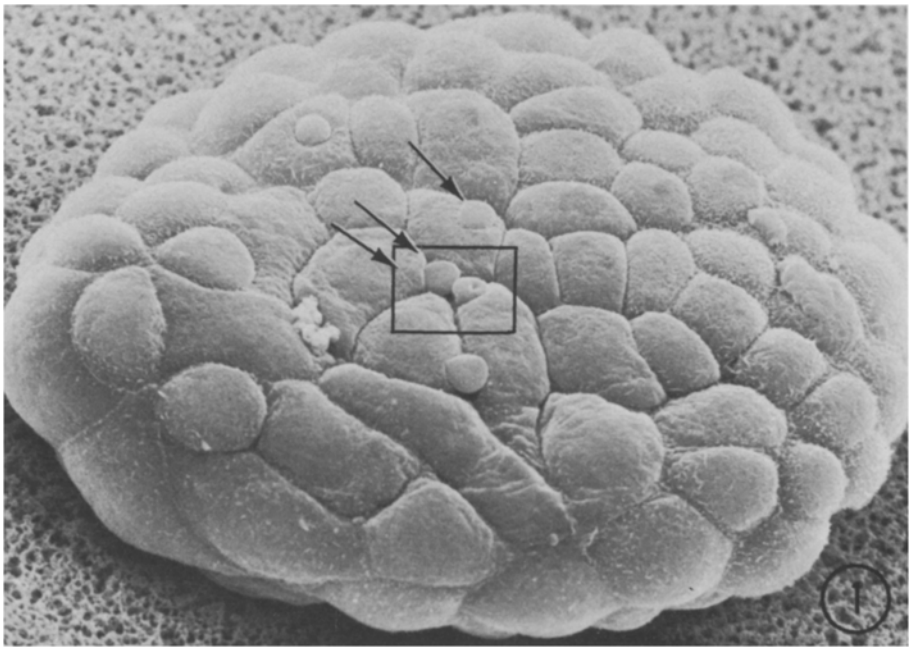

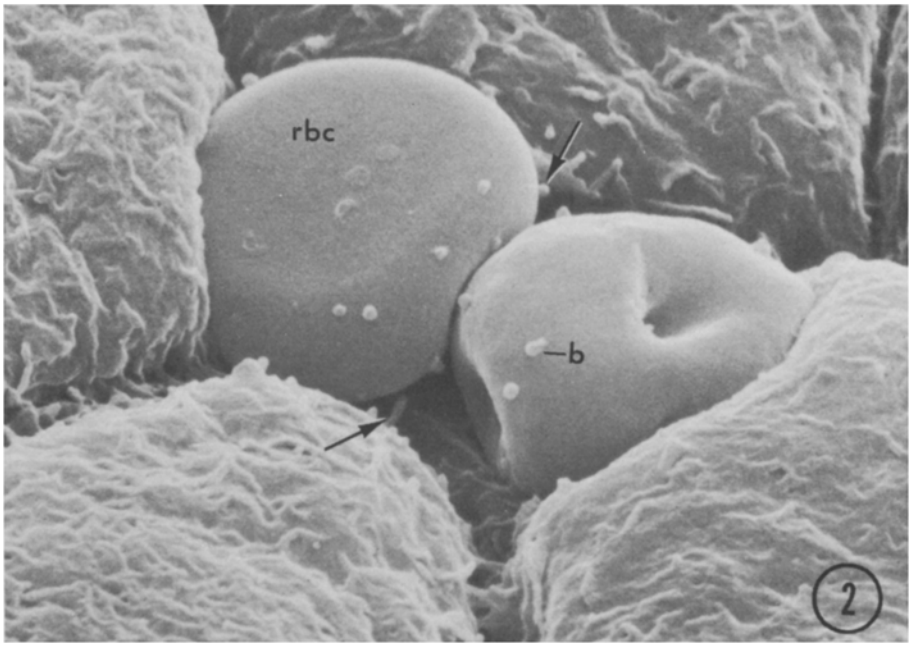

Fig. 1. Late mouse blastocyst. Several red blood cells (RBCs) are seen associated either with the surface (single arrow) or between (double arrows) trophoblast cells. ×1980.

Fig. 2. Higher magnification of area demarcated in figure 1. A few microvilli (arrows) are observed projecting intercellularly where 2 RBCs are located. The cell on the left is a normal shaped, although slightly sphered, discocyte. The cell on the right appears deeply invaginated. Note small blebs (b) on the surface of these sells. They are not likely due to intrinsic RBC membrane modification, but probably represent particulate matter which adhered to the RBC surface during routine preparation. ×17,600.

ingestion[14]. The craters resulting from adhering erythrocytes, described in this report, are different in location and, probably, function from concavities found on trophoblasts located at the abembryonic pole of day-5 normal rat blastocysts[15] and delayed-implantation mouse blastocysts[16–19]. In the latter instances, the craters were reported to result from imprinting of 'mushroom-like structures' of the uterine epithelium onto juxtapositional trophoblasts.

Several factors may account for the lower frequency of RBC association observed at this preimplantation stage of development. First, the embryos obtained by flushing of the uterus at the onset of implantation represent a population which are free for a relatively short period[20], or which are involved in the attachment phase of implantation. As noted above, it is during the later or invasive phases of implantation that phagocytic activity may increase and, therefore, be particularly evident. Second, the number of embryos, which are arrested during the course of an active process may be few in number. Third, the probability of having embryos capture RBCs from newly forming implantation sites or during rapid passage through the uterine scission site would be low. Nevertheless, from the assessment of surface morphology described in this report, the capacity for attachment of cells (the initial phase of phagocytosis) to the surface of late, preimplanted blastocysts, appears to be present at this early developmental stage.

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