Growth of secondary pulmonary or pulmonary and peritoneal tumour in animals carrying a 11-day primary thigh tumour. Control animals same only without thigh tumour

	Experimental animals		Controls	
	Perito-neum	Lung	Perito-neum	Lung
Tumour line				
Glioma: tumour incidence	9/10	2/20	10/10	18/20
Prostate: tumour incidence		2/10		10/10
Weights of tumour bearing lungs (range in g)		1.4–1.6		1.9–10.5
Average weight of tumour bearing lungs (g)		1.5±0.05		4.05±2.24*

Weight of normal lung 1.1–1.6 g, average 1.13±0.13 of animal weighing approximately 180 g. * $P < 0.001$.

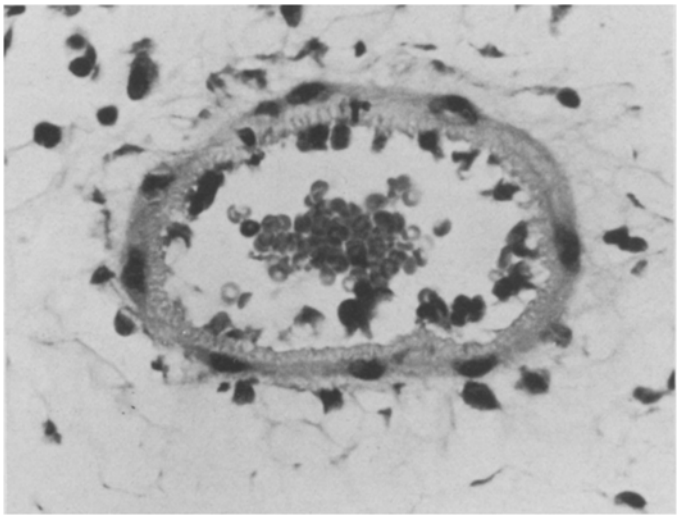

Fig. 2. Crossection of mesenteric vessel from above. Numerous tumour cell marginating and transmigrating the vascular wall. ×520.

lymphocyte sensitisation within striated muscle[4]. There was continued tumour growth in the thigh and peritoneum of animals which had rejected pulmonary tumour.

We therefore suggest that the absence of pulmonary deposits in experimental animals is due to the imperviousness of pulmonary vasculature.

As mentioned before, tumour vessels manifest an alpha, 2, macroglobulin mediated imperviousness to cellular elements[1]. It is therefore reasonable to assume that, in the case of large tumours, there is sufficient alpha, 2, macroglobulin in the tumour effluent also to bind to the vessels draining the tumour[5,6]. In the present series of experiments, it was the pulmonary vasculature which had thus acquired imperviousness.

The reverse was also found to hold true. The administration of an alpha, 2, macroglobulin synthesis inhibitor has been shown to enhance vascular permeability and to promote the haematogenous dissemination of tumour. According to Batson[7], paradoxical metastases develop as a result of tumour spread via the vertebral system of veins. This theory recently come under some criticism[8]. The present findings offer an alternative interpretation of this phenomenon. They show that, in the absence of lung metastases, the transpulmonary passage of tumour emboli could account for the emergence of tumour secondaries in viscera and bone, e.g. the development of liver and bone secondaries, in cases of primary prostatic cancer.

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Virus-like particles in the opossum submandibular gland

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Summary. Numerous inclusion bodies (virus-like particles) were observed in the lumina of the intercellular canaliculi, mucous tubules and intralobular ducts of the opossum submandibular gland. The particles are spherical in outline, show an electron dense core, and are surrounded by a peripheral membrane.

Inclusion bodies (virus-like particles) have been reported in a variety of tumors from several mammalian species, including man[1-6], and generally have been intranuclear or intracytoplasmic in location. During a study of postnatal development[7], we consistently observed numerous virus-like particles in the lumina of the mucous tubules and intralobular ducts of the opossum submandibular gland. These particles were observed in adult animals and in the majority of postnatal stages examined. The present study described the morphology of the virus-like particles observed.

Materials and methods. North American opossums (*Didelphis virginiana*) were used in this study. The animals were trapped in central Missouri and all appeared healthy and free of disease. The postnatal stages were obtained from captured wild animals or from captured animals bred in captivity. 150 animals were used in the study and were collected over a 3 year period. The pouch young opossums

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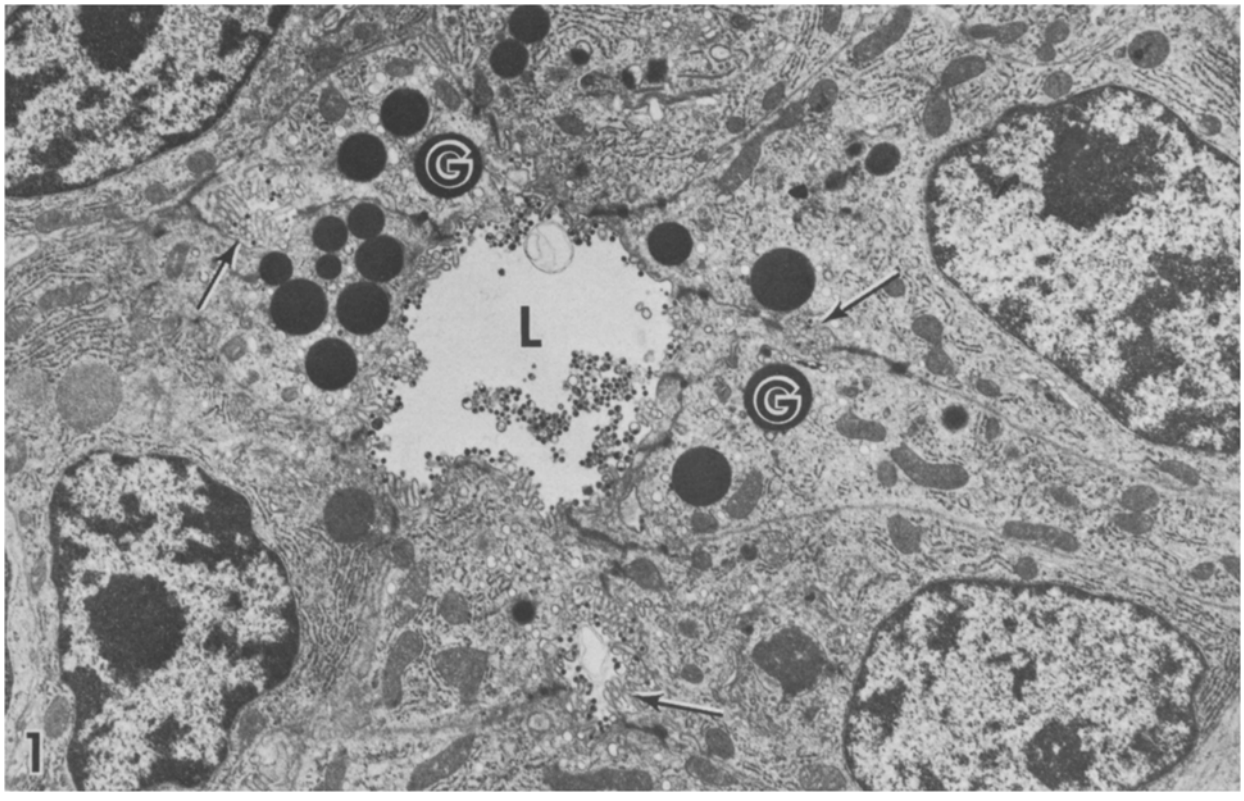

Fig. 1. A section through a mucous tubule of the opossum submandibular gland illustrates several secretory granules (G) in the cell apices. Numerous virus-like particles are found in the lumen (L) as well as in nearby intercellular canaliculi (arrows). 6.0 cm opossum ×1500

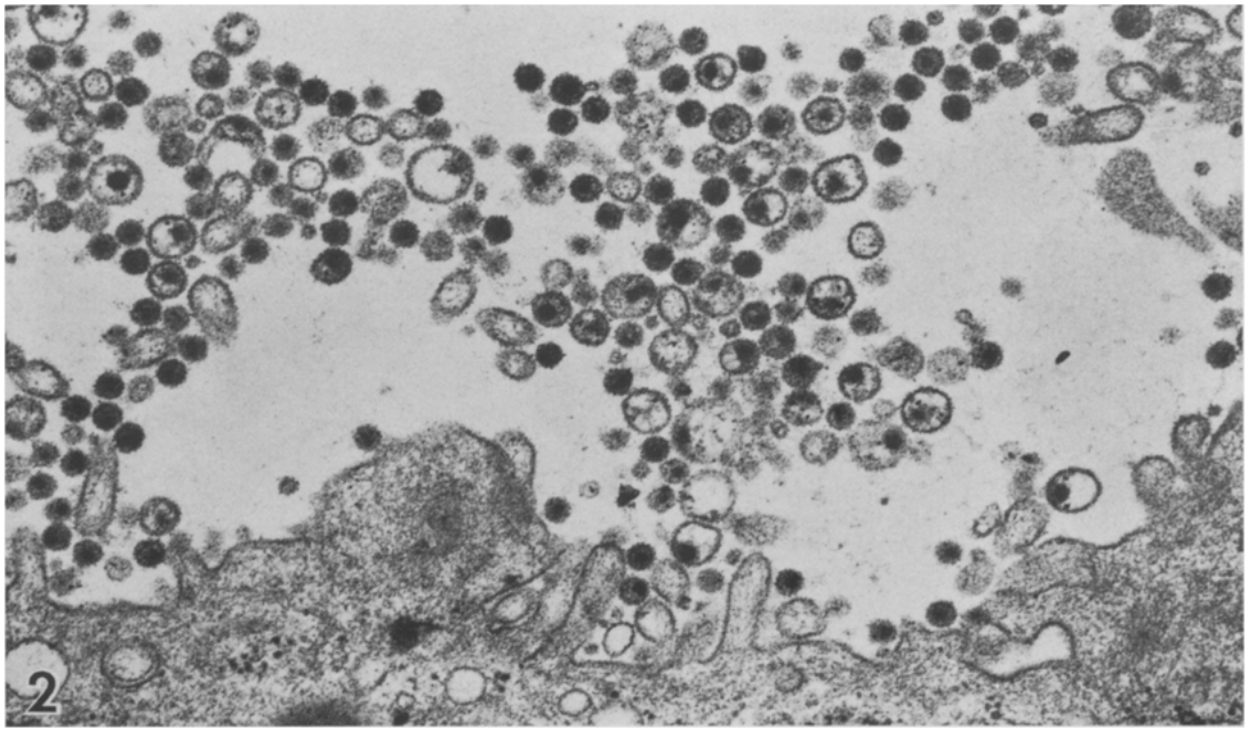

Fig. 2. Increased magnification of a portion of the lumen of a mucous tubule showing particles, many of which contain an electron dense core, each limited by a membrane. 6.0 cm opossum ×40,000.

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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