In accordance with the findings of Dahlström[2], the ligated proximal stump of the saphenous nerve revealed an accumulation of the noradrenaline transmitter accompanied by the thickening of the nerve fibres and by fluorescence (see figure 1), the intensity of which depended on the time of ligation. Besides weak background fluorescence, no accumulation of specific fluorescent material appeared in the contralateral normal saphenous nerve. The ligated arteries revealed the fluorescent sympathetic ground-plexus distributed in the deep adventitia between the elastic lamellae. Several fibres running between the muscle cells of the superficial media were found as well[5,6]. A normal shape and a normal degree of the intensity of fluorescence characterized the terminal ground-plexus, as visible in figure 2. No differences could be detected between the ligated and the normal contralateral artery.

In consequence, the mechanism of the varicosities performing the release, uptake, storage, synthesis and degradation of noradrenaline[4] reveals a system which stabilizes the intraneuronal level of noradrenaline. This stabilization is perfect and cannot even be destroyed by long-lasting ligation.

Vascular permeability in malignant disease

Rachel Stein-Werblowsky

General Hospital Bikur Cholim, 3–5, Strauss St., P.O. Box 492, Jerusalem (Israel), 19 July 1977

Summary. Experimental demonstration that vessels draining large tumours are impermeable to cellular elements. Thus the pulmonary vessels of animals bearing large, primary (other than gastro-intestinal) cancers can become impermeable to tumour emboli. This imperviousness prevents the establishment of secondaries in the lung and promotes the transpulmonary passage of tumor cells. This phenomenon may account for the development of paradoxical metastases.

Tumour vessels have been shown to be permeable to humoral agents but impermeable to cellular elements. This selective permeability has been attributed to the presence of tumour-associated, macrophage-repellent alpha, 2, macroglobulin on the intima of tumour vessels[1]. The present study extends this observation and shows that, under certain conditions, vessels draining large tumours are equally impervious to cellular elements, including tumour cells.

Materials and methods. White Wistar outbred rats weighing 180 g were obtained from the Hebrew University Animal Breeding Station, Jerusalem. 2 tumour lines were used. The 1st was a spontaneous glioma obtained from the Weizman Institute of Science, Rehovoth; the 2nd was an induced prostatic tumour. Both were maintained by serial passage. Tumour suspensions were prepared by mincing 1 vol. for tumour in 1 vol. of saline and passing it through a metal sieve. Amounts of 0.5 ml of this suspension were injected i.m. or i.p. For i.v. administration, a standard suspension was allowed to stand for 10 min at room temperature for the large fragments to settle to the bottom of the tube. The supernatant was aspirated via a tuberculin needle and injected in amounts of 0.25 ml into the femoral vein.

Tumours were injected into the thigh muscles and the animal challenged 5 or 11 days later with i.v. or i.v. and i.p. tumour.

The alpha, 2, macroglobulin synthesis inhibitor chloramphenicol[2] (Synthomycetine Succinate Abic, Ramat Gan) was administered in doses of 100 mg by i.m. injection on the day of and on the day following i.p. tumour inoculation. Tissues for histological examination were fixed in formol saline and stained with haematoxylin eosin.

Results. The tumours infused into the femoral vein of animals with a 5-day thigh tumour, took and grew in the lung in the same way as in the controls. However, i.v. tumour challenge of animals carrying an 11-day thigh tumour yielded few, and if any, very small, lung tumours (table). At the same time, there was no impairment of i.p. tumour growth in the experimental group.

An 8-day i,p, tumour usually presented as a lump of no more than 1.0 g on the omentum with an occasional small nodule on the mesentery. In animals receiving chloramphenicol simultaneously with i.p. tumour, there was a special pattern of tumour growth. Tumour nodules, all of equal size, appeared along the path of mesenteric vasculature (figure 1). Histological examination of such a vessel showed margination and transmigration of numerous tumour cells across the vessel wall (figure 2).

Discussion. The absence of pulmonary deposits in animals with large thigh tumours cannot be attributed to a cell mediated immunodestruction of pulmonary tumour emboli, for the following reasons: Large tumours abrogate, rather than enhance, cellular immunity[3]. There is no

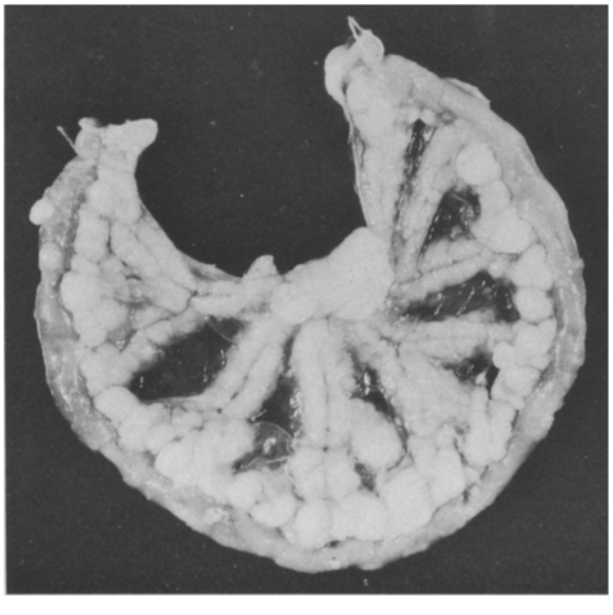

Fig. 1. Abdomen 8 days after i.p. inoculation of tumour suspension and i.m. administration of chloramphenicol. Tumour nodules of equal size distributed along mesenteric vascular channels.

1 R. Stein-Werblowsky, Experientia *33*, 78 (1977).
2 G. Mortara and C. Agostini, Path. Eur. *10*, 193 (1975).
3 P. J. Deckers, R. C. Davis, G. A. Parker and J. A. Mannick, Cancer Res. *33*, 33 (1973).

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. * $P < 0.001$.

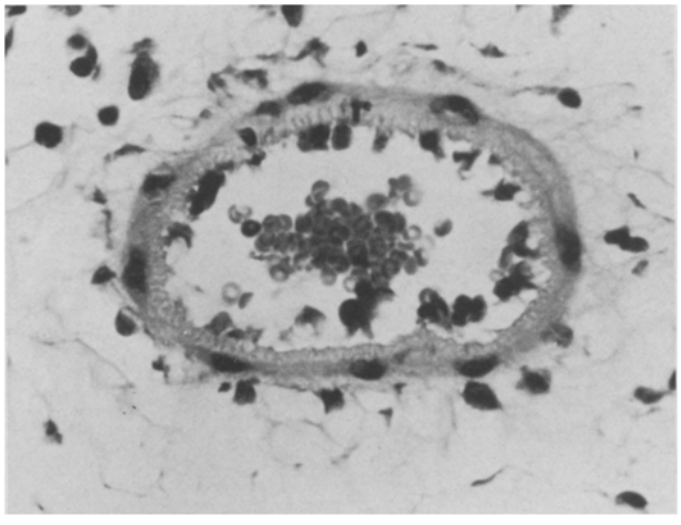

Fig. 2. Crossection of mesenteric vessel from above. Numerous tumour cell marginating and transmigrating the vascular wall. ×520.

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.

4 C. F. Barker and R. E. Billingham, J. exp. Med. *138*, 289 (1973).
5 A. H. Glasgow, J. D. Menzoian, R. D. Nimberg, S. R. Cooperband, K. Schmid and J. A. Mannick, Surgery *76*, 35 (1974).
6 C. G. Becker and P. C. Harpel, J. exp. Med. *144*, 1 (1976).
7 D. V. Batson, Anns. Surg. *112*, 138 (1940).
8 W. I. B. Onuigbo, Oncology *32*, 145 (1975).

Virus-like particles in the opossum submandibular gland

W. J. Krause, C. R. Leeson, J. H. Cutts and D. M. Sherman

Department of Anatomy, School of Medicine, University of Missouri, Columbia (Missouri 65201, USA), 1 August 1977

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

1 A. L. Chapman, W. J. Bopp, A. S. Brightwell, H. Cohen, A. H. Nielsen, C. R. Gravelle and A. A. Werder, Cancer Res. *27*, 18 (1967).
2 A. K. Shubladze, I. F. Barinsky, F. P. Filatov, E. P. Ugirumov, A. F. Bocharov, G. A. Delimnetova, T. A. Bektemirov and V. M. Ahdanov, Experientia *30*, 1156 (1974).
3 Y. Hashida and E. Yunis, Am. J. clin. Path. *53*, 537 (1970).
4 A. P. Lupulescu and G. L. Brinkman, Am. J. clin. Path. *56*, 553 (1971).
5 M. Tannenbaum and J. Lattimer, J. Urol. *103*, 471 (1970).
6 I. Tellez-Nagel and D. Harter, Science 154, 899 (1966).
7 C. R. Leeson, J. H. Cutts and W. J. Krause, J. Anat., in press.