lymphatics; they never traverse the entire mucosa towards the more superficial endometrial regions.

Discussion. Our integrated light and electron microscopic study agrees with the micro-anatomical results obtained in rabbits[6,10], rats[6] and mice[8,9]. They are, however, contradictory to those of Fabian[7] who described a very rapid filling (within a few seconds!) of endometrial lymphatics after intra-uterine injection of Patent Blue Violet. The uterine lumen is lined by epithelial cells that are closely connected with relatively impermeable tight junctions[13]. Tracer experiments with intra-luminal application of ferritin[14,15,16] or thorotrast[17] never demonstrated such a rapid trans- or intercellular passage of particles. However, Fabian noted that the injection of tracer was usually performed in post mortem isolated uteri. Erroneous interstitial injection or a post mortem increase of the epithelial permeability could result in an artefactual access and interstitial accumulation of the tracer. Since Fabian[7] never substantiated the results of injection experiments by morphological studies of the putative, tracer-filled lymphatic capillaries, her description of endometrial lymphatics in mice remains unconvincing.

The absence of an intrinsic endometrial lymphatic system in rats poses the problem of the clearance of fluid and proteins in the uterine mucosa. Because cyclical edemas are removed very efficiently under physiological conditions we presume that at least one (or more?) alternative clearance mechanism(s) is (are) active in the rat endometrium. Clearance along low-resistance connective tissue channels or prelymphatics on the one hand and venous drainage of fluid after interstitial proteolysis on the other hand are 2 such mechanisms suggested to be active in tissues lacking lymphatic capillaries[18,19]. The second part of this contribution will therefore investigate the presence of such non-lymphatic clearance mechanism(s) in the endometrium of the rat.

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Fluid and protein clearance in the rat endometrium. Part II: Ultrastructural evidence for the presence of alternative, non-lymphatic clearance mechanisms in the rat endometrium

F.J. Cornillie and J.M. Lauweryns[1]

Katholieke Universiteit te Leuven, School of Medicine, Laboratory of Histopathology, 12 Minderbroedersstraat, B-3000 Leuven (Belgium), 5 December 1983

Summary. In the rat endometrium, resident macrophages and exudate phagocytes ensure proteolysis by means of phagocytosis, macro- and micropinocytosis. Using exogenous tracer particles no ultrastructural evidence could be obtained for the occurrence of endometrial prelymphatics. It is suggested that the free tissue fluid may be drained via the fenestrated (probably venous) blood capillaries.

Key words. Rat, uterus; rat, endometrium; endometrium, rat; uterus, rat; microcirculation, endometrial; clearance, non-lymphatic; fluid-drainage physiology; lymph drainage; macrophages; phagocytes.

Absence of lymphatic capillaries from the superficial endometrium[2,3] brain[4-7], bone marrow[5,6], retina[5,6,8] and pulmonary interalveolar septa[9-11] has been shown in various species. This absence poses the problem of the clearance of interstitial proteins and fluid in these tissues. In the brain of rabbits and cats Casley-Smith et al.[7] demonstrated a local accumulation of interstitially injected carbon in the basement membrane-region of blood capillaries and in the tunica adventitia of larger vessels. They postulated that transport of proteins occurs in non-endothelialized, low resistance tissue channels, some of which conduct to lymphatic capillaries and therefore were named prelymphatics. Some preliminary morphological characterization of prelymphatics was provided by scanning electron microscope studies of corrosion cast specimens of the rabbit brain microcirculation[12].

Macrophages, however, play an important role in the clearance of interalveolar septa, as was shown with protein tracers such as carbon[13] and ferritin[14,15].

The present paper reports the results of a clearance study in the rat endometrium using colloidal ferritin and carbon as protein tracers and attempts to answer the question which clearance mechanism(s) is (are) present in this tissue, which lacks an intrinsic lymphatic drainage system.

Material and methods. 23 virgin WISTAR rats were used; the anesthesia and fixation procedures were identical to those described earlier[2]. Ferritin (horse spleen ferritin, 2 × crystalline, cadmium free, Fluka) was instillated into the uterine lumen at the ovarian end, using a tuberculin syringe, in 5 rats. Carbon (C11/1431a, Günther Wagner, Hanover) was administered in the same way to 8 animals. Usually 0.15 cc of a 10 x diluted

tracer suspension was injected. In 1 rat a higher dose of ferritin (i.e. 30 mg) was instillated. The animals were sacrificed at various intervals after injection of the tracer suspension (from 5 min to 19 days). 10 rats served as controls.

Results. In control rats some cells of the endometrial stroma accumulate ferritin particles in ferrisomes. These iron-storing cells may contain free intracytoplasmic ferritin particles. Iron-storing cells are present throughout the endometrium, although they are usually more frequent in the superficial areas. They never show mitosis and sometimes develop a rudimentary cilium.

Large pleomorphic phagocytes are present near some blood capillaries and glands. The number of these periglandular and perivascular macrophages is limited and they do not show any proliferation. They accumulate large amounts of hemosiderin and lipofuscin. Some of these cells show membrane defects and liberate residual material into the interstitium, contributing to a so-called defecation in situ.

Many monocytes leave the circulation during pre-estrus and estrus; in the interstitium they develop pseudo- and filipodia and differentiate into active phagocytes. They avidly engulf interstitial substances by means of macro- and micropinocytotic vacuoles and coated pits, the latter assumed to be involved in receptor-mediated endocytotic activity (fig. 1). They usually contain many lipid droplets and lysosomal inclusions.

After an intra-uterine instillation of ferritin or carbon, free particles are only seldom found in the endometrial extracellular interstitium. When 30 mg ferritin is administered, however, numerous electron dense tracer particles are present in the interstitium, but their topographical distribution is diffuse and they never accumulate along so-called low resistance tissue channels or prelymphatics. Carbon particles are phagocytosed by endometrial iron-storing cells. High numbers of carbon particles accumulate in perivascular and periglandular phagocytes (fig. 2). Exudate macrophages also incorporate carbon particles. This tracer never appears to accumulate in interstitial tissue channels or prelymphatics.

Discussion. The rat endometrium contains a very active mononuclear phagocytic system containing both resident and

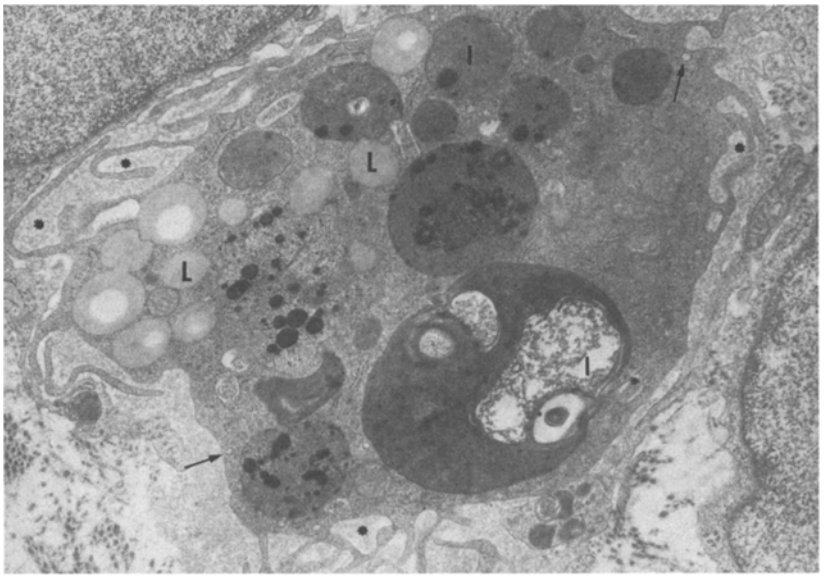

Figure 1. Endometrial exudate phagocyte with many lipid droplets (L) and lysosomal inclusions (1). Note the 'active' cell surface with many filipodia engulfing extracellular material (asterisks) and some coated vesicles (arrows). Pre-estrus, control animal; × 12,200.

Figure 2. Endometrial perivascular resident phagocyte with large accumulation of residual material including carbon particles (C). This macrophage exhibits a membrane defect and liberates electron dense particulate material in the interstitium (arrow). E, endothelium; SC, stromal cell. Pre-estrus, 19 days after carbon; × 9,000.

exudate phagocytes. Resident macrophages (i.e. the iron-storing cells, perivascular and periglandular phagocytes) probably develop from endometrial stromal cells. This is supported by the presence of rudimentary cilia in endometrial stromal cells[16] and the phagocytes described here. The absence of proliferation in these endometrial phagocytes also suggests they are an end-stage of differentiation of endometrial stromal cells.

Our morphological study reveals that exudate monocytes internalize and digest interstitial proteins. The presence of intracellular lipid droplets is in agreement with their high proteolytic activity[17,18]. Proteolysis reduces the colloid osmotic pressure in the interstitium[19]. This decrease is a prerequisite for the drainage of fluid from the tissue. Due to proteolysis a pool of free interstitial endometrial fluid remains. We postulate that this fluid may drain via the fenestrations and transendothelial channels of the endometrial blood capillaries. Ultrastructural studies of the rat endometrial microcirculation demonstrated this pore system in large, probably venous, capillaries[2,3]. Such a local extravascular circulation or ultracirculation seems to exist in the hypophysis, where it prevents an edema formation after ligation of the prelymphatic pathways[7]. In addition it is shown that venous drainage of proteins and fluid is rather important in primitive species such as Heterodontus portusjacksoni (Meyer), and Elasmobranch species in which lymphatics do not yet develop but in which the blood capillaries are intensely fenestrated[20].

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The peripheral innervation of the heart of *Eledone moschata* demonstrated by histofluorescence microscopy

G. Kling and R. Schipp

Institut für Allgemeine und Spezielle Zoologie, Stephanstr. 24, D-6300 Giessen (Federal Republic of Germany), 30 January 1984

Summary. A fluorescence histochemical investigation of the cardiac nerves of *Eledone moschata* has demonstrated that they contain catecholamines. This suggests that the cephalopod heart is supplied by a double innervation, where cholinergic and aminergic mechanisms work antagonistically. This is similar to the vertebrate cardiac innervation and therefore represents a convergent evolution.

Key words. Central heart; innervation; *Eledone moschata;* catecholamines; fluorescence histochemistry.

Cephalopods occupy an isolated position among the molluscan phyla with regard to their anatomy, locomotion, sense organs, behavior, embryology, and also their efficient circulatory organs[1]. The main pumping organ is the central heart, supported by rhythmical contractions of the paired branchial hearts and of some veins. The first anatomical and histological description of the octopod circulatory organs was given by Marceau[2], Isgrove[3], and Grimpe[4], and the high efficiency of the octopod cardio-vascular system has been shown in many physiological experiments[5-11]. In the possession of these highly evolved bodily functions, cephalopods are comparable to lower vertebrates, so that it is of interest to investigate whether the cephalopod central heart, like the vertebrate heart, shows a double innervation: on the one hand by inhibitory cholinergic fibers[11-14], and on the other hand by excitatory aminergic elements. It is reported that the cephalopod cerebellum contains biogenic amines[15-20] and peripheral aminergic nerves are also found[21-27]. We have investigated the central heart of *Eledone moschata* using the fluorescence histochemical method of Falck and Hillarp, as modified by Loren[28], for detection of monoaminergic nerve fibers within the octopod ventricle.

Materials and methods. Adult *Eledone moschata* (mantle-length 7–11.5 cm, weight 380–480 g) were captured in the Mediterranean sea near Banyuls-sur-Mer (France). After anesthetization in 1% ethanol-seawater, the animals were opened from the ventral side. The central heart was quickly dissected out and incubated for 10 min in 0.2 M phosphate buffer containing 2% glyoxylic acid, 0.5% formaldehyde and 28% $MgSO_4$. The hearts were then deep-frozen in Freon 11, cooled by liquid nitrogen and dried for 4 days at −40 °C in a Combitron CM 30 (Leybold-Heräus) freeze-drier. The tissue was vacuum-embeded in paraffin after treatment with p-formaldehyde vapor at 80 °C for 1.5 h 10 20-µm sections were observed with a Leitz DIALUX fluorescence microscope, fitted with a BP 390–490 excitation filter and a LP 515 barrier filter. Black and white prints were obtained from Agfachrome 50L original color slides. Additionally, histological standard methods and Bodians silver impregnation were applied on formol and Bouin-solution fixed organs.

Results and discussion. The central heart of *Eledone* lies in the dorsal body cavity and receives oxygenated blood from the efferent branchial vessels via enlarged veins, so-called auricles. It pumps blood through the cephalic aorta, the posterior aorta and the genital artery into the various regions of the body[3,29]. As in other octopods, the ventricular wall consists of a peripheral epithelium (= epicardium) which represents the residue of the pericardium, which is almost totally reduced in octopods[4,30]. This is followed by a solid connective tissue layer and a mass of ventricular muscle (= myocardium)[2,31,32]. An incomplete ventricular septum represents a relic of the paired origin