DMSO has also proved its merits as a solvent of metabolic inhibitors in experiments with yeasts. On a synthetic glucose medium, both in static cultivation and in cultivation on a shaker, it did not influence to any appreciable extent the course of growth curves of the yeasts *Saccharomyces cerevisiae* and *Candida albicans* in concentrations up to 5%. Only at about a concentration of 10%, prolongation of the lag phase and decrease in the rate of growth was observed. However, not even 10% concentration of DMSO proved sufficient to stop the proliferation of cells of *C. albicans* (Figure 3). In 1% concentration it did not influence the intensity of respiration nor change the respiration quotient. In the same concentration it did not influence the incorporation of adenine-^{14}C and leucine-^{14}C either (Table II). DMSO has proved likewise useful in studying the influence of isothiocyanates and antibiotics on the protoplasts of *S. cerevisiae* where in 1% concentration it did not influence respiration (Figure 4), incorporation of adenine-^{14}C and leucine-^{14}C in its protoplasts in a hypertonic synthetic medium[8].

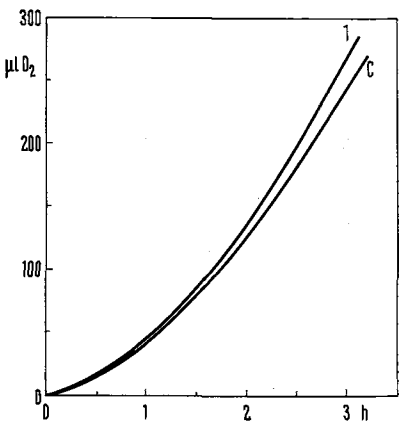

Fig. 4. Oxygen consumption by *S. cerevisiae* sferoplasts in synthetic medium[10] containing 0.6 *M* KCl[8]. C, control; 1, % DMSO. Measured manometrically at 28 °C.

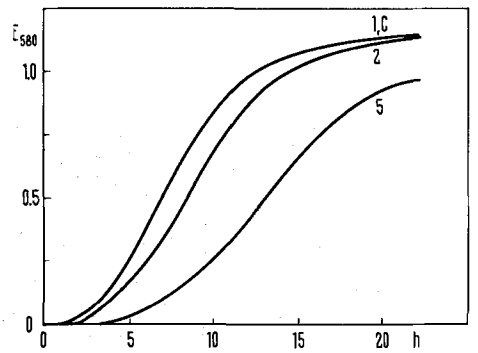

Fig. 5. Growth *E. coli* B on synthetic medium at 37 °C under aeration. C, control; 1, 2 and 5 represent final concentration of % DMSO.

DMSO has also proved effective as a solvent when studying the influence of inhibitors on metabolic processes in bacteria. As it appears from Figure 5, in 1% concentration it did not influence proliferation of the bacteria *E. coli* B. In a concentration of 5% its inhibitory influence had already become manifest. When investigating biologically effective substances against *M. tuberculosis* H_{37}Rv, solutions of these substances in DMSO could likewise be utilized to advantage[9].

As we were able to show, certain protozoa are particularly sensitive to the presence of organic solvents in the culture medium used. For instance, dimethylformamide and ethanol in 5% concentration will irreversibly stop mobility of the protozoa *Euglena gracilis*. DMSO in a peptone medium in 5% concentration will only cause a reversible loss of mobility of *E. gracilis* cells. After several hours their mobility will become entirely or partially restored. In a concentration under 2% DMSO has no inhibitory influence on the mobility of *E. gracilis* cells.

Zusammenfassung. Es wird gezeigt, dass 1–2%iges Dimethylsulfoxyd sich als unschädliches Lösungsmittel für antibakterielle Substanzen besonders gut eignet.

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The Relative Antiquity of Fenestrated Blood Capillaries and Lymphatics, and their Significance for the Uptake of Large Molecules: an Electron Microscopical Investigation in an Elasmobranch

Lymphatics remove large molecules from the tissues. Fenestrated blood capillaries may also remove large molecules[1–4]. The probable mechanisms involved have been discussed elsewhere[2,3].

One of the very few studies of the relative effectiveness of the blood vessels and the lymphatics in the removal of large molecules concluded that the blood capillaries are the major mechanism for the removal of extravascular protein from skin[5]. Recently, SZABO[6] has found that in the cortex and in the medulla of the kidney, where fenestrae are very numerous[1], the blood removes about 100 times more protein than the lymph does[6]. In the skin, the blood and lymph systems are about equally effective. Here there are few fenestrae[1,7] and hence many of the molecules removed by the blood may enter the capillaries via the small endothelial vesicles[1,8–10].

In privative fish, the absence of lymphatics has made it difficult to understand how the tissues are cleared of large molecules[11]. In spite of earlier confusion, it is now considered that true lymphatics are absent in most species of elasmobranchs[12,13], including the Port Jackson shark, *Heterodontus portusjacksoni* (Meyer), which we studied. (We also injected colloidal carbon into the intestinal wall, and found no lymphatics.) Pieces of intestine, kidney,

pancreas and ciliary body were fixed with glutaraldehyde and osmium tetroxide, embedded in araldite and stained with lead citrate and uranyl acetate.

Fenestrated blood capillaries, usually possessing diaphragms, were found in all the tissues (Figures 1–3). The endothelium of the capillaries resembled that in the mammals[1], with 3 exceptions. The basement membranes often appeared less substantial (Figures 1–3). While the intercellular junctions all appeared to possess *zonulae occludentes*, the cells were often less closely applied in the other parts of the junctions (Figure 1). (It is likely that both of these features are reflections of the low blood pressures in *Heterodontus*.) The third difference is probably caused by the venous pressures often being negative[11]: the ablumenal endothelial plasma membranes in the venous limbs of capillaries and in venules often had many connective tissue fibrils attached to them, sometimes on to small endothelial projections. These are not found in mammalian blood vessels, but are found in mammalian lymphatics, where they serve to maintain the patency of the vessels against tissue pressures which are higher than those in the vessels[14]. In contrast with mammalian lymphatics[14,15], however, no completely open junctions were seen.

In the intestinal villi there were many particles (~20 nm) resembling lipoproteins[15] (Figure 3). They were in vesicles in the epithelial cells, in the lamina propria, in the fenestrae and small vesicles of the endothelium, and in the

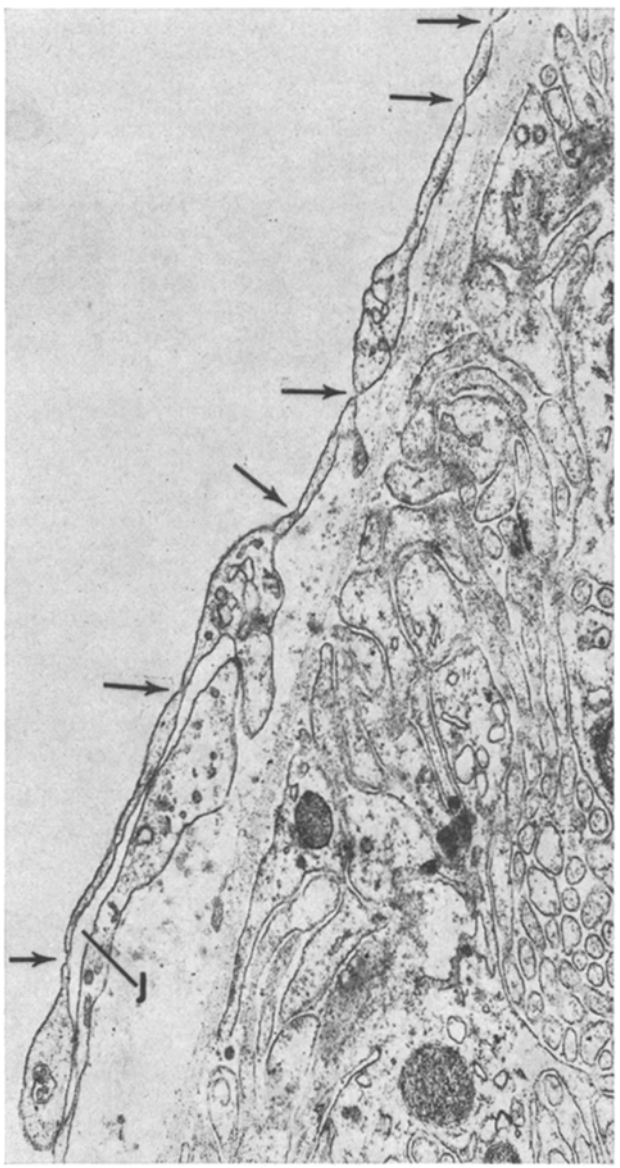

Fig. 1. Capillary in the kidney. There are a number of fenestrae (arrows). A junction (J) between 2 endothelial cells shows 4 regions where the cells are closely apposed, but they are quite widely separated along the rest of the junction. The poorly developed basement membrane of the capillary may be compared with that around the renal tubule cell. × 12,000.

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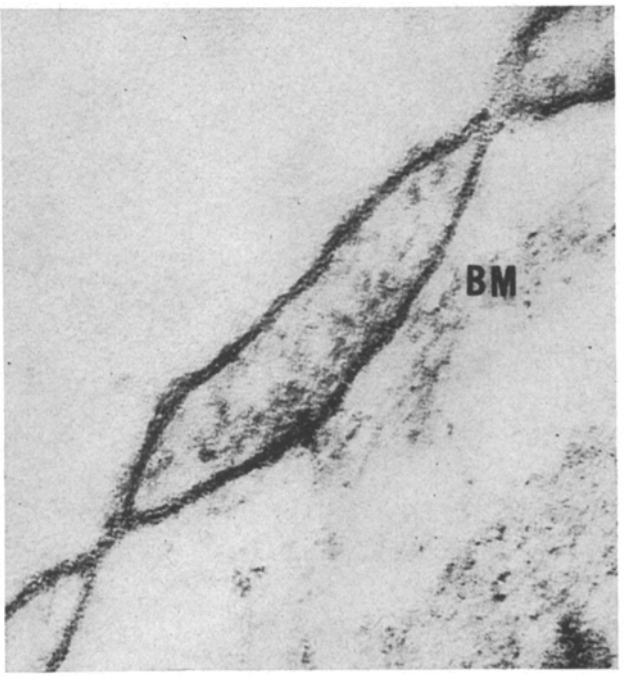

Fig. 2. Capillary in the kidney. 2 fenestrae are visible. One shows the fenestral diaphragm clearly; the other is probably insufficiently contained in the section, so that any diaphragm is obscured by the obliquely sectioned plasma membranes around the fenestra. The basement membrane (BM) is very tenuous. × 150,000.

lumens of the capillaries and venules. Where the capillaries were close to the arterioles, there were few fenestrae and the particles were relatively rare near the vessels and in their lumens. The venous limbs of the capillaries had many fenestrae and the particles were plentiful both adjacent to the vessels and in their lumens. The differences in the distributions of the particles, near the vessels and in their lumens, is consistent with their being swept towards and into the venous limbs by the extravascular fluid passing from the arterial to the venous ends of the capillaries in accordance with Starling's hypothesis. (In mammals the much larger chylomicra similarly accumulate around the lacteals[15].) While it is impossible to say with certainty, it would seem, from the apparent ease with which the particles enter the capillaries, that most of them pass via the fenestrae rather than via the slow system of small vesicles[8–10]. (Generally the vesicles cannot provide a net removal of extravascular proteins because the concentrations of these in most of the body are lower than those in the blood[8, 10].)

In the ciliary body the fenestrae were much more frequent in the capillaries in the ciliary process than in those in the muscle. The capillaries in the process are close to the veins; those in the muscle are close to the arteries. Hence it appears that there also, as in the gut[2], the adrenal[4], the rete of the renal medulla[16] and the swim bladder[17], and the skin[7], fenestrae are concentrated on the venous limbs of capillaries. This, together with their possession of diaphragms, are likely to be of fundamental importance for their uptake of large molecules from the tissues[2–4].

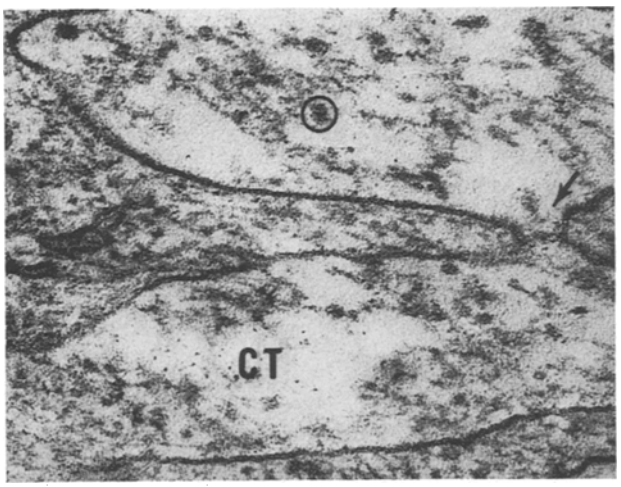

Fig. 3. Venous limb of a capillary in an intestinal villus. There are numerous particles (circles) which resemble lipoproteins as seen in mammals. They are present in the connective tissue (CT) and lumen. One is very close to a fenestra (arrow). × 100,000.

Since *Heterodontus* has fenestrae but no true lymphatics, it seems conclusive that fenestrae antedate the lymphatic system. It also appears that they allow large molecules to enter the blood capillaries on their venous limbs[3]. We are left, then, with the problem of why the lymphatic system developed and what its important functions are.

In some regions the lymphatics are indubitably important for the clearance of particles and large molecules, particularly those larger than the fenestrae. E.g. in the skeletal muscle and in all the regions of the body where fenestrae are infrequent, especially bordering mesothelium lined cavities. It is likely that they are still of importance, even in the presence of fenestrae, if there is much lymph flow, e.g. in the villi of the mammalian gut during digestion. However, they are by not means essential for the uptake of large molecules.

DRINKER[18] suggested that the lymphatics function primarily as spillways to remove the large accumulations of extravascular fluid which occur in active muscles. It may be that the lymphatics developed for this reason in the higher fish, with their higher blood pressures. Then they could also remove large molecules during periods of high lymph flow[19].

Résumé. Le requin n'a pas de vrais vaisseaux lymphatiques, mais ses capillaires sanguins sont fénestrés. On suppose que les grandes molécules sont éliminées des tissus par ces fenestrae.

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Depolarization of Tooth Pulp Primary Afferent Fibers in the Medulla oblongata

The depolarization of primary afferent fiber terminals in the spinal cord and medulla oblongata has been the subject of intensive investigation, and evidence has accumulated to show that it is causally related to pre-synaptic inhibition[1, 2]. Depolarization of fast-conducting cutaneous fibers can be produced by stimulation of other fibers belonging to the same afferent group[3].

The aim of this study was to see whether an analogous presynaptic control mechanism exists at central terminals of the afferent fibers supplying the tooth pulp. These fibers consist of slow-conducting fibers with maximum velocity 30–45 m/sec[4] and it is generally recognized that pain is the only modality of perception they subserve[5].

Material and methods. The experiments were carried out on 16 cats anesthetized with Nembutal. Animals were fixed in a stereotaxic holder, the posterior fossa was exposed and part of the cerebellum removed to gain access to the medulla oblongata. The infraorbital nerve was exposed and a bipolar stimulating electrode placed close to its point of exit from the infraorbital foramen. Two cavities were then prepared in the upper and lower canine teeth and filled with Ringer agar gel. Tungsten wire