The organisms are motile, rod-shaped in the vegetative state measuring 4–5 μ in length and 0.6–0.8 μ in diameter. As the transition occurs from vegetative state to commitment to sporulation the cells become swollen and assume a spindle-shape. With the progress of sporulation the refractile body, which appears initially at the terminal end of the organism, moves and is located centrally in the mature spore (Figure 1). The electron micrograph shown in Figure 2 of a thin section of a refractile body bears cytological evidence to the fact that the refractility is indeed due to spore formation and not to the accumulation of lipoid material[3]. In the cells containing the immature spore there can be noticed a granular area corresponding to the position where the parasporal inclusion usually develops. When the spore matures, however, the parasporal body is not intact inside the sporangium but released in the medium. During that time an active proteolytic enzyme is secreted in the medium. Preliminary investigations have shown that the enzyme exhibits differences in properties from those that have been isolated during the sporulation of *B. subtilis* and *B. licheniformis*[4,5]. It is interesting to speculate whether the parasporal inclusion is indeed a pro-enzyme which is activated under conditions of sporulation in vitro and secreted as a proteolytic enzyme[6].

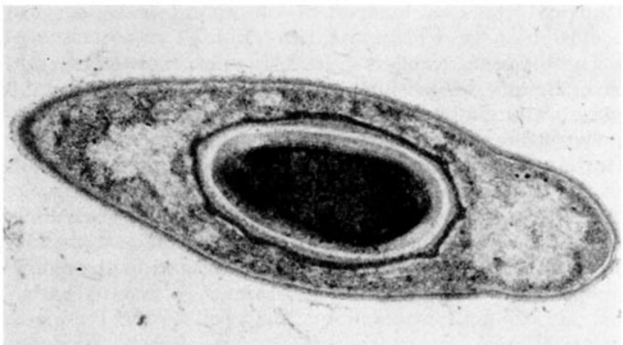

Fig. 2. Electron micrograph of a refractile spore intact within the sporangium. Spores were fixed in osmium tetroxide and counterstained with lead hydroxide after sectioning. Pictures were taken at a magnification × 27,000 and enlarged.

Zusammenfassung. Durch Behandlung mit Chloroform ist es möglich, *Bacillus popilliae* in flüssigem Medium zur Sporenbildung zu veranlassen. Diese Bakterien können zur Bekämpfung von Insekten, der *Popilliae japonica*, benützt werden.

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Department of Microbiology, Louisiana State University, Baton Rouge (Louisiana 70803, USA), 8 July 1968.

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[6] Our grateful thanks are due to Mrs. JOANNE PARKER who helped us with the electron micrograph.

STUDIORUM PROGRESSUS

Lymphatic Drainage of the Brain

Investigators studying the pathomechanism of cerebral edema are aware of the difficulties inherent in the production of an experimental edema of the brain. Experimental brain tumours, experimental brain injuries, or freezing of the brain surface, and recently administration of triethyl tin, are common methods, though admittedly rough procedures that finally result in various degrees of edema.

Obviously, nobody has tried to produce a cerebral edema by obstructing lymph flow of the brain, since a well-known fact for first-grade students of medicine is that the brain lacks lymphatics. In this paper, the strange fact will be discussed that, unexpected as it is, by means of obstruction of the cervical lymphatic pathways, a massive cerebral edema can be produced with great regularity in various experimental animals, characterized by gross anatomical, histological, functional and clinical signs.

Connections between the CNS and the lymphatic system. 'With regard to the lymphatic pathways, it is generally agreed that there are no lymphatics within the CNS or meninges'[1].

On the other hand, the presence of lymphatics in the nasal cavity, in the orbita and around the jugular foramen, in the leptomeningeal sheaths around cranial nerves is well known. Different tracer substances injected intrathecally regularly appear in the cervical lymph glands. Nevertheless, no functional significance is ascribed to this lymphatic drainage in the fluid exchange of intracranial structures, since, in the experiments of COURTICE and SIMMONDS[1], the rate of absorption of labelled protein and erythrocytes was not affected by ligation of the main cervical lymphatics.

The above experimental results appear to be supported also by sophistication: (a) according to the present view, the main role of lymphatics is the re-transport of proteins escaped from blood capillaries from the tissues into the blood stream; (b) brain tissue is vigorously protected from the entrance of plasma proteins by the blood-brain-barrier (BBB); hence, there is no need of a lymphatic system; (c) in full accordance, neither classical morphology nor electron microscopy reveals any lymph vessels in the brain; their absence was repeatedly confirmed in the course of our own studies also.

Recent studies, however, revealed that the protection of the brain by the BBB is not an absolute one: (a) a very limited but by no means negligible amount of plasma protein molecules is steadily escaping the blood capillaries and entering the brain substance[2]; (b) several areas of

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[2] F. H. SKLAR, E. F. BURKE and T. W. LANGFITT, J. appl. Physiol. *24*, 79 (1968).

the brain, the so-called circumventricular organs: pineal body, area postrema, hypophyseal stalk etc. are not protected at all by a BBB; (c) in several pathological cases, e.g. inflammatory processes, tumours, cerebral hemorrhage etc. an important amount of plasma proteins have access to the brain tissue. (d) It should furthermore be recalled, that the connective tissue and musculature of intracerebral blood vessels represents a not negligible volume, that constantly needs a lymphatic drainage, just like any other vascular tissues of the organism.

One of the basic laws of pathology is that stagnating proteins in tissues exert a deleterious effect. Thus, if proteins have access to brain substance, they would be harmful unless a re-transport or local catabolism takes place.

Experimental occlusion of cervical lymphatic pathways. The basic approach of lymphology is similar to that of classical endocrinology, studying physiological functions by learning the consequences of extirpation. The facts described in the preceding paragraph seem to justify the study of the possible results of an extensive surgical occlusion of cervical lymphatics.

By injecting various dyestuffs into the tongue and intrathecally, respectively, it is easy to delineate the lymph vessels and glands of the neck. (Having gained the necessary routine, previous dye injections become superfluous.) In the course of our earlier studies, cervical lymph vessels and glands were simply ligated, carefully spearing the arteries, veins and nerves. It is a well-known fact, however, that lymphatico-lymphatic anastomoses rapidly develop after ligation of lymph vessels, and in the course of our studies we actually learned that several patho-

Table I. Some consequences of cervical lymphatic blockade

Animal	Type of surgery	Sign
Dog, cat, rat	Ligation	Lymphedema of the muzzle[3]
Dog, cat, rat	Ligation	Apathy, depression[3]
Cat, rat	Ligation	Reversible decrease in conditioned reflex activity[4,5]
Rat	Extirpation	Lasting decrease in conditioned reflex activity
Dog, cat, rat	Ligation and extirpation	Decreased convulsion threshold[3,6]
Dog, cat, rat	Ligation and extirpation	Increased barbital sensitivity[3,4]
Dog, cat, rat	Ligation	Edema of retina and papilla nervi optici[3,7].
Rat	Ligation	Decreased acethylcholin esterase activity of hippocampus. Enlarged perinuclear cisternae and swollen mitochondria in nerve cells. Vacuoles in endothelial cells of brain capillaries. Destruction of myelin sheaths. Increased lysosomal activity[8,9]
Rat	Extirpation	As after ligation, plus: extremely swollen glial cells; fluid-filled spaces between distended lamellae of capillary basement membranes

[3] *Lymph and the Lymphatic System* (Proc. Conf. Lymph. and lymph. Syst.; Ed. H. S. MAYERSON; C. C. Thomas, Springfield, Illinois 1968).
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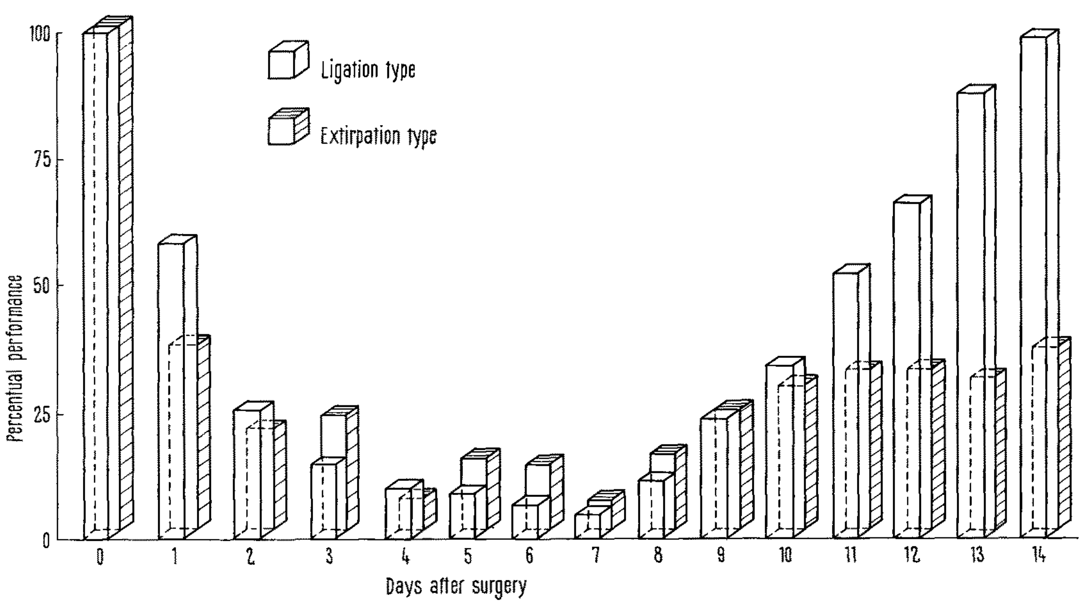


Fig. 1. Inhibition of conditioned reflex activity after surgical obstruction of cervical lymphatics. Reflex activity 100% before surgery (0). Both types of surgery induce a sharp decrease. In ligation type the original activity is regained in a fortnight. After extirpation type there is no recovery during the time of observation. Rat.

physiological consequences of cervical lymphatic ligation (Table I) are spontaneously normalized. On the other hand, the possibility could not be ruled out that toxic products of necrotizing, ischemic ligated lymph glands were absorbed and might be partly responsible for some of the consequencies of this surgery. Therefore, we later developed a new technique: instead of ligation, we now extirpate the cervical lymph glands and vessels. Consequences of this type of surgery are more severe (in the terms of electron microscopy) and there is no rapid spontaneous recovery of clinical signs (Figure 1).

Consequences of cervical lymphatic blockade. Since 1962, the pathophysiological and structural alterations of the CNS after ligation of cervical lymphatics were described in detail in a series of papers and lectures. They are summarized in Table I.

Lymphostatic hemangiopathy. In our first studies, the fine structure of the area postrema was investigated electron microscopically after the ligation type of surgery. The area postrema is, even under normal conditions, quite rich in connective tissue elements; and, as it was pointed out previously, it is devoid of the BBB, i.e. its tissues are steadily inundated by protein molecules due to the lymph. Several days after surgery, a significant increase in the size of the normally present space between the 2 layers of the basement membrane could be observed. It should be recalled that, under normal conditions, the basement membrane of area postrema capillaries is divided into 2 layers, the internal one lying close to the endothelial cell, the external one exhibiting a folded appearance. Under normal conditions the width of the gap measures 0.1–0.5 μ. After a cervical lymph blockade, the width of the gap increases to 1–3 μ, and seems to be filled with edema fluid (Figures 2 and 3).

Both in the area postrema and in capillaries of other regions of the rat brain, conspicuous vacuoles of various sizes became apparent in the cytoplasms of endothelial cells, usually many times larger than pinocytotic vesicles in general.

When using the extirpation type of cervical lymphatic surgery, structural alterations of capillary basement membranes became apparent throughout the brain.

Under normal conditions, basement membranes have a compact appearance, their external surface being surrounded by glial end feet. Several days after extirpation of cervical lymphatics, basement membranes of many capillaries become split into 2 ill-defined layers, resulting here and there in fluid-filled tissue spaces between these 2 layers (Figures 4 and 5). The above tissue spaces are sometimes in a close continuity with perivascular glial profiles that, in turn, exert signs of an extreme hydration (swelling). Not infrequently glial cytoplasms appear as empty spaces, containing loose structural elements, e.g. 1 or 2 mitochondria etc. only. In the perikaryal region of

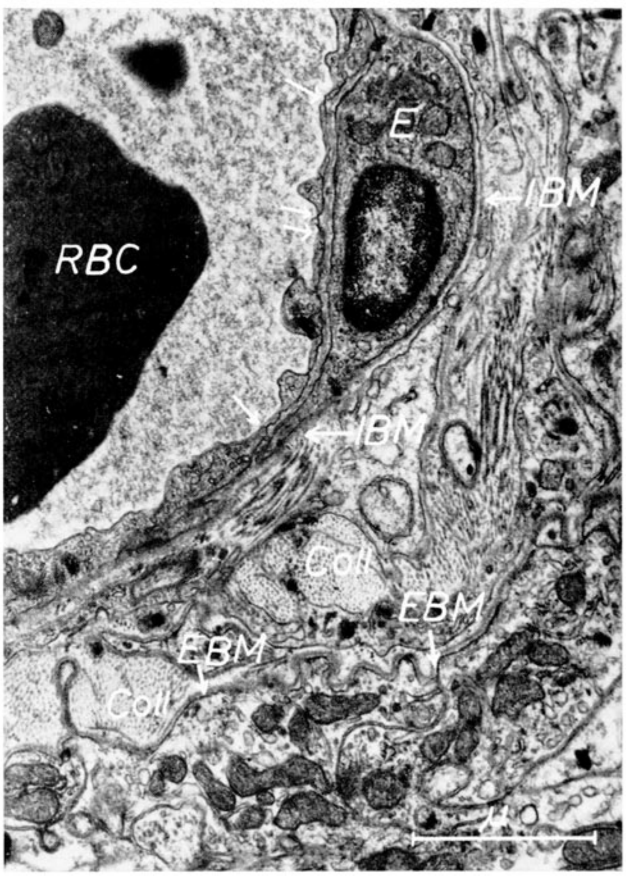


Fig. 2. Area postrema capillary in a normal rat. Note the 2 layers of the basement membrane (IBM, EBM) and the collagenous fibres in the space between them. E, endothelial cell; RBC, red blood cell; Coll, collagen.

Fig. 3. Area postrema capillary of a rat subjected to an experimental lymphostatic encephalopathy. Note the fluid-filled, dilated space between the 2 layers of the basement membrane (BM). E, endothelial cell.

such glial cells also the perinuclear cistern is extremely dilated.

Prelymphatic-lymphatic[10] *draining system in cerebral blood vessels.* Based on our studies, we feel justified in developing a new theory of cerebral fluid and protein drainage.

Figure 6 represents 2 types of tissues. Type B is the prototype of tissues equipped with lymphatics, e.g. subcutaneous connective tissue. Type A represents brain tissue, devoid of lymph vessels. In both cases, blood vessel walls contain lymphatics among their vasa vasorum. Surgical occlusion of regional lymphatics at point R results in edema in both cases. In tissue B, this lymphedema can be readily explained by the abolishment of lymphatic protein uptake at the lymph capillary level and by insufficient protein catabolism of connective tissue cells.

In the brain, lacking an extracellular space, glial cells play the role of connective tissue ground substance. Lymph capillaries are absent from the brain.

Yet, under normal conditions, in between the lamellae of the basement membranes ot brain capillaries, a constant prelymphatic drainage of a film consisting of water, protein and other 'lymph-due' substances, takes place towards larger blood vessels.

This is the only explanation of the appearance of fluid-filled lakes between the distended lamellae of the basement membrane and of the edema, resulting after a cervical lymph blockade.

Dislocation of basement membranes is never circular, but extends only to a part of it. It seems that the main prelymphatic pathways would be limited to well-described areas of the basement membrane structures. In the walls of larger blood vessels, both electron and light microscopic observations show the appearance of intramural gap formation, filled with edema fluid, corresponding to the so-called VIRCHOW-ROBIN spaces. Further work is needed to locate the exact anatomical point where the first real endothelium-lined vasa lymphatica vasorum show their appearance. In the edematous tissue of the neck of animals subjected to a cervical lymphatic blockade, the anatomical connection has already been demonstrated[11] (Figure 7).

Intracellular edema of blood capillary endothelial cells is a sign of their involvement in prelymphatic fluid movements via blood stream → pinocytotic uptake → basement membrane → glia → nerve cell and vice versa. Structural alterations of nerve cells and myelin sheaths, i.e. the pathomechanism of lymphostatic encephalopathy are summarized in Table II.

Conclusion. In striking contrast to the prevailing textbook opinion, paradoxically, lymphatic drainage plays a role of paramount importance in the fluid circulation of the brain devoid of lymphatics.

By severing lymphatic connections of the brain in the cervical region, an experimental disease entity, lymphostatic encephalopathy arises with great regularity in various experimental animals, characterized by edema of

[10] According to the present view in Lymphology, lymphatics are endothelium-lined vessels; lymph is fluid contained by such vessels, a pathway leading tissue fluid in direction to lymph vessels may be called a prelymphatic pathway.

[11] I. SCHNEIDER, M. SIMON, Ö. T. ZOLTÁN and M. FÖLDI, Arch. klin. exp. Derm. *232*, 367 (1968).

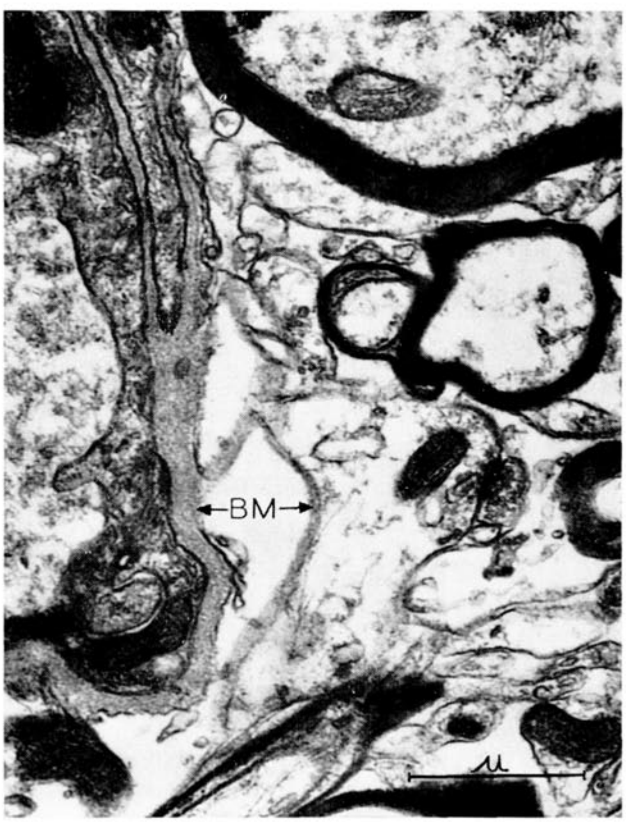


Fig. 4. Displacement of the basement membrane (BM) of a brain capillary (in the caudate nucleus) after extirpation of cervical lymphatics. Rat.

Fig. 5. Extremely swollen pericapillary glial process (Gl) after extirpation of cervical lymphatics. Also note the explosion-like displacement of the basement membrane (arrow). Rat.

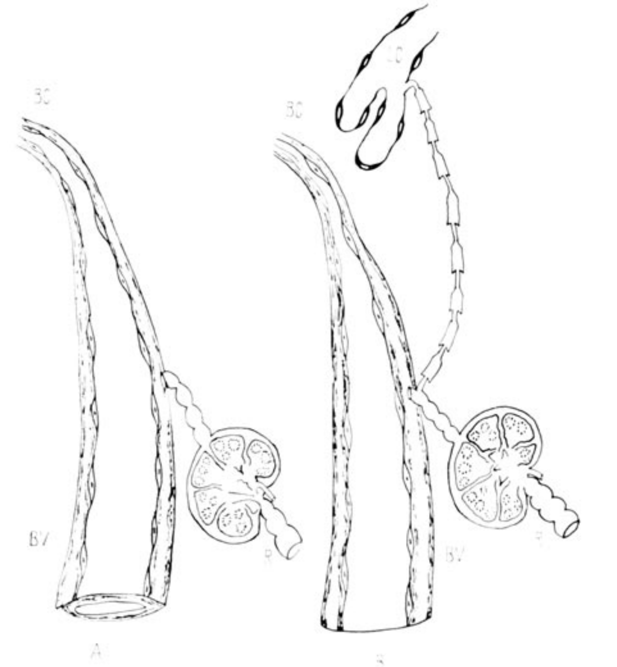


Fig. 6. See text. BC, blood capillary; BV, blood vessel; LC, lymph capillary.

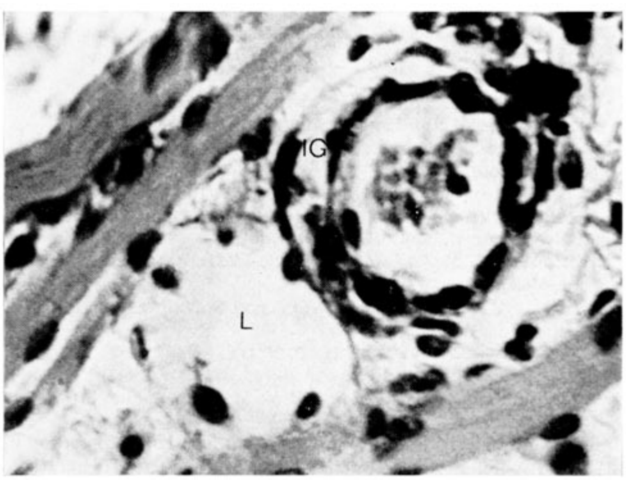
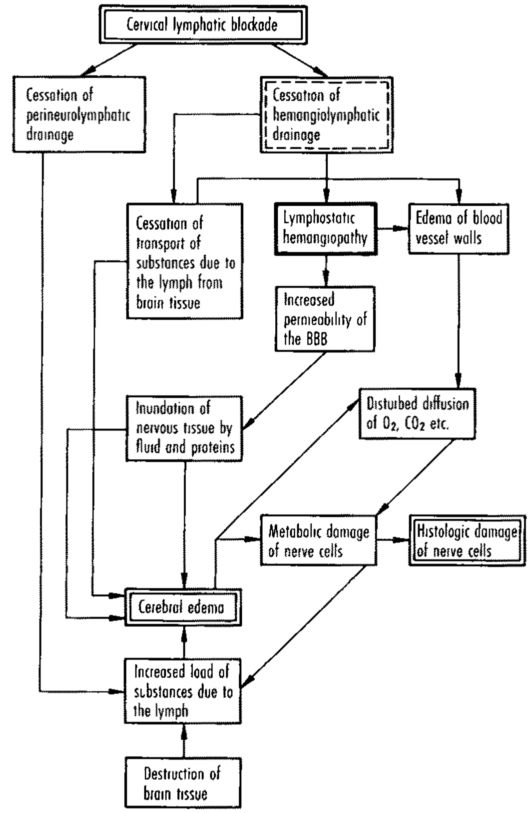


Fig. 7. Intramural gap (IG) in the wall of a vein. Close apposition of a dilated lymph vessel (L). From the edematous subcutaneous tissue of the neck after cervical lymphatic blockade. Dog. Haematoxylin-eosin staining. × 1100.

brain, lesions of nerve cells and myelin sheaths on the one hand, and functional impairment of the brain on the other. Limited space of this present article prevents us from describing the therapeutical approach of lymphostatic encephalopathy and from discussing lymphostatic encephalopathy in man. We are referring to pertaining publications[12,13].

Finally, we should like to stress one consequence of our studies. As in the case of tissue B, the appearance of an edema of unknown ethiopathology, a search for primary or secondary lymphatic involvements is mandatory: there is no edema without some type of lymph circulation insufficiency. The same question should be raised in all

Table II. Pathomechanism of lymphostatic encephalepathy



cases of cerebral edema or protein accumulation in the brain. This means the necessity of a lymphologic breakthrough into the fields of neurology and neuropathology.

Zusammenfassung. In krassem Gegensatz zur heute herrschenden Auffassung spielt eine Lymphdrainage beim Flüssigkeitskreislauf des Gehirns eine ausschlaggebende Rolle. Werden die zervikalen Lymphbahnen verschlossen, so entsteht eine lymphostatische Enzephalopathie, welche u.a. durch ein Hirnödem, Ganglienzell- und Myelinveränderungen sowie durch Beeinträchtigung der Funktion des Zentralnervensystems gekennzeichnet ist. Es wird ein Durchbruch der Lymphologie in das Gebiet der Neurologie und Neuropathologie gefordert und darauf hingewiesen, dass bei der Pathologie des Hirnödems die Rolle der Lymphzirkulation nicht ausser acht gelassen werden sollte.

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