

## The Demonstration of the Lymphatic Pathways of the Pacinian Corpuscles in the Mesojejenum of the Cat

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### Summary

The possibilities of a combination of modified injection techniques are described. This allows the lymphokinetic effect of a drug to produce a definite improvement in the morphological demonstration of the lymphatics.

The investigation of the lymphatic system needs to be considered more or less solely in relation to its dynamics. Up to the present time basic information, not only on the morphology, but also on the physiology and pathology, has been obtained by the use of dyestuffs. There have only been sporadic reports about substances which have a pharmacodynamic action on the lymphatic system. In this connexion, there is nothing reported about any structural changes in the lymphatic pathways. The object of this present paper is to widen the scope of our current knowledge.

### Literature

The investigations pursued so far, since the discovery of the powerful intrinsic activity of the lymphatics, allow one to take a new view of the lymphatic system. In 1622 *Asellius* (1) had already observed a spontaneous rhythmic activity in the lacteals of the dog and this was confirmed by *Hewson* (24). Likewise *Heller* (23) described active contractions in the mesenteric lymph vessels of mice. Subsequently similar observations were made in other species of animal. However, statements to the contrary also appeared e. g. *Florey* (15) and *Yoffey* and *Courtice* (35) were not able to report any spontaneous contractions in dogs. In man *Todd* and *Bowman* (34) were the first to report slow contractions of the thoracic duct after mechanical stimulation and *Kinmonth* and *Taylor* (25) saw spontaneous rhythmic movement in the absence of any external stimula-

tion. Also in later investigations (26) spontaneous contractions with a frequency of 2–5/min. occurred in the large pelvic lymphatics. For success in demonstrating spontaneous contractions of the lymphatics the type of anaesthetic agent used and its mode of administration are of considerable importance (33). Thus, *Hauck* (21) found no contractility in guinea-pigs anaesthetised with pentobarbitone, while *Mislin* (33) obtained good vascular activity in animals anaesthetised with ether after isolating the mesenteric lymphatics; however, when evipan-sodium was used the results were variable.

Furthermore, changes in temperature and variations in the pressure relationships are often regarded as responsible for the absence of vascular pulsation (33). *Málek* (29) also stressed the difficulty of analysing the factors which are responsible for the movement of lymph. According to *Mislin* (33) the mechanisms of this complex lymphatics drainage are manifold and can be divided into three lymphodynamic transport systems:

1. Extravascular lymph drainage (extravascular circulation and lymph formation),
2. Extramural lymph drainage (mechanical effect on the vessels from without),
3. Vasomotor (vascular) lymph drainage (spontaneous rhythm of the lymphatics).

The so-called "compensating lymphatic drainage" (18) depends, according to *Mislin* (33), on the constant functional interplay of these different mechanisms of lymph drainage.

### Material and Methods

For this investigation Pacinian corpuscles were obtained from the mesentery of 10 male and 10 female cats of about 1–3 years old, who had been killed with nembotal (pentobarbitone sodium).

For the demonstration of the lymphatic system the previously described injection techniques (9, 10, 11, 13) followed immediately after the killing of the animal. The previously used techniques for showing the Pacinian corpuscles were also used (14), although in this instance in a modified form and with several solutions. They were injected with a 2.5% solution of patent blue violet<sup>1</sup>, with Japan ink and also with Venalot<sup>2</sup> and a 0.1% solution of fluorescein sodium<sup>3</sup>. Immediately after opening the abdominal cavity the Pacinian corpuscles in the mesojejunum and also the lymphatics in their vicinity and near the root of the mesentery were injected orthograde and retrograde, under the stereo-microscope and using an extremely fine cannula. An injection of Venalot was then given intravenously, or even directly into the Pacinian corpuscles, in view of the lymphokinetic effect of this preparation (20). Indeed, an increase in lymph transport of 1.7 to 2.6 times can be demonstrated in animal experiments (19).

### Results and discussion

Although the Pacinian corpuscles are the main subject of the investigation, we must also take into account the lymph capillaries in their vicinity, the lymphatics within the mesentery, and the removal of their contents. Thus, after the injection of patent blue violet or Japan ink, it is transported away from the Pacinian corpuscles in the lymph capillaries or lymphatics which run from here to the periphery and ultimately into the jejunal lymph nodes. As shown by observation, the lymph capillaries do not form a closed network, although as everybody knows their beginning was generally described as closed (33). *Földi* (16) saw cup-shaped open lymph capillaries arising in an oedematous lung, and in the small intestines the lymphatics begin at the tip of the villi and then pass vertically through into the deeper layers (12).

<sup>1</sup> Makers: Byk-Gulden, Constance

<sup>2</sup> Makers: Schaper and Brümmer, Salzgitter-Ringelheim. "Venalot" is a Coumarin, (5, 6-Benzo-a-pyron)-rutin derivative

<sup>3</sup> Makers: E. Merck, Darmstadt

In general today it is predominantly electron microscopic findings which are referred to, in statements about the structure of the lymph capillaries, among which the papers of *Casley-Smith* (2, 3, 4, 5, 6, 7) and *Casley-Smith* and *Florey* (8) should be particularly noted. Nevertheless it should not go unnoticed here that as long ago as 1939, *Held* (22) had already read a paper "Regarding the basal lymphatic portals (Lymphpforten)". On the basis of paraffin sections stained with molybdenum haematoxylin he wrote that "the lymph capillaries are provided with openings into the tissue clefts and are thus able to take up the (tissue) fluid directly. . . . The lymph vessels pass through the tunica propria of the basement membrane of the mucous membrane and expand in the epithelium as intercellular spaces. My preparations leave no room for doubt about this. The endothelial cells of the lymphatics which have richly branching processes on their outer surfaces and anastomose with the connective tissue cells are connected with the protoplasm of the lowermost epithelial cells. Their transition I propose to call the basal lymphatic portals ("Lymphpforten") and from these very intercellular spaces there then arises fine channels, some rising vertically and some flat, bending and branching. An important part of this arrangement is that the intercellular spaces of the epithelium are closely related to the deep lymphatics, via the basal lymphatic portals". This summary should be retained however, with the comment, that such findings already existed nearly forty years ago. As has already been shown (14), without injection the lymph capillaries leaving the Pacinian corpuscles completely escape detection. Only after injection with patent blue violet and Japan ink. (Fig. 1) are they easily identified. A characteristic property of the lymph capillaries is their great capacity to dilate, which in certain organs enables them to form extensive storage spaces for the lymph (13), although here this function is not normally particularly prominent. However, it should be noted here, that the lymphokinetic effect can be demonstrated after an intravenous injection of Venalot, in

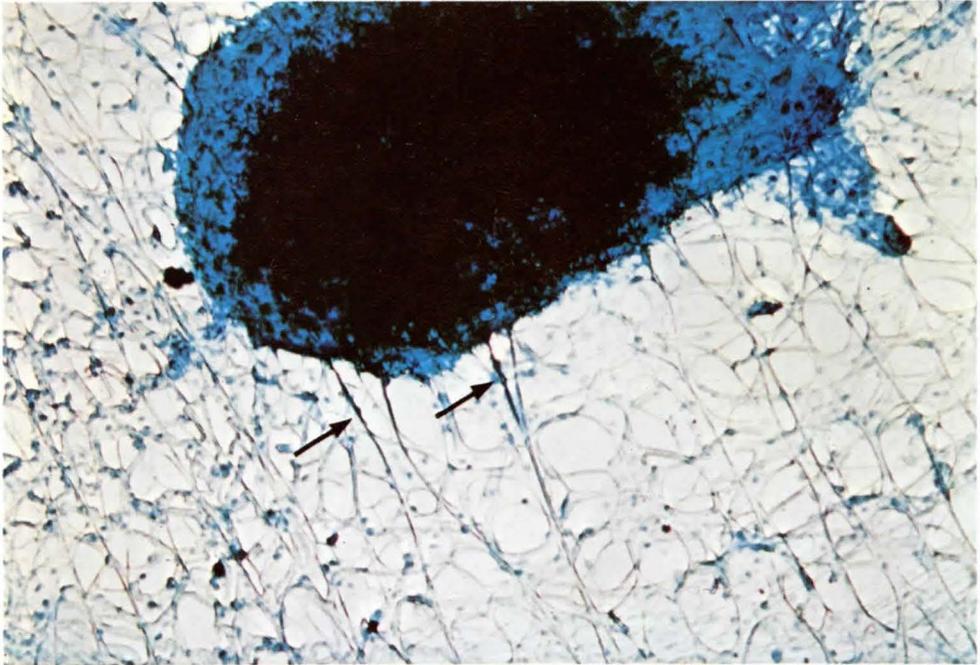


Fig. 1 Pacinian corpuscles injected with patent blue violet and subsequent retrograde injection of Japan ink into a peripherally lying lymphatic which has been outlined by the blue. Here also the beaded appearance of the lymph capillaries can be seen (arrow).

that there is a dilatation of the lymph capillaries or lymphatics, due to the increased lymph flow (Fig. 2). In order to produce a clearer effect for photographic reproduction, small amounts of fluorescein sodium are added to the dyestuff. Also a more intense filling of the Pacinian corpuscles is seen, so that here also it is natural to speak of an 'organ with short "prelymphatic pathways"', in contrast to organs lying outside the parenchyma with long "prelymphatic pathways", in contrast to organs lying outside the parenchyma with long "prelymphatic pathways." It seems appropriate here to attempt an actual definition: By lymphatics, we understand endothelial-lined vessels in which lymph flows and which discharge into the venous system. Preformed connective tissue pathways, which conduct the interstitial fluid in the direction of the lymph capillaries, belong to the prelymphatic system (17). Because of the raised pressure of the injection of patent blue violet into the Pacinian corpuscles, in association with Venalot, there evidently follows very quickly an opening

up of the lymph capillaries and escape of dye into the tissue clefts which however, is no longer apparent after ten minutes. A similar injection of Japan ink cannot be seen in the surrounding connective tissue. It is known that the lymph capillaries are only permeable in one direction for colloidal substances, but in two directions for fluid and diffusible substances. It is by this that the lymph in the capillaries becomes concentrated.

From the so-called capillary network (33) arise the very thinwalled efferent lymph vessels, from which any retrograde flow of lymph is prevented by infundibular or semilunar (bicuspid) valves. The lymphatic channel is divided into numerous intervalvular segments. These valves appear as endothelial folds at regular intervals, so that on account of this segmentation the vessels have rather the appearance of a rosary. Each intervalvular section (valve segment) was called by *Mislin* a lymphangion (30, 31, 33). He deduced from this that there were two types of lymph vessels, firstly vessels in which the



Fig. 2 Widening peripherally of a lymph capillary coming from a Pacinian corpuscle after the application of the pharmacological agent, in combination with patent blue violet and fluorescein sodium.

extramural forces played the main part in lymph transport, which are only conducting vessels, and those which show rhythmic spontaneous contractions and represent specialised transport vessels. In spite of contrary opinions, this assumption has been confirmed by more recent investigations (33).

In order, at the same time to provide experimental proof of the inherent automatic action of the lymph vessels draining the Pacinian corpuscles, in my own series of experiments isolated flat preparations of mesentery were taken and, in addition, completely isolated lymphatics connected to their Pacinian corpuscles were examined at room temperature (22° C). The chain of segments consisted of about ten lymphangions of which each single lymphangion contains a cuff of muscle, which is markedly reduced in the vicinity of the valves. The actual region of the valves is completely free of muscle. The musculature of the lymph segments is extremely richly innervated, with as a rule, one single nerve

fibre going to each muscle fibre (32). *Kubik* and *Szabó* (28) found sensory end organs of the Pacinian type adjacent to and surrounding the lymph vessels.

Having removed a very tightly filled Pacinian corpuscle together with its related lymph vessels, a very fine cannula was put into its centre, it was emptied of its contents and thus made to collapse. Simultaneously with this withdrawal of fluid it was slowly replaced with patent blue violet.

With increasing pressure within the particular lymphangion as a whole, an irregular pulsating lymph vessel was observed, and if the pressure was kept constant a frequency of pulsation between 20–25/min. was achieved. Even *Kubik* (27) was unable to find such uniform rhythmic flow in the entire lymphatic system. Injection with Japan ink produced lower values of 15–20/min. After this Venalot was injected. This showed the most rapid pulsation with a frequency of 30/min. with a dilatation of the lymphatics and a conse-

quent increase in the flow of lymph. This finding, also recorded in the previous series of experiments, shows that by the administration of Venalot there is a shortening of the transit time of the flow of dyestuff, by about a half. Accordingly, this preparation has a threefold myotropic effect on the lymphatics. Thus, the pulse rate, the amplitude and the tone of the lymphatics were considerably increased (32).

Even with isolated specimens which were kept for up to five days in the refrigerator at 4° C, after injection of the dye into the Pacinian corpuscles an active pulsation of the individual lymphangions appeared after 4–5 hours at room temperature. By moistening the specimen with Venalot before the injection of the dye, active pulsation could be seen after only three hours, with removal of the dye.

### References

- 1 *Asellius, G.*: De lactibus sive lacteis venis quarto vasorum mesaraicorum genere novo invento Gasp. Asellii Cremonensis Anatomici Ticinensis. Dissertatio qua sententiae Anatomicae multae, nel perperam receptae convelluntur, nel parum perceptae illustrantur. Mediolani, apud Jo. Baptistam Bidellium (1627)
- 2 *Casley-Smith, J. R.*: Identification of chylomicra and lipoproteins in tissue sections and their passage into jejunal lacteals. *Cell Biol Commun.*, 15 (1962) 259
- 3 *Casley-Smith, J. R.*: Endothelial permeability: the passage of particles into and out of diaphragmatics. *Quart. J. exp. Physiol.*, 49 (1964) 383
- 4 *Casley-Smith, J. R.*: Endothelial permeability. II. The passage of particles through the lymphatic endothelium of normal and injured ears. *Brit. J. exp. Path.*, 46 (1965) 35
- 5 *Casley-Smith, J. R.*: The functioning of the lymphatic system under normal and pathological conditions: Its dependence on the fine structures and permeabilities of the vessels. In: Rüttimann, A., *Progress in Lymphology. Proceedings of the International Symposium on Lymphology Zurich, Switzerland, July 19–23, 1966*, Georg Thieme Verlag, Stuttgart, 1967.
- 6 *Casley-Smith, J. R.*: The fine structure, properties fine and permeabilities of the lymphatic endothelium. *Experientia (Basel)*, Suppl. 14 (1967) 19
- 7 *Casley-Smith, J. R.*: The structure of normal large lymphatics: How this determines their permeabilities and their ability to transport lymph. *Lymphology*, 1 (1969) 15
- 8 *Casley-Smith, J. R. and H. W. Florey*: The structure of normal small lymphatics. *Quart. J. exp. Physiol.*, 46 (1961) 101
- 9 *Fabian, G.*: Zur Darstellung der Lymphkapillaren mittels Patentblau-Violett und Tusche. *Berl. Münch. Tierärztl. Wschr.*, 82 (1969) 113
- 10 *Fabian, G.*: Experimentelle Untersuchungen über die enterale Resorption des Vitalfarbstoffes Patentblau-Violett und von Japanischer Tusche. *Z. Angew. Zool.*, 59 (1972) 17
- 11 *Fabian, G.*: Zur Aufnahme des Vitalfarbstoffes Patentblau-Violett und von japanischer Tusche in die Lymphkapillaren der Darmzotte. *Zbl. Vet. Med. A*, 19 (1972) 52
- 12 *Fabian, G.*: Untersuchungen morphologisch faßbarer Vorgänge der enteralen Resorption, der Persorption und des lymphatischen Abtransportes. *Z. Angew. Zool.*, 60 (1973) 183
- 13 *Fabian, G.*: The Lymphatic Vessels of the Uterus during the Sexual Cycles of the Mice. *Lymphology*, 9 (1976) 78
- 14 *Fabian, G.*: Pacinian Corpuscles and their Relationship to the Lymphatic System. *Lymphology*, 10 (1977) 27
- 15 *Florey, H.*: Observations on the contractility of lacteals. Part II. *J. Physiol.*, 63 (1927) 1
- 16 *Földi, M.*: Physiologie und Pathologie des Lymphkreislaufes. *Verh. dtsh. Ges. inn. Med.*, 66 (1961) 531
- 17 *Földi, M.*: Lymphostatische Krankheitsbilder. *Herz/Kreisl.*, 2 (1970) 217
- 18 *Földi, M.*: Physiologie und Pathophysiologie des Lymphgefäßsystems. In: *Handbuch der Allgemeinen Pathologie Bd. 3/6* (1971) 239
- 19 *Földi, M., A. G. B. Kovách, L. Varga und Ö. T. Zoltán*: Die Wirkung des Melilotus-Präparates Esberiven auf die Lymphströmung. *Arzneim. Forsch.*, 16 (1962) 1/99
- 20 *Gregl, A.*: *Lymphographie und Pharmakolymphographie*. Gustav Fischer Verlag, Stuttgart, 1975.
- 21 *Hauck, G.*: Functional Aspect of the Topical Relationship between Blood Capillaries and Lymphatics of the Mesentery. *Pflügers Arch.*, 339 (1973) 251
- 22 *Held, H.*: Über basale Lymphpforten. *Verh. Anat. Ges. 46. Vers. Anat. Anz. Erg. z. Bd. 87* (1939) 224
- 23 *Heller, A.*: Über selbständige rhythmische Contractionen der Lymphgefäße bei Säugethieren. *Z. med. Wiss.*, 7 (1869) 545
- 24 *Hewson, W.*: *Experimental inquiries: Inquiry into the properties of the blood. An appendix, relating to the discovery of the lymphatic system in birds, fish, and the animals called amphibious.* J. Johnson, London, 1774.
- 25 *Kinmonth, J. B., G. W. Taylor*: Spontaneous rhythmic contractility in human lymphatics. *J. Physiol.*, 133 (1956) 3
- 26 *Kinmonth, J. B., G. W. Taylor*: Chylous reflux. *Brit. med. J. I*, (1964) 529

- 27 *Kubik, I.*: Die hydrodynamischen und mechanischen Faktoren in der Lymphzirkulation. *Acta morph. Acad. Sci. hung.*, 2 (1952) 95
- 28 *Kubik, I., J. Szabó*: Die Innervation der Lymphgefäße im Mesenterium. *Acta morph. Acad. Sci. hung.*, 6 (1955) 25
- 29 *Málek, P.*: Pathophysiological and radiological aspects of lymphovenous anastomoses. In: *New trends in basic lymphology*, p. p. 197–204. Birkhäuser, Basel (1967)
- 30 *Mislin, H.*: Experimenteller Nachweis der autochtonen Automatie der Lymphgefäße. *Experientia*, 17 (1961) 29
- 31 *Mislin, H.*: Zur Funktionsanalyse der Lymphgefäßmotorik beim Meerschweinchen (*Cavia porcellus* L.). *Revue Suisse de Zoologie*, 68 (1961) 228
- 32 *Mislin, H.*: Die Wirkung von Cumarin aus *Melilotus officinalis* auf die Funktion des Lymphangioms. *Arzneim.-Forsch. (Drug Res.)* 21 (1971) 852
- 33 *Mislin, H.*: Die funktionelle Organisation der vasomotorischen Lymphdrainage. In: *A. Gregl, Lymphographie und Pharmakolymphographie*. Gustav Fischer Verlag, Stuttgart (1975)
- 34 *Todd, R. B., W. Bowman*: *The physiological anatomy and physiology of man*. Blanchard and Lea, Philadelphia (1857)
- 35 *Yoffey, J. M., F. C. Courtice*: *Lymphatics, lymph and lymphoid tissue*. Edward Arnold, London (1956)

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- 27 *Kubik, I.*: Die hydrodynamischen und mechanischen Faktoren in der Lymphzirkulation. *Acta morph. Acad. Sci. hung.*, 2 (1952) 95
- 28 *Kubik, I., J. Szabó*: Die Innervation der Lymphgefäße im Mesenterium. *Acta morph. Acad. Sci. hung.*, 6 (1955) 25
- 29 *Málek, P.*: Pathophysiological and radiological aspects of lymphovenous anastomoses. In: *New trends in basic lymphology*, p. p. 197–204. Birkhäuser, Basel (1967)
- 30 *Mislin, H.*: Experimenteller Nachweis der autochtonen Automatie der Lymphgefäße. *Experientia*, 17 (1961) 29
- 31 *Mislin, H.*: Zur Funktionsanalyse der Lymphgefäßmotorik beim Meerschweinchen (*Cavia porcellus* L.). *Revue Suisse de Zoologie*, 68 (1961) 228
- 32 *Mislin, H.*: Die Wirkung von Cumarin aus *Melilotus officinalis* auf die Funktion des Lymphangioms. *Arzneim.-Forsch. (Drug Res.)* 21 (1971) 852
- 33 *Mislin, H.*: Die funktionelle Organisation der vasomotorischen Lymphdrainage. In: *A. Gregl, Lymphographie und Pharmakolymphographie*. Gustav Fischer Verlag, Stuttgart (1975)
- 34 *Todd, R. B., W. Bowman*: *The physiological anatomy and physiology of man*. Blanchard and Lea, Philadelphia (1857)
- 35 *Yoffey, J. M., F. C. Courtice*: *Lymphatics, lymph and lymphoid tissue*. Edward Arnold, London (1956)

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