3 *Askar, O., K. A. Kassem:* The lymphatics of the leg in deep venous thrombosis. Brit. J. Radiol. 42 (1969), 122
4 *Blalock, A., C. S. Robinson, R. S. Cunningham, M. E. Gray:* Experimental studies on lymphatic blockage. Arch. Surg. 34 (1937), 1049
5 *Cockett, F. B., D. E. E. Jones:* The "Blowing-out" Syndrome. Lancet 1953/I, 17
6 *Goodwin, W. E., J. J. Kaufman:* The renal lymphatics. I. review of pertinent literature. Urol. Surg. 6 (1956), 305
7 *Haeger, K.:* Venous and lymphatic disorders of the leg. Bokforlager Universitet Och Skola, Lund, 1966
8 *Job, T. T.:* Lymphatico-venous communications in the common rat and their significance. Amer. J. Anat. 24 (1918), 467
9 *Kindal, F., E. Mannheimer, L. Pfleger-Schwarz, B. Thurnher:* Lymphangiographie und Lymphadenographie der Extremitäten. Thieme, Stuttgart 1960
10 *Kinmonth, I. B., R. A. R. Harper, G. W. Taylor:* Lymphangiography, a technique for its clinical use in the lower limbs. Brit. med. J. 1 (1955), 940
11 *Lee, F. C.:* The establishment of collateral circulation following ligation of the thoracic duct. Bull. Johns Hopkins Hosp. 33 (1922), 21
12 *Patterson, R. M., C. L. Ballard, K. Wasserman, H. S. Mayerson:* Amer. J. Physiol. 194 (1958), 120
13 *Pentecost, B. L., J. I. Burn, A. Davies, J. S. Calnan:* Brit. J. Surg. 53 (1966), 630
14 *Pick, J. W., B. J. Anson, H. W. Burnett:* Quart. Bull. Northw. Univ. med. Sch. 18 (1944), 307
15 *Retik, A. B., A. D. Perlmutter, J. H. Harrison:* Communications between lymphatics and veins involving the portal circulation. Amer. J. Surg. 109 (1965), 20
16 *Rusznyak, I., M. Foldi, H. W. Burnett:* Lymphatics and Lymph circulation. Pergamon Press, London 1960
17 *Silvester, C. F.:* On the presence of permanent communications between the lymphatic and venous systems at the level of the renal veins in adult, South-American monkeys. Amer. J. Anat. 12 (1912), 447
18 *Threefoot, S. A., M. T. Kossover, D. N. Aiken:* Radioscopic detection of lymphatico-venous communications in living animals. J. Lab. Clin. Med. 65 (1965), 688
19 *Threefoot, S. A., M. V. Kossover, W. T. Kent, B. F. Hatchett, J. E. Pearson, C. Cabrera-Gil:* Factors stimulating function of lympho-venous communications. Angiology 18 (1967), 682

Omar Askar, M. D., Professor of Surgery, Kasr El-Aini, Cairo University, Cairo/Egypt

The Treatment of Acute Lymphoedema with Pantothenic Acid and Pyridoxine:

An electron microscopical investigation

J. R. Casley-Smith

(Dept. Zoology, University of Adelaide, South Australia)

M. Földi, Ö. T. Zoltán

(2nd. Dept. of Internal Medicine, University Medical School, Szeged, Hungary)

Lymphology 2 (1969), 63–71

Lymphoedema which is caused by impedance of lymph outflow from a region, is becoming recognised as the origin, or a contributing factor of many diseases. It has been shown that surgical obstruction of the cervical lymphatics results in *Lymphostatic Encephalopathy*. This is characterised by striking central nervous signs and a series of morphological alterations in the brain and the eye, accompanied by a lymphoedema of the muzzle (1–8). Surprisingly, it was found that these signs and alterations can be largely and safely prevented by treatment with high doses of Pantothenic acid and Pyridoxine (9, 10). This has been established in a number of species, including the rat, the dog and man.

The electron microscope was used to help investigate how this treatment works. Observations were made on the lymphatics in the tongues of normal rats, of some whose cervical lymphatics had been ligated, and of others with a similar operation but with the treatment as well. The tongue was chosen since it is drained by the same

lymphatics as is the brain, yet it possesses the normal, small, "initial" lymphatics which are not present in the brain. The plentiful supply of lymphatics in the fascial planes of the tongue made it easy to find enough of them. Preliminary experiments on the skin of the cheek showed similar results to those reported here, but a relative paucity of lymphatics made the findings less reliable.

Methods

The cervical lymphatics of 6 rats (180–200 gms.) were occluded by passing ligatures around the lymph nodes (1). Three of the rats were given subcutaneous injections of 125 mg/kg of Pantothenic acid* and 25 mg/kg of Pyridoxine** on the day of the operation and on the next two days. In the 3 untreated rats the oedema of the muzzle and the tongue was maximal on the third day. At that time pieces of the tongues of both groups of rats were excised for electron microscopic analysis. The tongues of 5 normal rats were also studied. The tissues were fixed in phosphate-buffered 4% formaldehyde (with 7% sucrose) for 1 hour at 4 °C and post-fixed in 2% Osmium tetroxide for 12 hours. The blocks were embedded in araldite, sectioned, and stained with Lead citrate (pH 10) and Uranyl acetate by conventional methods.

Results

Macroscopically and with the light microscope the tongues of the three operated, non-treated animals appeared quite oedematous when compared with those of the normal animals, or of the treated ones. The tongues of the three operated, but treated, rats were very similar to normal tongues.

Under the electron microscope the tongues of each group of animals showed differences between the groups, but were fairly homogenous within the groups. Naturally some variations were observed from one animal to another within a group and from one site to another within the one tongue: such variations are normal. They did not obscure the considerable differences between the normal and the operated animals, nor between the operated ones with and without treatment.

The fine structure of all five normal tongues showed no oedema; the lymphatics (Figs. 1–3) were similar to those observed in other regions (11–14). As would be expected, since the tongue is in fairly constant mild activity, these vessels were rather more dilated, contained rather more protein, and had rather more open endothelial intercellular junctions than are found in small lymphatics in quiescent regions – e.g. the pinna of the ear (2). However they were not nearly as dilated, nor did they have the protein content and the open junctions associated with lymphatics in very active (12) or injured regions (11, 12, 14). In fact, they were sligthly more similar to the quiescent regions than they were to the very active ones. In this they resembled the lymphatics of the normal foot-pad of the rat (14). There was the usual tenuosity of the basement membrane, the paucity of zonulae adhaerentes and occludentes, and the presence of many fine fibrils connecting the endothelial cells with the connective tissues.

* Panthenol, Jenapharm.
** Pyridoxine, Egypt.

There was considerable oedema in the tongues of all operated, untreated animals (Figs. 4–6). There was much plasma protein in the connective tissues and in the lumen of the widely dilated lymphatics. There were many collections of protein in what appeared to be "pre-formed" channels through the connective tissue, lying between the bundles of collagen and leading to the lymphatics (Fig. 4). These are a kind of "pre-lymphatic pathway". Open endothelial junctions (Figs. 5, 6) were considerably more frequent than in the normal animals.

The tongues of all three animals having undergone lymphatic ligation, and treatment with pyridoxine and pantothenic acid, had features resembling both of the other two groups (Fig. 7, 8). Their lymphatics were wider than normal, but were not early as much dilated as those of the untreated animals. They did not appear to have more than the normal proportion of open junctions. There was some excess protein present in the connective tissues and in the lymphatic lumens, but not nearly as much as in the untreated animals.

Discussion

The present results confirm the earlier findings about the morphology of lymphatics in normal and in oedematous tissues (11–14). They also confirm the effectiveness of this treatment of lymphoedema.

It is evident that in lymphoedema, when there is either a relative or an absolute impedence to lymph out-flow, the lymph will accumulate in the lymphatics. These will dilate, first centrally at the site of the blockage, then the dilatation will spread centrifugally until the small, initial, lymphatics are dilated. The raised intra-lymphatic pressures, augmented by the contractions of the adjacent muscles and by those in the walls of the larger lymphatics, will eventually force open the poorly supported (11, 12) intercellular junctions in the initial lymphatics. As the lymph accumulates the cells will eventually be forced so far apart that the junctions do not seal during the transient tissue compressions. Hence they will become incompetent, will not act as efficient inlet-valves, and the lymph will escape back into the tissues. These will then swell too and macroscopic oedema will result. Most of the small molecules, however, will escape back into the blood vessels under the influence of the raised tissue hydrostatic pressures. The large molecules cannot do this since they are too large to pass through the closed junctions of the blood vessels (12, 13, 15, 16) and they remain in the tissues and the lymphatics, retaining the oedema-forming water by their osmotic action. It is evident, therefore, that the pathogenesis of lymphoedema, and of the oedema caused by increases in blood vascular permeability are different:

In lymphoedema the dilatation of the lymphatics precedes the tissue oedema; the small, initial, lymphatics are dilated by the accumulating lymph and their junctions are pushed apart. In the oedema arising from increased vascular permeability the tissue oedema precedes the dilatation of the lymphatics which are pulled open by the tensions in the fine fibrils attached to the cells. Thus the cells are pulled apart and their junctions opened (14). (No doubt once a lymphoedema has spread to the tissues the increased tensions in the fibrils will also prevent the lymphatics from collapsing and their junctions from closing.) It is apparent that in lymphoedema it is the protein in the tissues which causes the oedema to appear and to remain. Mere

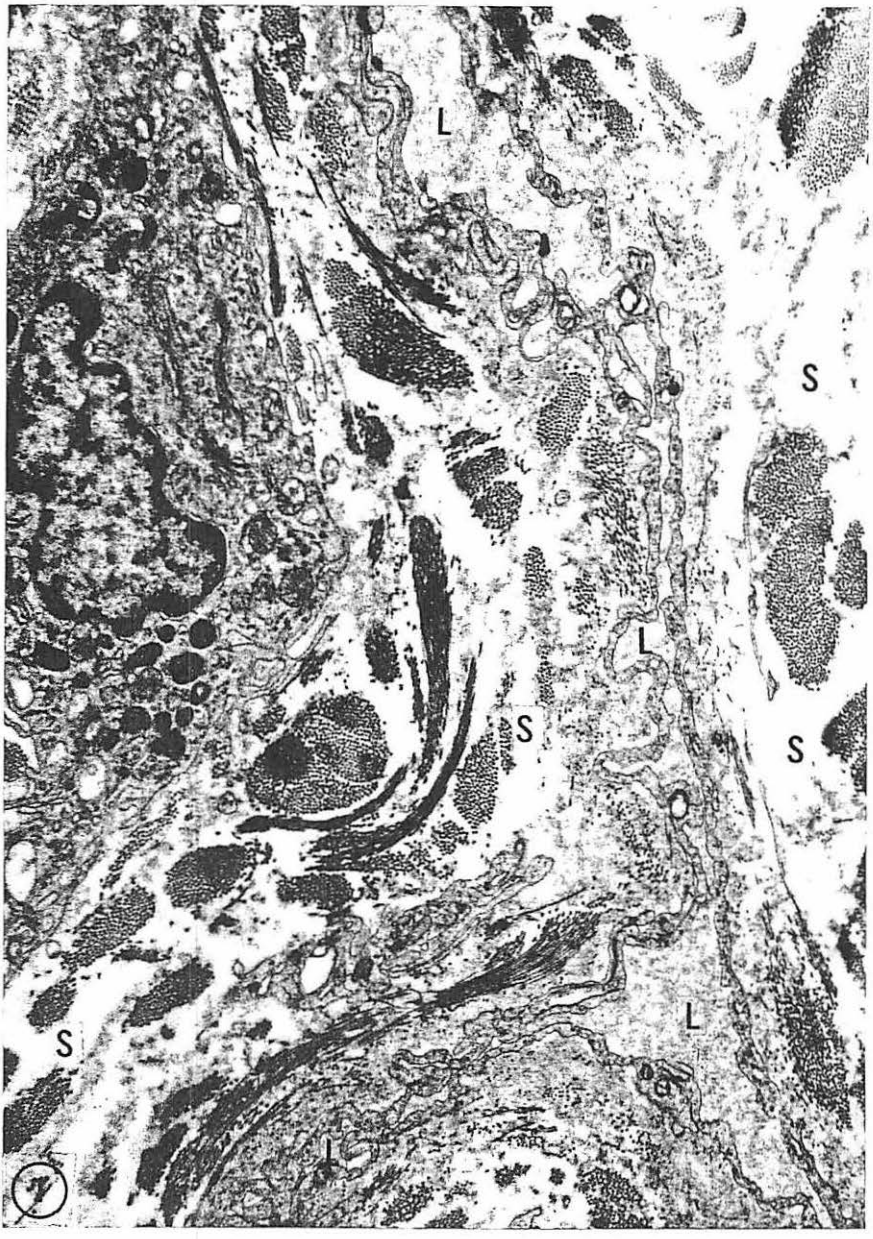
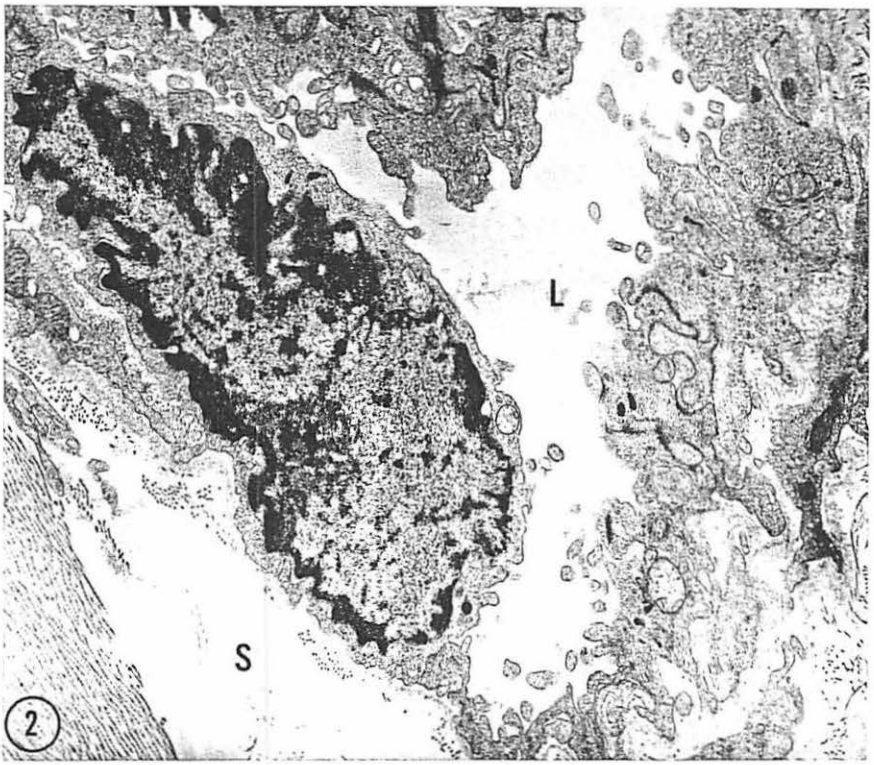


Fig. 1 Normal lymphatic (LLLL); showing its collapsed state, and its normal content of protein. There is very little protein in the connective tissue spaces (S), although some is concentrated near the lymphatic. 2,500x.



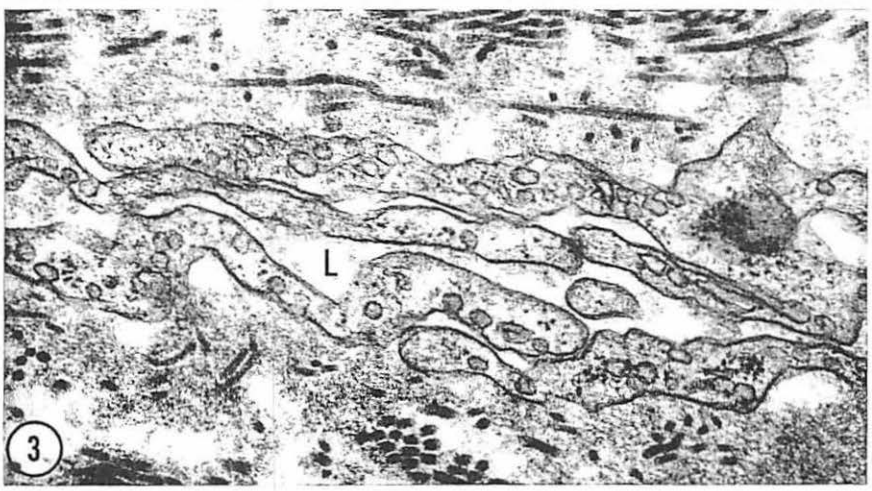


Fig. 2 Normal lymphatic (L), slightly dilated, but showing much infolding of the endothelium, with closed junctions. The connective tissue space (S) is free of protein, as in the lumen. 5,000x.

Fig. 3 Normal lymphatic (L), which is quite collapsed. The junctions are slightly open over parts of their lengths. Again, some concentration of protein is visible near the vessel, but not further away. 40,000x.

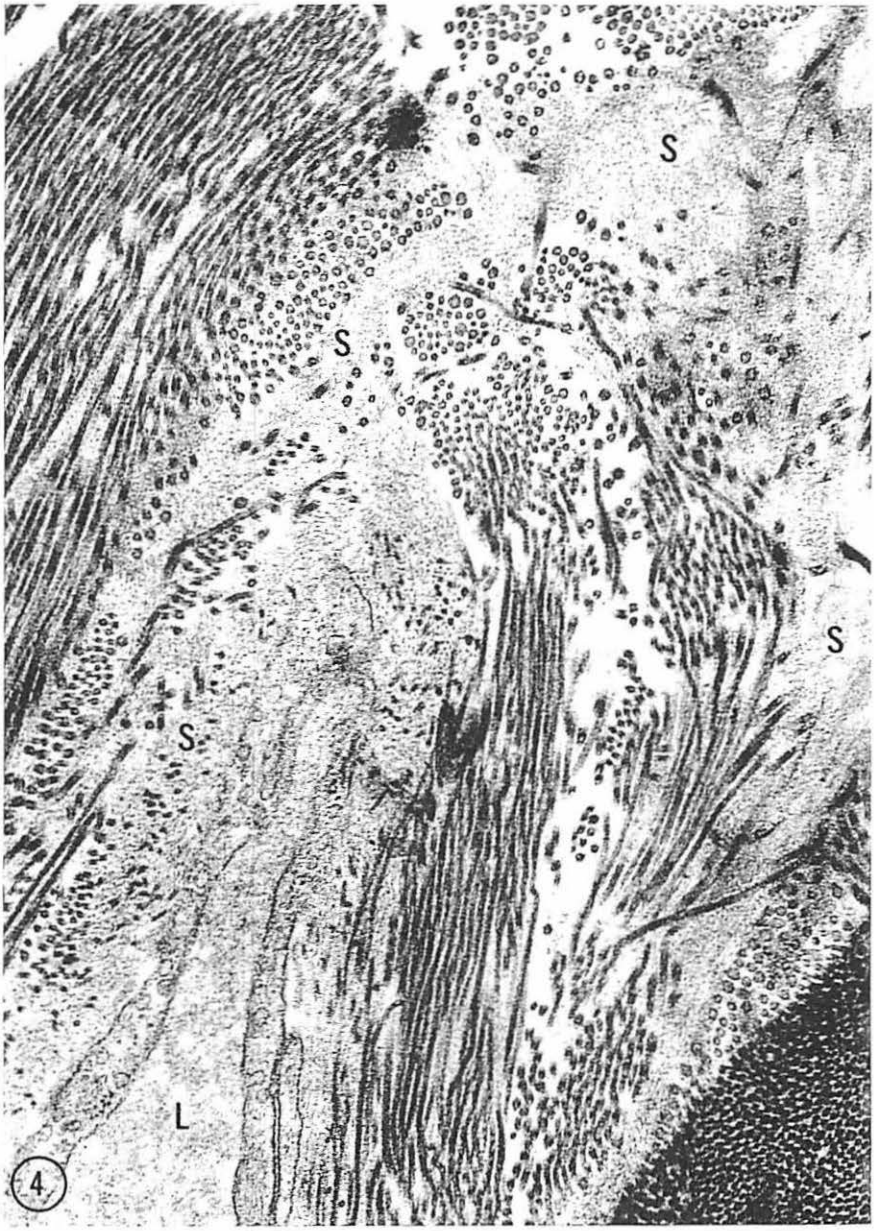


Fig. 4 Lymphatic (L) in oedematous tongue. There is much protein in the connective tissue spaces (S), which lead to the lymphatic. These are probably one type of "prelymphatic pathway." The visible portion of the lymphatic does not look very dilated; the remainder was. 25,000x.

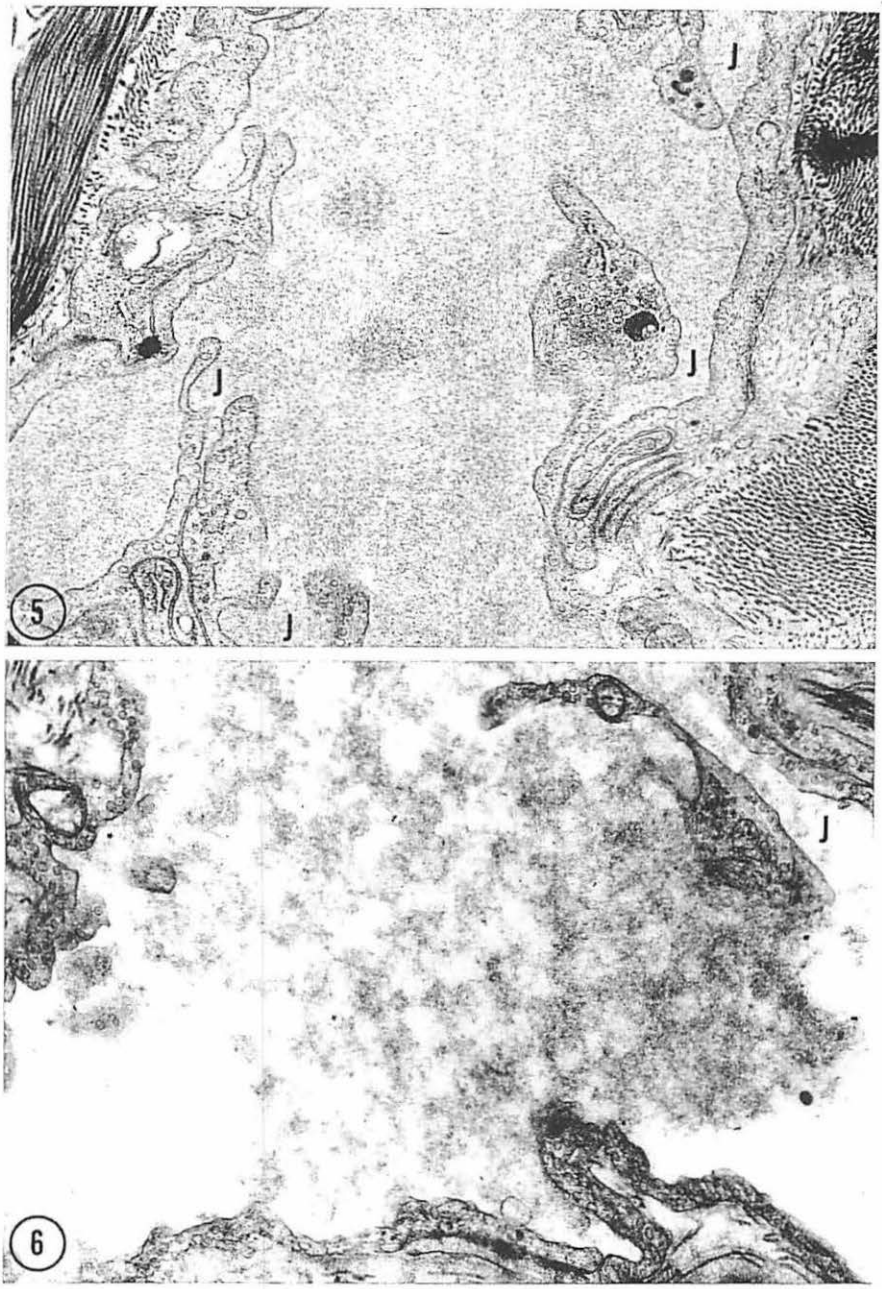


Fig. 5 A widely dilated lymphatic in an oedematous tongue. There are a number of open and partly open junctions (J). There is much protein in and around the vessel. 20,000x.

Fig. 6 A very widely dilated lymphatic, in an oedematous tongue, with an open junction (J) and contained protein. 20,000x.

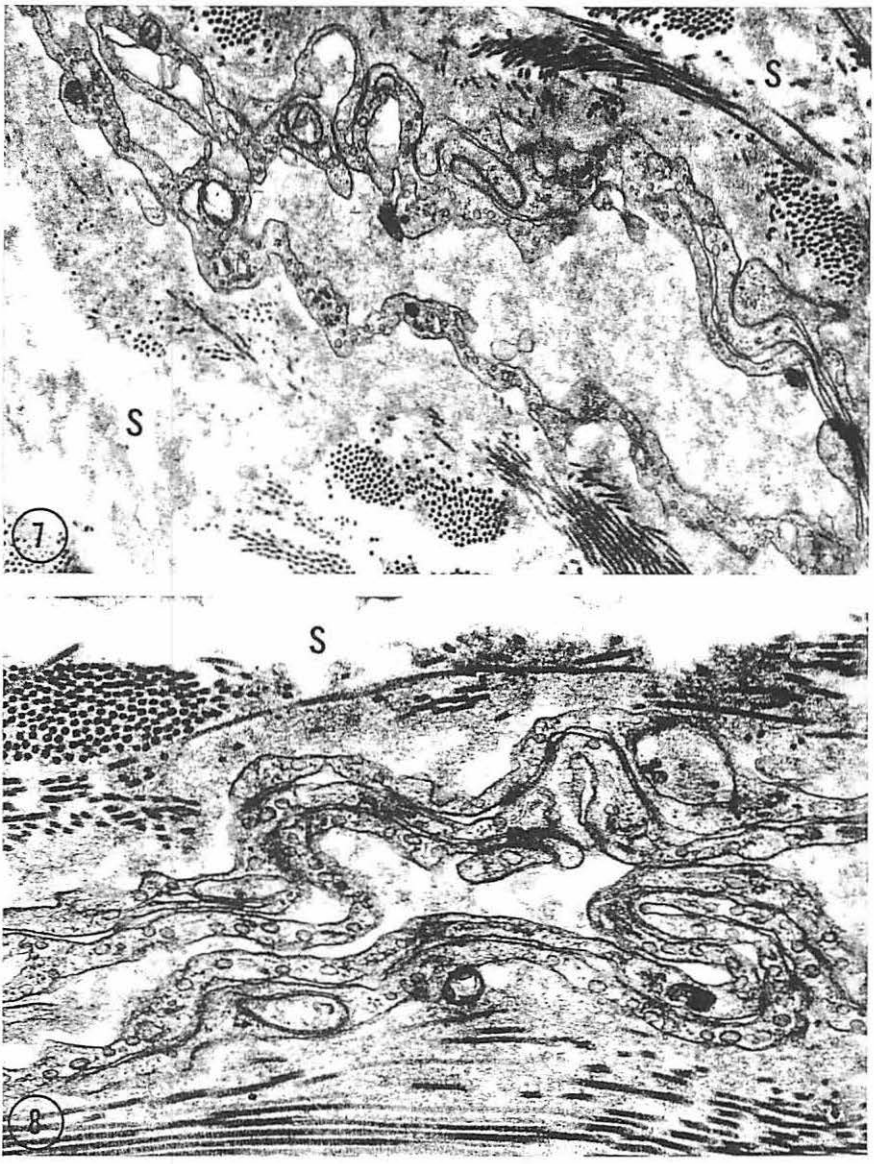


Fig. 7 A moderately collapsed lymphatic in a treated tongue. There is some protein in the lumen and near the vessel, but some of the connective tissue spaces (S) are fairly free of it. Most of the junctions are quite closed. 15,000x.

Fig. 8 A collapsed lymphatic in a treated tongue, with little protein in the lumen. There is some near the vessel, but none in an adjacent space (S). The junctions are all closed, except for two very minor openings in the centre of two of them. 25,000x.

blockage of the lymphatics will not produce oedema. There has to be trapped protein to retain the water. Similarly, treatment of such an oedema must either reduce the amount of protein reaching the tissues, or somehow facilitate its removal.

It is evident that the treatment considered in these experiments does reduce the amount of protein in the tissues, removing the oedema, and thus allowing the lymphatics to become less dilated and their junctions to close. The three most likely explanations are that the treatment prevents much of the normal leakage of protein through the blood capillary walls, that it causes collateral lymphatics to open more rapidly or to function more effectively, or that it increases the catabolism of the accumulated proteins in the tissues. From what is known of the passage of proteins through continuous, non-fenestrated, capillaries, it would seem that the first possibility is very unlikely (12, 16). The second possibility also appears most unlikely. Thus the third may be the correct explanation. Certainly such large doses of very active chemicals can greatly alter many cellular metabolic paths. It is possible that this is what happens when these coenzyme-A precursors are given in such large amounts.

Summary

Normal and lymphoedematous rat tongues were examined with the electron microscope. Lymphoedema was associated with dilated lymphatics, with many open intercellular junctions, and with much protein in the tissues. Treating the lymphoedema with large doses of pyridoxin and pantothenic acid greatly reduced these abnormalities.

References

1 *Földi, M.:* Diseases of Lymphatics and Lymph Circulation. Thomas, Springfield 1968

2 *Földi, M.:* Lymphogenous Encephalopathy. In: Mayerson, H. S.: Lymph and the Lymphatic System. Thomas, Springfield 1968

3 *Földi, M., B. Csillik, F. Joó, Ö. T. Zoltán:* Electron microscopic alterations in the central nervous system in experimental lymphogenic encephalopathy. I. Angiol. 4 (1967), 50

4 *Csillik, B., M. Földi:* Severe alterations in myelin structure in experimental lymphogenous encephalopathy. Experientia 2 (1967), 835

5 *Földi, M.:* Lymphogenous Encephalopathy - Lymphostatic Cerebral Haemangiopathy. Acta med. Acad. Sci. Hung. 25 (1968), 299

6 *Földi, M., B. Csillik, Ö. T. Zoltán:* Lymphatic Drainage of the Brain. Experientia 24 (1968), 1283

7 *Csanda, E., M. Földi, F. Obál, Ö. T. Zoltán:* Cerebral Oedema as a Consequence of Experimental Cervical Lymphatic Blockage. Angiologica 5 (1968), 55

8 *Földi, M., E. Csanda, Ö. T. Zoltán, I. Dobranovics:* Oedema of the Optic Nerve and the Retina as a Consequence of Experimental Cervical Lymphatic Blockage. Angiologica 4 (1967), 431

9 *Földi, M., E. Csanda, B. Csillik, A. Jáki, I. Madarász, F. Obál, Ö. T. Zoltán:* Verhütung der Symptome des „cerebralen Lymphoedems" mit einer Pantothensäure-Pyridoxinbehandlung. Angiologica 2 (1965), 133

10 *Földi, M., F. Obál, A. Kahán, A. Wagner, E. Csanda, E. Börcsök:* Lymphogene Encephalopathie. Acta paediat. Acad. Sci. hung. 8 (1967), 171

11 *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

12 *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 "Progress in Lymphology", Ed. A. Rüttimann. Thieme, Stuttgart 1967

13 *Casley-Smith, J. R.:* An electron microscopical study of the passage of ions through the endothelium of lymphatic and blood capillaries, and through the mesothelium. Quart. J. Exp. Physiol. 52 (1967), 105

14 *Casley-Smith, J. R.:* Electron microscopical observations on the dilated lymphatics in oedematous regions and their collapse following hyaluronidase administration. Brit. J. Exp. Path. 48 (1967), 680

15 *Cotran, R. S., G. Majno:* Studies in the permeability of endothelium and mesothelium. Protoplasma 63 (1967), 45

16 *Majno, G.:* Ultrastructure of the vascular membrane. In "Handbook of Physiology". Section 2; Circulation, III. (1965), 2293

J. R. Casley-Smith, Dept. Zoology, University of Adelaide, South Australia,
M. Földi, Ö. T. Zoltán, 2nd. Dept. of Internal Medicine, University Medical School, Szeged, Hungary