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On the Pathomechanism of Development of Postsurgical Lymphedema.

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Summary

Lymphangiographic, histological, electronenmicroscopic, biochemical and lymph pressure studies were carried out in a group of 26 dogs after the operation for production of persistent postsurgical lymphedema. The purpose of the study was to follow the history of development of obstructive lymphedema, and to chronologically document alterations in the limb lymphatics. The most interesting findings were: lack of correlation between the intensity of edema and radiological changes which usually preceded the appearance of edema, lack of lymph hypertension, low protein concentration, permanent patency of interendothelial junctions of lymph capillaries and ineffectiveness of the muscular pump in forcing the interstitial fluid into the lymph capillaries and propelling the lymph along the lymph collectors.

Postsurgical lymphedema of extremities remains a serious and not uncommon complication of radical surgery for tumors. It occurs most commonly after radial mastectomy, groin dissection, and hysterectomy with postoperative radiotherapy. The mechanism of development of postsurgical lymphedema has not been thoroughly investigated, thus the principles of numerous surgical methods of treatment remain uncertain and results of treatment unsatisfactory. An increasing number of patients with postsurgical lymphedema in our department prompted us to investigate experimentally the process of development of obstructive lymphedema, with its radiological, histological, lymph flow and pressure, and biochemical aspects.

Material and methods.

Twenty-six dogs, both sexes, weighing 15-18 kg were used for experiments. In all animals an operation for production of obstructive lymphedema imitating postsurgical lymphedema in man was performed (3, 4, 8). The operation was carried out on one hind limb, the other one served as a control. The procedure consisted of excision of a segment of femoral lymph vessels toghether with a circular strip of skin, subcutaneous tissue, muscular fascia, intermuscular connective tissue, and periosteum in the upper thigh of the dog. A 2 cm wide gap was left between the skin edges to be healed by granulation. Through a small incision in the popliteal region the popliteal lymph node was removed. The following studies were carried out postoperatively in the 3rd or 4th week, and every 6 months thereafter: 1. Limb volume measurements, with displacement techniques, 2. Skin with subcutaneous tissue, and muscle water content, 3. Direct lymphangiography of the operated limb, with Lipiodol-Ultra-fluid, Laboratoires *André Guerbet*, F 93 Saint-Quen, France., 4. Direct lymphangiography with aqueous contrast medium Uromiro 60 percent, Bracco Industria Chimica S.p.a., Milano, Italia, for study of the permeability of lymph vessels. 2.5 ml of Uromiro were injected in 30 sec. into the paw lymphatic vessel, and serial X-ray film taken after 1, 3, 5 and 10 min. 5. Phlebography, 6. Calf skin biopsy for stereomicroscopic investigation of the dermal lymphatic plexus, according to the method of *Zerbino*, 10.

7. Calf skin and lymph vessel biopsy for light microscopy, 8. Calf skin biopsy for electron microscopy, 9. Lymph pressure measurement by direct cannulation of thigh lymphatic vessels. *Elema*-Mingograph 81 (*Elema*-Sweden) recorder with pressure transducer was used for all pressure measurements, 10. Interstitial fluid pressure measurements a. in *Guyton's* capsules implanted under the calf skin 4 weeks before the study (6), and b. by micro-droplet technique, 11. Lymph vessel compliance, by intralymphatic infusion of dextran in the paw and simultaneous pressure recording in the thigh lymphatics. Increase in pressure in mmHg per 1 ml of increase in volume was a measure of compliance, 12. Lymph protein concentration, 13. Lymph coagulation and fibrynolysis.

Results

Clinical course of lymphedema

A transient edema of the whole limb developed soon after the operation in all dogs, to subside almost completely within 4-6 weeks (acute lymphedema). In the next period there was no edema for at least 7-8 months (latent lymphedema), when it began to reappear. By the end of the 1st year 25 percent of dogs had evident lymphedema (chronic lymphedema) (Fig. 1). That percentage went up to 35 percent after 2 years, to 55 percent after 5 years, and to 61 percent after 6 years (Fig. 2).

Fig. 1. Dog with postsurgical lymphedema of the right hind limb.

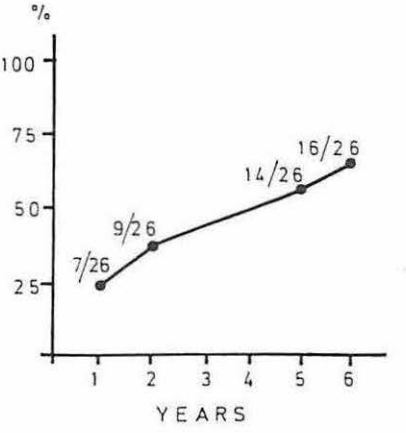
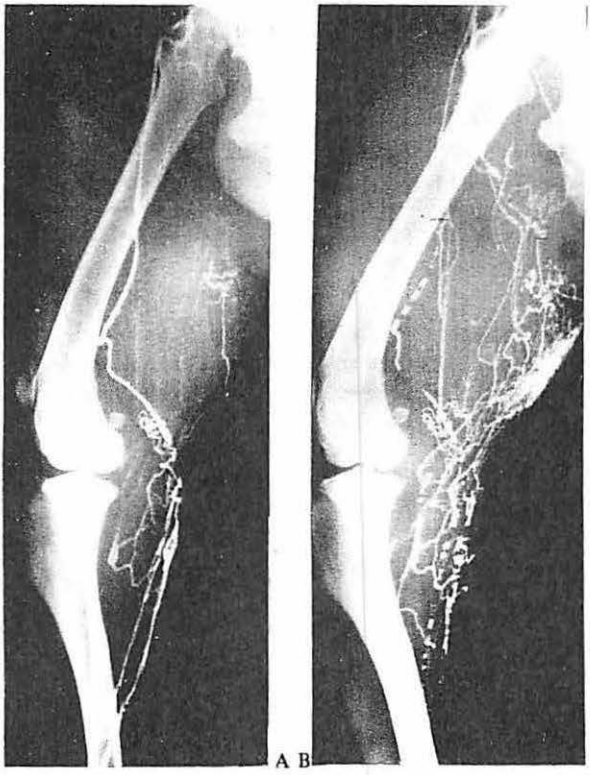


Fig. 2. Yearly rate of incidence of lymphedema after the operation for production of experimental lymphedema in 25 dogs.



A B

Fig. 3 Lymphangiograms of dogs hind limb performed 9 (A) and 14 (B) months after the operation for production of lymphedema. Increasing dilatation of lymph vessels, but clinically no edema.

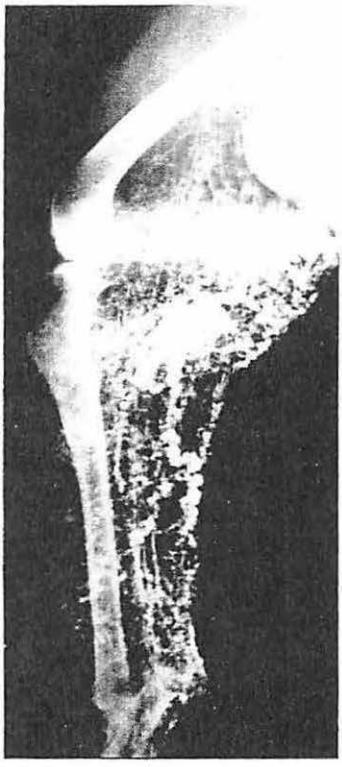


Fig. 4 Typical lymphangiogram in a dog with chronic lymphedema of 4 years duration.

Limb volume measurements.

In the 3rd week of acute stage of lymphedema limb volume increased by 125± 72 ml (10 percent of normal limb volume). In the stage of latent lymphedema no significant differences of the volume were found, as compared with the normal limb. In the stage of chronic lymphedema the increase in volume of the lymphedematous limb was over the control on the average 250 ml (20 percent increase); The measurements were taken at different time of duration of edema, and only the mean value can be presented.

Skin and muscle water content.

Average water content of the normal skin and subcutaneous tissue was 0.69± 0.07g/1g of tissue, of the normal muscle 0.71± 0.07g/1g of tissue. In the chronically edematous limb skin water content increased by 0.09 g amounting to 0.78± 0.08g/1g of tissue, and that of the muscle remained unchanged.

Lymphangiography with Lipiodol-Ultra-fluid.

Lymphangiography performed during the period of acute lymphedema revealed slight dilatation of main lymphatic trunks. There was additional opacification of small tortuous

vessels in the calf and popliteal region. These vessels have never been visible on normal lymphangiograms. A network of fine lymphatics was seen in the thigh bridging cut ends of femoral lymphatics.

Most unexpected were the lymphangiographic findings in the period of latent lymphedema Although clinically no edema was present at that time lymphangiograms showed typical radiological signs of lymph stasis. During the first 3–4 months a network of dilated tortuous lymphatics was seen in the lower part of the calf and in the thigh below the site of operation. Subsequent lymphangiograms performed at 6–12 months intervals, with the same amount of Lipiodol, revealed continuing dilatation of lymphatics, with destruction of calces, dermal backflow, and retention of Lipiodol in the vessels for several days (Fig. 3).

The difference in lymphangiographic patterns of the latent and chronic stage of lymphedema was only a quantitative one. When edema appeared lymphangiographic patterns consisted of tremendous dilatation of lymphatics, retrograde filling of vessels, valve incompetency, accumulation of contrast medium in the dependent parts of the limb (Fig. 4)

Lymphangiography with aqueous contrast medium.

This technique helped to determine the permeability of main lymph vessels to the highly diffusive low molecular contrast medium. In acute lymphedema contrast medium transsudated almost immediately through the lymphatic wall giving an angora – like lymphangiographic picture (Fig. 5B). In the period of latent lymphedema aqueous contrast medium was retained within the dilated lymph vessels, to transsudate slowly only after several minutes (Fig. 5C). In chronic lymphedema the contrast medium was also retained in lymph vessels, giving a sharp outline of the lymphatic network (Fig. 5D).

Phlebography.

No abnormalities were found on phlebograms. Femoral vein remained patent. There were no radiological sings of venous stasis.

Stereomicroscopic picture of skin lymphatics in transparent tissue specimens.

No special changes could be found, with this technique, in calf skin lymphatics in acute and latent stage of lymphedema. In the stage of chronic lymphedema lymphatics of the skin were markedly dilated, there was herniation of the intima through the separated collagen fibers of the outer layer of the vessel wall, with formation of "varicosities" (Fig. 6A). A dense meshwork of dilated irregular lymphatics could be seen in the skin (Fig. 6B), some of them protruding through the epithelium to form small vesicles on the surface of the skin.

On a transverse section of the thigh the dilated lymph vessels were located not only in the skin but also in the subcutaneous tissue, on the muscular fascia, intermuscular septa, and on the periosteum (Fig. 7).

Histology of skin and lymph collectors.

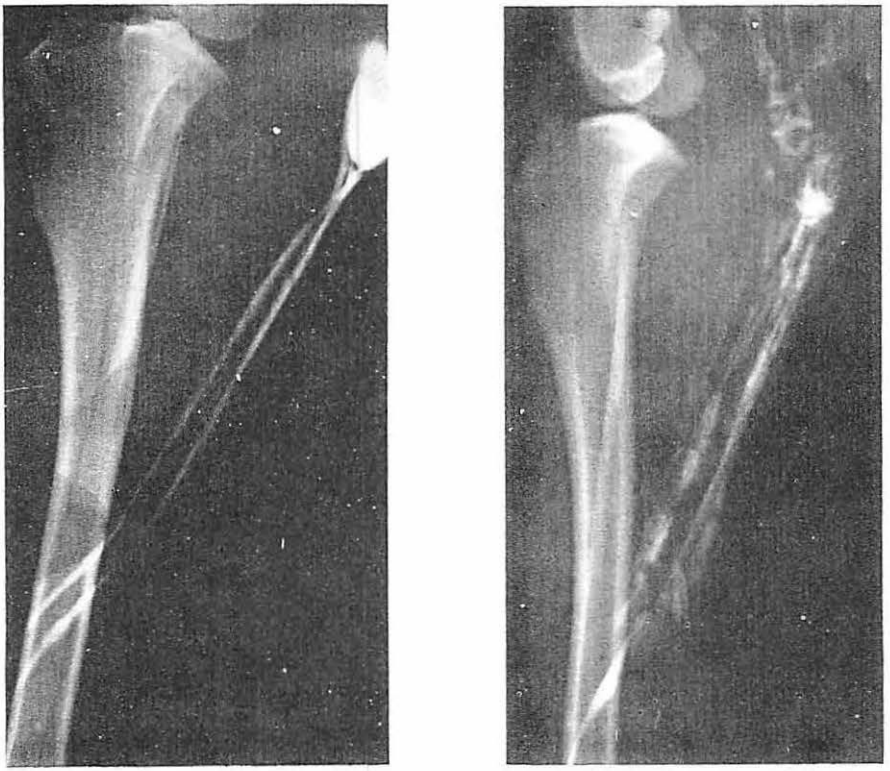
Skin. The period of latent lymphedema was characterized by dilated lymphatics in the subpapillary plexus, swelling of collagen fibers, and their separation by the interstitial fluid (Fig. 8A) Focal accumulations of mononuclear cells could be seen around the vessels.

In chronic lymphedema the typical pattern consisted of numerous lakeshaped channels lined by endothelium (Fig. 8B, C), surrounded by layers of compact collagen bundles, and scattered infiltrates of mononuclear cells around the lymphatics.

Lymph collectors. There was marked dilatation and thinning of the vessel wall in the stage of acute lymphedema. In the latent stage lymphatic walls were becoming thickened, the muscular elements undergoing atrophy, and many new collagen fibers being deposited underneath the intima (Fig. 9B). In chronic lymphedema the wall of lymphatic collectors was entirely without muscular fibers. No boundary between the vessel wall and adjacent tissues could be distinguished (Gif. 9C).

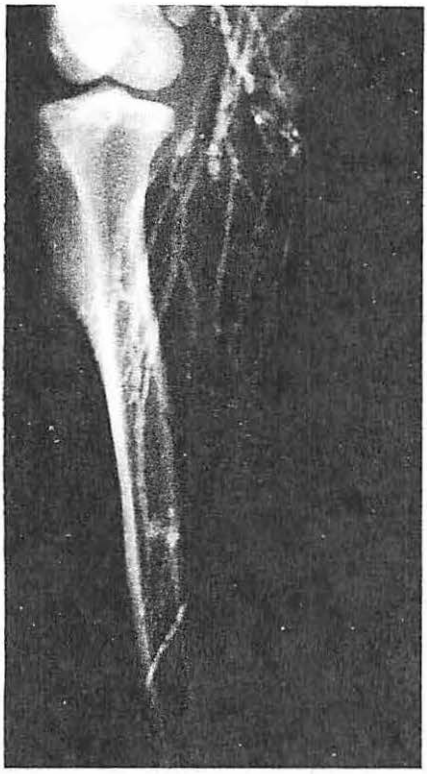
Electronmicroscopy of skin lymphatics.

The most typical changes were observed in dogs with lymphedema of 2 years duration. The interendothelial junctions of lymph capillaries remained permanently open, enabling the to-and-fro fluid movement (Fig. 10A). There was accumulation of proteinaceous fluid underneath the endothelium and also between the collagen fibers (Fig. 10B). Many small cisternae filled with proteinaceous fluid could be seen around the lymph capillaries. The most important finding was a thick basement membrane increasing in thickness and density with duration of lymphedema (Fig. 10C).



A B

Fig. 5 Lymphangiograms carried out with aqueous contrast medium. Note high permeability of the lymphatic wall to the low molecular contrast medium in normal vessels (A), and in acute lymphedema (B), whereas almost no transsudation in latent (C), and chronic lymphedema (D).



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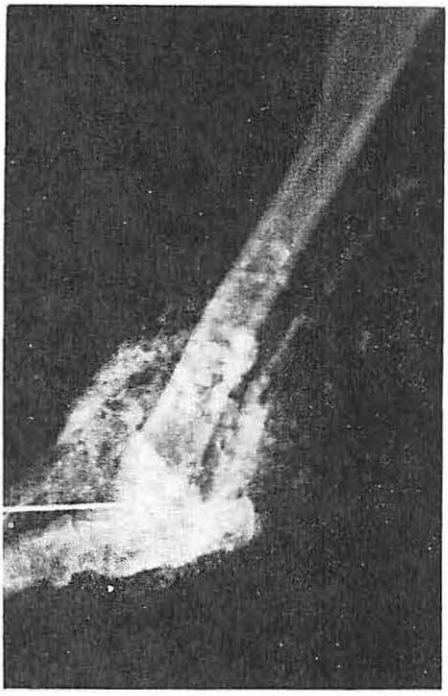

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Lymph and interstitial fluid pressure (Fig. 11, Table 1).

Lymph pressure measured in normal thigh lymphatics in the resting limb ranged from + 1.5 to + 6.5 mmHg, increasing to 30 and above that level during passive movements of the limb.

Table 1 Lymph and interstitial fluid pressure in various stages of lymphedema (6 dogs).

	pressure in mmHg			
	at rest		during passive movements	
	lymph vessel	Guyton's capsule	lymph vessel	Guyton's capsule
Normal	+ 1.5 – + 6.5	– 4.0 – – 8.0	+ 4.0 – + 33.0	– 11.0 – – 30.
Acute lymphedema	0 – + 4.0	– 2.0 – + 4,0	+ 4.0 – + 10.0	– 2.5 – + 2.5
Latent lymphedema	+ 1.5 – + 5.9	– 2.0 – – 3.6	+ 10.0 – + 14.0	– 5.6 – – 7.0
Chronic lymph-edema	+ 1.2 – 4.0	– 0.5 – – 5.0	+ 2.5 – + 10.0	– 1.0 – – 10.0
			fluctuations	

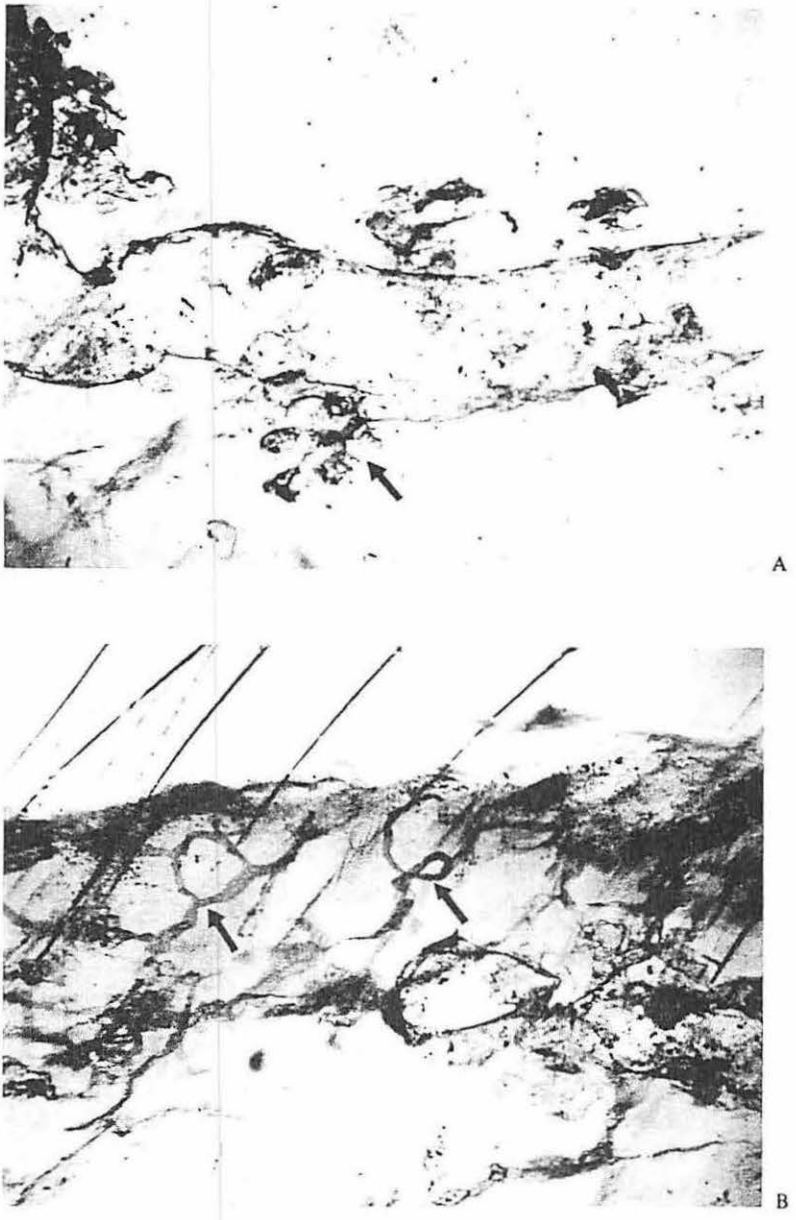


Fig. 6 Stereomicroscopic picture of lymph vessels in a dog with chronic lymphedema. A. subcutaneous vessel with "varicosities" (arrow), B. network of dilated dermal lymphatics (arrows).

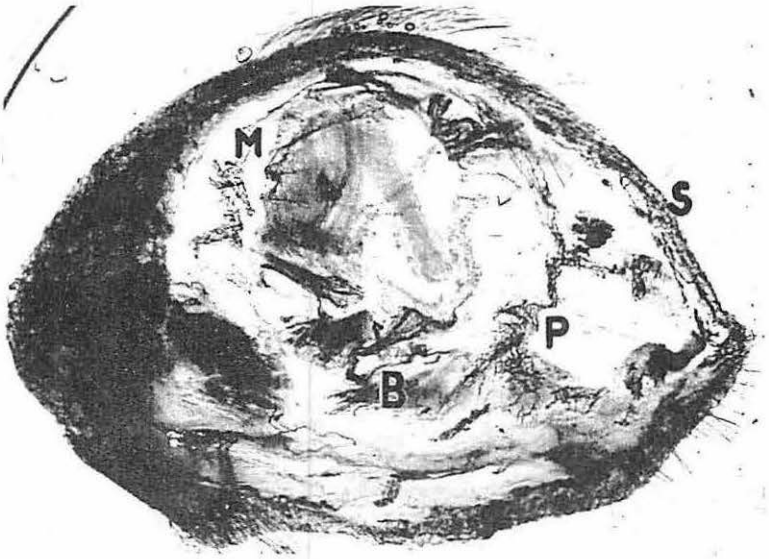


Fig. 7 Stereomicroscopic picture of a transverse section of the calf in a dog with chronic lymphedema. Dilated networks of lymphatics in the skin (S), muscular fascia (M), periosteum (P), and neurovascular bundle (B).

In the stage of acute lymphedema resting lymph pressure did not exceed normal values, whereas during movements it increased only to + 10 mmHg, decreasing rapidly after cessation of movements. This was due to dynamic valve incompetency.

In the stage of latent lymphedema pressure remained within the same limits as in normal limbs.

In the stage of chronic lymphedema resting pressure did not exceed that of normal limb. During a single passive movement of the limb it rose to 2.5 - 10.0 mmHg, to drop immediately after relaxation of the muscles. Major fluctuations of the pressure could be observed (Fig. 11).

The interstitial fluid pressure measured in Guyton's capsules was negative in normal limbs, with the range of – 4 to 7 mmHg. During limb exercise it decreased to as low as, – 30 mmHg. In acute lymphedema interstitial pressure was positive in most cases, but remained at low levels around 4.0 mmHg. During the exercise it did not become negative.

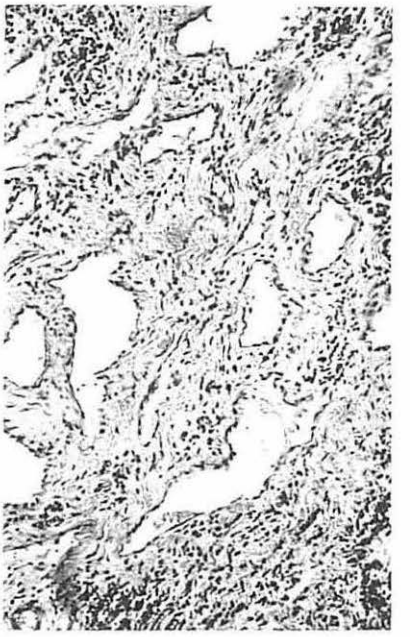
Latent period of lymphedema resembled that of the normal limb.

In the chronically lymphedematous limb resting interstitial fluid pressure was also negative but oscillating around O. During the exercise it had a slight tendency to decrease, but not to such a degree as in normal limb. There were major fluctuations of the interstitial fluid pressure depending on the contraction or relaxation of the limb muscles.

Lymph vessel compliance.

In the normal limb lymph vessel compliance was 0.25 ml/mmHg, in chronically lymphedematous limb it ranged between 2.0 and 3.8 ml/mmHg (Fig. 12).

A



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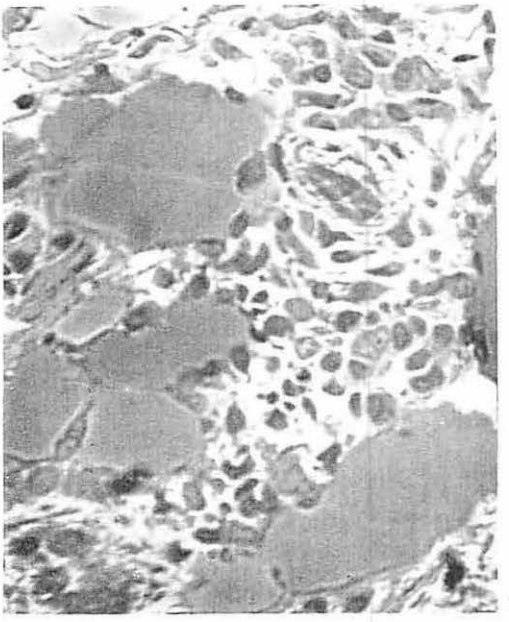
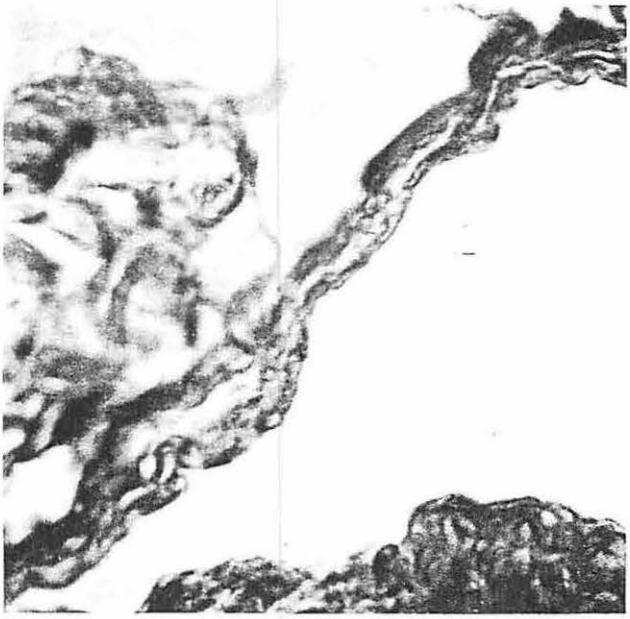


C

Fig. 8 Histological appearance of the skin. A. Latent lymphedema clinically no edema. Bundles of collagen fibers separated by edema fluid, mononuclear cell infiltrates. H.E. x 250. B. Chronic lymphedema of 2 years duration. Fibrosis of the skin, irregular shape, new lymphatic channels, mononuclear cell infiltrates H.E. x 100. C. Chronic lymphedema of 4 years duration. Lymphatic capillaries form multiple, dilated intercommunicating spaces H.E. x 600.



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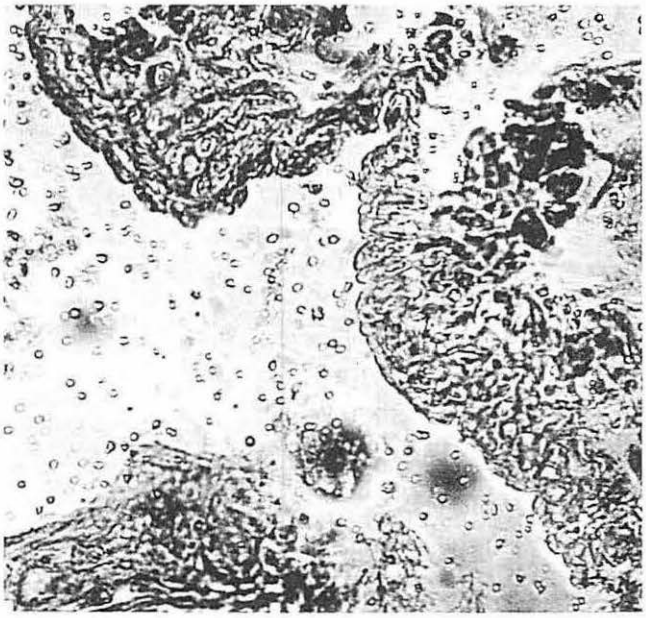


B

Fig. 9 Histological appearance of a lymphatic collector. A. Normal vessel H.E. x 250, B. Latent lymphedema. Clinically no edema, but evident thickening of the vessel wall, H.E. x 250, C. Chronic lymphedema. Vessel wall entirely fibrotic. No boundary between the vessel wall and surrounding tissue, H.E. x 150

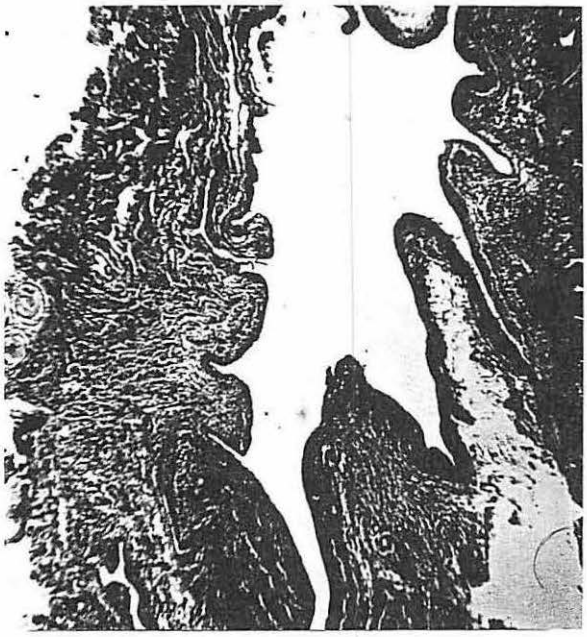


Fig. 9 C

Lymph protein concentration.

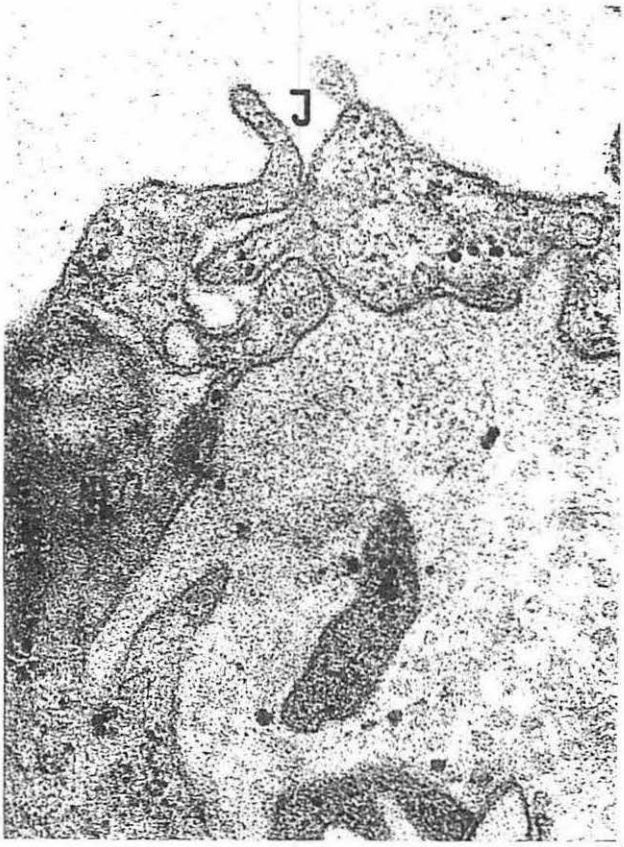
Protein concentration in the lymph collected from calf lymph vessels (Table 2) was almost twice as high as that of the normal limb and amounted to 2.26 g/100 ml in the stage of chronic lymphedema. Paper electrophoresis did not reveal any special differences in albumin and globulin concentration between the serum, normal, and stagnant lymph.

Lymph coagulation and fibrinolysis.

The concentration of lymph coagulation factors corresponded to the lymph protein level, irrespective of the stage of lymphedema. Only fibrinogen level remained at a lower level (Table 3).

Table 2 Total protein in the lymph in various stages of lymphedema.

Stage of lymphedema	No. of cases	Total protein g/100 ml	S.D.
Normal	10	1.46	±0.54
Acute	8	2.26	±0.79
Latent	5	2.04	±0.37
Chronic	10	1.35	±0.69
Serum	10	6.91	±2.69



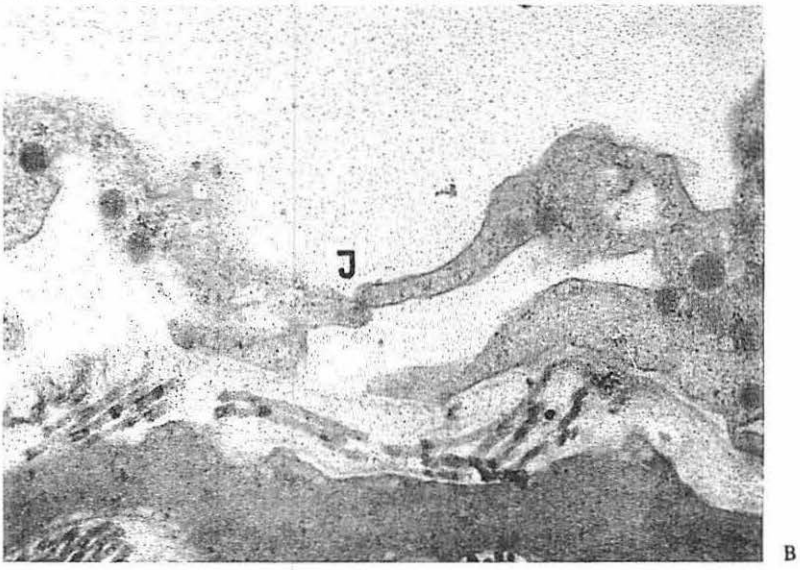
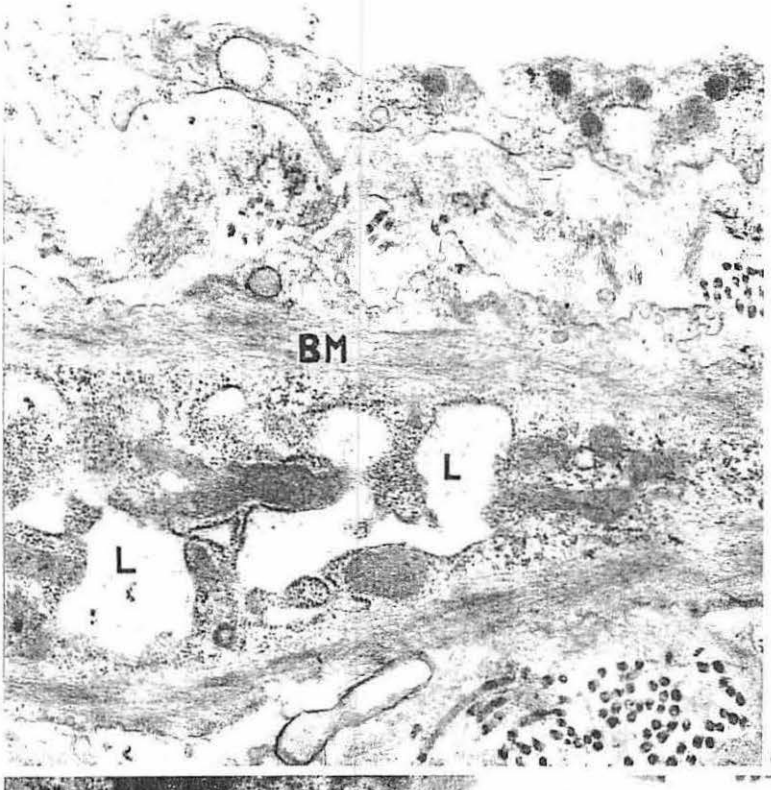


Fig. 10 Electron micrographs of skin lymph capillaries in chronic lymphedema. A. Permanently open interendothelial junctions (J), x .30000. B. Accumulation of fluid underneath the endothelium. Note the same density of proteinaceous fluid outside and inside the capillary, x.40000. C. Thick basement membrane like structure (BM) which may be a local accumulation of protein in the ground substance gel. Small lake (L) of proteinaceous fluid around the lymphatic capillary, x.22000. D. Skin fibroblasts surrounded by interstitial fluid. Note wide endoplasmic reticulum channels, probably active formation of collagen, x.30000.



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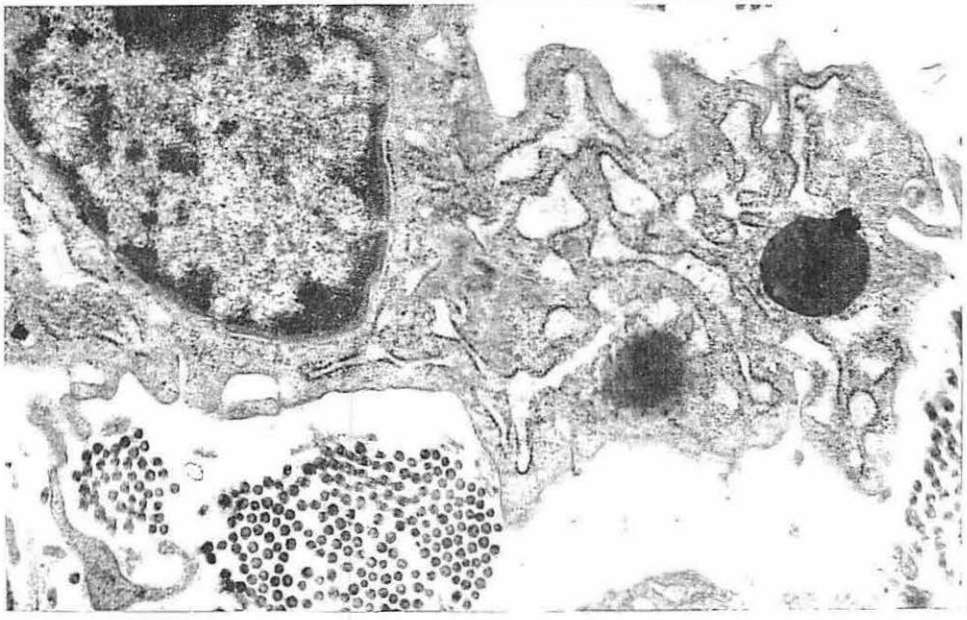

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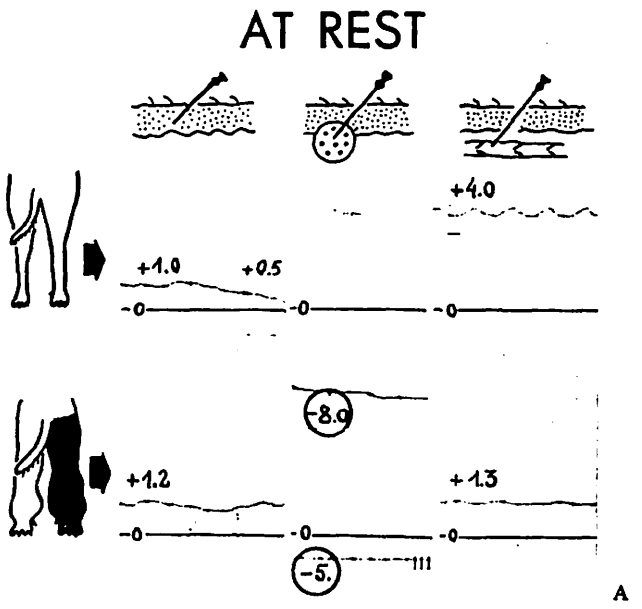


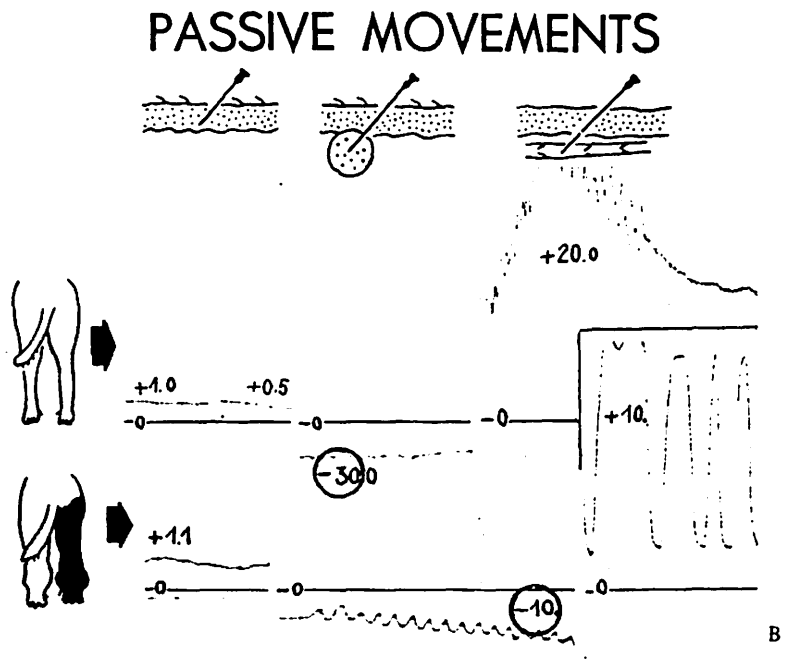


Fig. 11 Interstitial fluid (microdroplet, Guyton's capsule techniques) and intralymphatic pressure in normal and chronicalyy lymphedematous limb, at rest and during limb exercise.

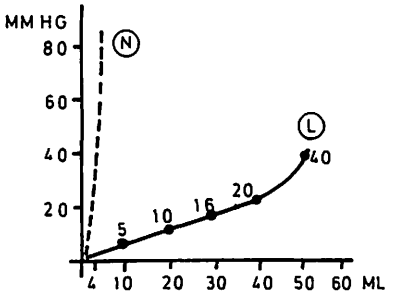


Fig. 12 Pressure-volume relation of lymphatic vessels in a normal (N) and chronically lymphedematous (L) limb of a dog.

Table 3 Coagulation and fibrinolysis in the lymph obtained from the calf lymphatics in the stage of chronic lymphedema (mean of 10 dogs).

	Lymph	Percent of plasma values	Plasma
Calcium clotting time	124"	60	40 – 60"
Prothrombin time	25"	30	8"
Thrombin time	50"		10"
Factor V	36"	24	15"
Factor VII	49"	27	18"
Fibrinogen mg/100 ml	51	10	300 – 500
Plasminogen index	0.15		1.0
Totai protein g/100 ml	1.7	28	5.6

Discussion

The purpose for the study was to follow the natural history of experimental postsurgical lymphedema and to answer several clinically important questions, as: a. what morphological changes develop in lymph collectors, capillaries, and interstitial space structures, b. is there any correlation between the intensity of edema and the extent of lymphangiographic changes, c. is there permanent hypertension in the lymph vessels and interstitial space, d. why does the postsurgical lymphedema not develop until several months to years after excision of lymph vessels and nodes?

The first step in our studies was to find a reproducible experimental method for production of chronic lymphedema, imitating postsurgical lymphedema in man. The method of *Drinker* (5) did not suit for our purposes as lymphedema produced by that author was primarily of an inflammatory and not obstructive type, and had no counterpart in clincial conditions. This prompted us to try our own method based on simple surgical interruption of all lymphatics of the limb (8, 9). A similar method was described in 1967 by *Danese* (3,4).

The first important finding was that simple mechanical interruption of lymph vessels of the limb was sufficient for the development of chronic lymphedema. But a period of

minimum 7–8 months was necessary for that process, in some cases even of several years.

The second finding was that marked lymphangiographic changes preceded the outbreak of lymphedema. After the operation slight transient edema could be seen in all animals. It subsided completely in 4–6 weeks time. In the following months or even years there was no edema of the limb. Strangely enough, major morphological and lymphangiographic alterations continued to proceed in the limb. Lymphangiograms performed at 6 months intervals revealed growing lymph stasis, despite of abscence of edema.

The third important finding was that lymph collectors became fibrotic and consequently have lost their normal permeability, what was proved by lymphangiograms with aqueous contrast medium.

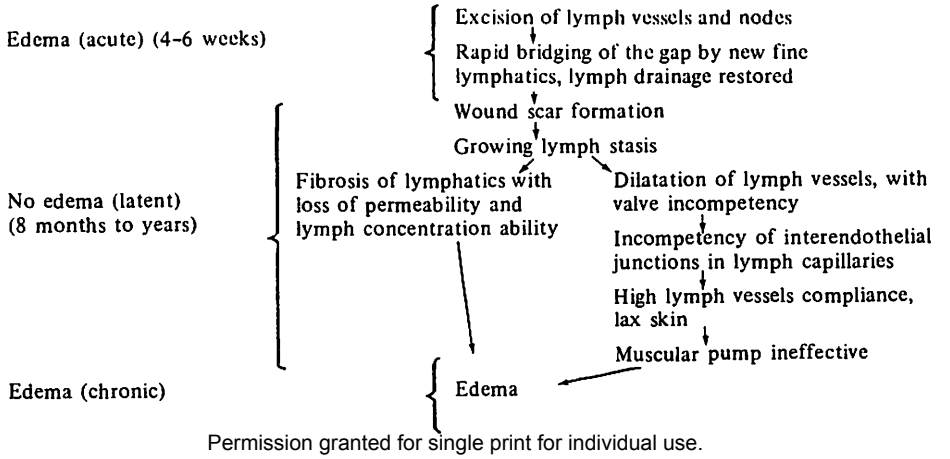
Electron micrographs of skinlymph capillaries revealed permanent incompetency of interendothelial junctions what has made the interstitial space and lymph capillaries and vessels one common freely intercommunicating space (1, 2). The mechanism of thickening of the basement membrane remains unclear.

Another unexpected observation was that there was no lymph hypertension in limbs with chronic lymphedema. Resting pressures in the limbs remained within normal limits. During limb movements pressures did not increase so much as in normal limbs, what might be explained by valve incompetency and high lymph vessel compliance.

Interstitial fluid pressures remained in chronic lymphedema slightly negative, but very close to zero. They decreased only slightly during the limb activity. This has been an additional evidence of valve and interendothelial junctions incompetency. High compliance of lymph vessels together with valve incompetency and permanent patency of interendothelial junctions have made the muscular pump ineffective in forcing the interstitial fluid, unidirectionally, from the interstitial space into the lymph capillaries, and propelling the lymph along the lymph vessels.

Low protein concentration of the lymph in chronically edematous limb, similar to that of the interstitial fluid, was probably caused by mixing of interstitial fluid and lymph due to permanent patency of interendothelial junctions, and also by loss of protein concentration ability in the fibrotic impermeable lymphatics (7, 11).

The most difficult and challenging question to answer was, why does postsurgical lymphedema develop only months or years after excision of lymph vessels and nodes? This we try to explain, basing on own observations, listing chronologically the events which take place in the limb after excision of lymphatics:



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BOOK REVIEW

Erkrankungen des Lymphsystems – Grundlagen, Diagnostik, Therapie
1971, 250 pp. 83 ill. DM 84,– (Baden-Baden, Brüssel: Gerhard Witzstrock)

This book is the third monograph on lymphatics, its disorders and diseases by Professor *Földi*. It summarises the up to date knowledge and experience of a quarter of a century of original clinical and experimental studies and advances by this world wide known pioneer.

The first chapters are devoted to the anatomy, physiology and pathology of the lymphatic system. New and original aspects are displayed and the many relationships of this universal circulatory organ are described, from clinical forms to the level of the electron microscope.

In a second part, the diagnostic procedures (radiological, isotopes, biopsies, pharmacodiagnostics), for lymphvessels and for lymphnodes, are demonstrated by *M. Collard*, including indications, contraindications, results, complications.