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Investigations into the Lymphatic Vessels and their Valves in the Fore-limb of the Pig

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Summary

Measurements were carried out on the lymphatics of the fore-limb of the pig, the values recorded in tabular form and further classified. In this way lymph transport was considered, particularly through the lymphokinetic action of a drug, and by using patent blue violet.

Although nowadays it is usually claimed that the anatomical, physiological and patho-physiological conditions of the lymphatic system are quite well-known, it is still worth mentioning that few investigations have been made into the pharmacology and the pharmacotherapy (7). Thus, research into the pharmacodynamic process in the lymphatics, represents an important task for the solution of diagnostic and therapeutic problems (20).

In the course of this investigation into the question of the lymphokinetic effect of a drug (Venalot) on transport in the lymph vessels, and by using a dye, it proved possible to make definite statements about functional changes in the valves, which are an important element in the lymphatic system.

Literature

The lymphatics extend throughout the whole body as a second system of tubes which runs along side of the blood vessels. They contain the lymph, a fluid which is subject to quite wide fluctuations in its constitution. Thus, the content of the lymphatics in the extremities quite closely resembles blood plasma, although it is more watery and has a lower protein content. This is in complete contrast to the intestinal lymphatics which, with the digestion of resorbed fat, contain a turbid, milky emulsion, the chyle (30). In addition, it is possible to detect so-called macro-corpuscular elements, which are in the micron range and are designated by *Volkheimer* in the literature by the term persorption (10, 11, 12, 13, 15, 47, 48, 49, 50, 51, 52).

In spite of the anatomical relationship between the lymphatics and the venous system they have a different function, which is of particular medical interest. This is, that with the lymph in the lymphatic pathways pathogenic agents, toxins and carcinoma cells can be trapped by the lymph nodes which are placed in between. The difference also consists in that they contain particles of various molecular sizes, which have been removed from the tissues and transported there (30). Also, in acute and chronic infections in an organ, the lymphatics react by going into spasm, which is transmitted up to the next group of lymph nodes and can be transmitted to further lymphatics which are coming to this group of lymph nodes, from other organs and tissues. Because of this a reduction in the lymph drainage results and the sequel is disturbances of cellular metabolism, which are ultimately designated as a remote lymphogenic effect (31). This inflammatory oedema develops as a result of combined reactions and interactions of the terminal blood and lymph capillaries (25). In general the lymphatic system can be regarded as a particularly important member of the defensive system (reticuloendothelial system) of the body (39).

Material and methods

The focal point of this research has been the investigation of lymph transport with, and without, Venalot[1], in conjunction with a 2.5 % solution of patent blue violet[2]. This has definitely shown that, on average, lymph transport is 2.490 : 1 more rapid with Venalot. In addition, measurements have been undertaken on the lymph vessels which have yielded results about their valves as described below, and thus distinguish the concept of the lymphangions.

We investigated 100 pigs' forelimbs, of which 27 left (lt) and 23 right (rt) were injected with Venalot, and 22 left (lt) and 28 right (rt) had no injection of Venalot. The individual forelimbs varied in length from 19.60 to 34.40 cm, the distance being measured proximally from the point of injection of the patent blue violet in the 3/4 interdigital cleft. In one group (Tab. 1) an injection of one ampule of Venalot (2 ml) was given into the median or anterior common interosseous artery at the proximal cut end, and the effect was assessed during the next ten minutes. In the other group (Tab. 2) no injection of Venalot was given. In order to obtain further information about the time of lymphatic transport, patent blue violet was injected into all the limbs in the interdigital cleft between the third and fourth digits. The time at the start of the injection was noted and also when the dye appeared in the lymphatic vessels at the proximal end of the fore-limb. This distance was measured in centimetres and later converted, in order to given comparable values. The lymphatics which had been outlined by the patent blue violet were almost always visible through the skin of the limb and to complete the investigation a strip of skin about 10 cm wide, in their vicinity, was removed over the whole length. This skin was afterwards fixed, histologically processed and finally cleared in oil of wintergreen (40). Patent blue violet was chosen for this investigation, because as is well-known it has an affinity for the lymphatic system (6, 9, 46).

[1] Venalot is a coumarin (5,6-Benzo-a-pyrone)-rutin derivative. Manufacturer: Schaper & Brümmer, Salzgitter-Ringelheim

[2] Manufacturer: Byk-Gulden, Constance

Results

The investigations undertaken here (Tab. 1, 2, 3) were carried out on specimens cleared in oil of wintergreen. The following values were measured and calculated; the total length in mm of the section of lymphatic comprising ten lymphangions, length of a lymphangion in mm, greatest and smallest length of a lymphangion in mm, range of variation in the length of the lymphangions in mm, arithmetic mean of the greatest and smallest length of a lymphangion in mm and the diameter in the middle of a lymphangion in mm. All these values were recorded in tables and can thus be compared with each other, so that in particular (with the help of patent blue violet) one can consider lymph transport with Venalot (Tab. 1) and without Venalot (Tab. 2). The total figures in these two groups show that apart from the already mentioned undoubted accelerated lymph flow resulting from the administration of Venalot, there is approximately a one third increase in the diameter of the lymphangion, corresponding to the diameters of 0.697 mm as against 0.523 mm. This has also contributed to the increase in the flow of lymph, as has already been shown in the mesojejunum (14).

Besides the actual measurements on the lymphatics of the fore-limb, further observations can also be made here on the experimental confirmation of the inherent automatism of the lymphatics, which has already been confirmed by other investigations (14). Isolated pieces of skin injected with patent blue violet, as well as completely isolated lymph vessels, were investigated at room temperature (20 °C). The segment of vessel consisted of about ten lymphangions. Each individual lymphangion contains a cuff of muscle, which is markedly reduced in the vicinity of the valves. The actual region of the valve is completely free of muscle.

It was possible to infer from these observations, that within the individual lymphangions there was, on the whole, an irregularly pulsating lymph vessel with a frequency between 15 and 20/min. Even *Kubik* (21) failed to find any regular rhythmic flow in the entire lymphatic system. *Leonhardt* (24) reported contraction waves of 10–12/min. After the injection of Venalot a much more rapid pulsation was seen

Table 1 Measurements in the fore-limb of the pig (with administration of Venalot)

Number	Record number	Section of lymph vessel in mm	Length of a lymph-angion in mm	Greatest length of a lymph-angion in mm	Smallest length of a lymph-angion in mm	Range of variation in length of a lymph-angion in mm	Mean of the greatest and smallest length of a lymph-angion in mm	Diameter of a lymph-angion in mm
1	1 lt	12.846	1.285	1.423	1.154	0.269	1.289	0.654
2	2 lt	11.538	1.154	1.231	1.077	0.154	1.154	0.692
3	3 rt	11.769	1.177	1.308	1.038	0.270	1.173	0.692
4	4 rt	11.692	1.169	1.269	1.077	0.192	1.173	0.769
5	11 lt	11.115	1.112	1.269	0.962	0.307	1.116	0.654
6	12 lt	12.462	1.246	1.423	1.115	0.308	1.269	0.769
7	14 rt	11.885	1.189	1.308	1.077	0.231	1.193	0.615
8	15 rt	11.654	1.165	1.269	1.038	0.231	1.154	0.654
9	21 rt	11.654	1.165	1.308	1.115	0.193	1.212	0.654
10	22 lt	12.654	1.265	1.385	1.154	0.231	1.270	0.769
11	23 rt	11.885	1.189	1.308	1.077	0.231	1.193	0.731
12	24 lt	11.769	1.177	1.269	1.115	0.154	1.192	0.692
13	31 lt	12.462	1.246	1.385	1.115	0.270	1.250	0.692
14	33 lt	10.000	1.000	1.269	0.654	0.615	0.962	0.654
15	34 rt	11.769	1.177	1.308	1.077	0.231	1.193	0.731
16	35 lt	10.115	1.012	1.115	0.885	0.230	1.000	0.692
17	36 lt	10.923	1.092	1.192	1.000	0.192	1.096	0.654
18	37 lt	10.192	1.019	1.154	0.885	0.269	1.020	0.654
19	38 lt	10.346	1.035	1.154	0.885	0.269	1.020	0.654
20	40 rt	11.962	1.196	1.269	1.115	0.154	1.192	0.769
21	51 rt	8.538	0.854	1.077	0.692	0.385	0.885	0.654
22	52 rt	11.192	1.119	1.231	0.923	0.308	1.077	0.769
23	53 rt	8.769	0.877	1.077	0.731	0.346	0.904	0.654
24	55 rt	8.808	0.881	1.038	0.731	0.307	0.885	0.615
25	56 rt	7.923	0.792	0.962	0.654	0.308	0.808	0.692
26	67 lt	11.077	1.108	1.192	0.962	0.230	1.077	0.692
27	68 rt	13.962	1.396	1.500	1.231	0.269	1.366	0.769
28	69 lt	12.500	1.250	1.423	1.115	0.308	1.269	0.692
29	70 lt	10.000	1.000	1.115	0.885	0.230	1.000	0.692
30	71 rt	10.615	1.062	1.154	0.885	0.269	1.020	0.692
31	72 lt	12.154	1.215	1.346	1.115	0.231	1.231	0.769
32	74 rt	12.038	1.204	1.346	1.115	0.231	1.231	0.654
33	76 rt	11.500	1.150	1.231	0.962	0.269	1.097	0.692
34	87 lt	13.692	1.369	1.462	1.269	0.193	1.366	0.615
35	89 lt	12.500	1.250	1.385	1.115	0.270	1.250	0.731
36	90 lt	13.192	1.319	1.462	1.192	0.270	1.327	0.577
37	91 lt	12.038	1.204	1.346	1.077	0.269	1.212	0.731
38	92 rt	12.769	1.277	1.462	1.154	0.308	1.308	0.769
39	93 lt	13.077	1.308	1.462	1.154	0.308	1.308	0.692
40	94 rt	10.308	1.031	1.154	0.923	0.231	1.039	0.654
41	95 rt	12.885	1.289	1.462	1.154	0.308	1.308	0.731
42	96 rt	11.885	1.189	1.346	1.077	0.269	1.212	0.654
43	97 rt	12.885	1.289	1.462	1.154	0.308	1.308	0.769
44	103 lt	12.962	1.296	1.423	1.154	0.269	1.289	0.769
45	104 lt	12.231	1.223	1.423	1.115	0.308	1.269	0.692
46	105 rt	11.423	1.142	1.231	1.038	0.193	1.135	0.769
47	106 lt	13.038	1.304	1.462	1.154	0.308	1.308	0.769
48	107 lt	11.269	1.127	1.269	1.038	0.231	1.154	0.654
49	108 lt	11.385	1.139	1.269	1.000	0.269	1.135	0.731
50	109 lt	10.808	1.081	1.192	0.962	0.230	1.077	0.654
Total		578.115	57.815	64.580	51.346	13.234	57.976	34.842
Arithmetic mean		11.562	1.156	1.292	1.027	0.265	1.160	0.697

Table 2 Measurements in the fore-limb of the pig (no administration of Venalot)

Number	Record number	Section of lymph vessel in mm	Length of a lymph-angion in mm	Greatest length of a lymph-angion in mm	Smallest length of a lymph-angion in mm	Range of variation in length of a lymph-angion in mm	Mean of the greatest and smallest length of a lymph-angion in mm	Diameter of a lymph-angion in mm
1	6 rt	12.808	1.281	1.462	1.154	0.308	1.308	0.577
2	10 lt	10.654	1.065	1.154	0.962	0.192	1.058	0.615
3	16 rt	10.846	1.085	1.192	0.962	0.230	1.077	0.538
4	17 lt	11.500	1.150	1.269	0.962	0.307	1.116	0.577
5	18 lt	11.462	1.146	1.231	1.038	0.193	1.135	0.615
6	19 lt	11.346	1.135	1.269	1.038	0.231	1.154	0.538
7	20 rt	12.423	1.242	1.385	1.115	0.270	1.250	0.538
8	26 lt	10.692	1.069	1.154	0.923	0.231	1.039	0.538
9	27 lt	11.192	1.119	1.231	0.962	0.269	1.097	0.577
10	28 lt	11.077	1.108	1.231	1.000	0.231	1.116	0.538
11	29 rt	11.577	1.158	1.308	1.038	0.270	1.173	0.500
12	30 lt	12.808	1.281	1.423	1.154	0.269	1.289	0.615
13	41 lt	9.923	0.992	1.192	0.731	0.461	0.962	0.577
14	42 rt	14.538	1.454	1.808	0.923	0.885	1.366	0.615
15	43 rt	13.808	1.381	1.500	1.231	0.269	1.366	0.577
16	44 rt	11.538	1.154	1.231	0.923	0.308	1.077	0.500
17	45 lt	8.269	0.827	1.000	0.654	0.346	0.827	0.577
18	46 lt	12.808	1.281	1.462	1.115	0.347	1.289	0.500
19	47 rt	11.500	1.150	1.269	1.000	0.269	1.135	0.538
20	48 rt	11.385	1.139	1.269	0.962	0.307	1.116	0.500
21	49 lt	12.385	1.239	1.423	1.115	0.308	1.269	0.615
22	57 lt	9.538	0.954	1.192	0.731	0.461	0.962	0.423
23	58 rt	11.769	1.177	1.308	1.038	0.270	1.173	0.538
24	59 lt	9.769	0.977	1.192	0.731	0.461	0.962	0.500
25	60 rt	9.577	0.958	1.154	0.731	0.423	0.943	0.462
26	61 lt	12.538	1.254	1.423	1.077	0.346	1.250	0.577
27	62 rt	10.308	1.031	1.462	0.692	0.770	1.077	0.538
28	63 rt	9.154	0.915	1.115	0.731	0.384	0.923	0.423
29	64 rt	10.500	1.050	1.308	0.846	0.462	1.077	0.462
30	65 lt	11.154	1.115	1.269	0.962	0.307	1.116	0.385
31	66 rt	11.923	1.192	1.423	1.115	0.308	1.269	0.615
32	77 lt	12.846	1.285	1.423	1.154	0.269	1.289	0.500
33	78 rt	11.808	1.181	1.462	0.962	0.500	1.212	0.500
34	79 rt	12.115	1.212	1.346	1.038	0.308	1.192	0.423
35	80 lt	12.000	1.200	1.346	1.077	0.269	1.212	0.577
36	81 rt	11.462	1.146	1.231	1.000	0.231	1.116	0.577
37	82 rt	11.615	1.162	1.346	1.038	0.308	1.192	0.462
38	83 rt	11.154	1.115	1.231	0.962	0.269	1.097	0.385
39	84 lt	11.500	1.150	1.269	1.000	0.269	1.135	0.538
40	85 rt	11.308	1.131	1.231	1.038	0.193	1.135	0.500
41	86 rt	12.500	1.250	1.385	1.115	0.270	1.250	0.462
42	88 rt	12.923	1.292	1.462	1.154	0.308	1.308	0.538
43	98 rt	11.000	1.100	1.308	0.962	0.346	1.135	0.500
44	99 rt	11.154	1.115	1.308	0.962	0.346	1.135	0.500
45	100 lt	10.923	1.092	1.192	1.000	0.192	1.096	0.423
46	101 rt	10.500	1.050	1.154	0.923	0.231	1.039	0.615
47	102 rt	11.115	1.112	1.269	1.000	0.269	1.135	0.538
48	112 rt	9.615	0.962	1.077	0.846	0.231	0.962	0.346
49	113 lt	11.000	1.100	1.192	1.038	0.154	1.115	0.577
50	114 lt	11.077	1.108	1.231	1.000	0.231	1.116	0.500
Total		568.384	56.842	64.772	48.885	15.887	56.942	26.149
Arithmetic mean		11.368	1.137	1.295	0.978	0.318	1.139	0.523

Table 3 Total measurement in the left and right fore-limb of the pig, in Table 1 (given Venalot) and Table 2 (not given Venalot)

Total measurement of the length of a lymphangion in mm from 27 left (lt) fore-limbs, with administration of Venalot	31.836	
Arithmetic mean of the length of a lymphangion in mm from 27 left fore-limbs, with administration of Venalot		1.179
Total measurement of the length of a lymphangion in mm from 23 right (rt) fore-limbs, with administration of Venalot	25.978	
Arithmetic mean of the length of a lymphangion in mm from 23 right fore-limbs, with administration of Venalot		1.129
Total measurement of the length of a lymphangion in mm from 22 left (lt) fore-limbs, not given Venalot	24.647	
Arithmetic mean of the length of a lymphangion in mm from 22 left fore-limbs, not given Venalot		1.120
Total measurement of the length of a lymphangion in mm from 28 right (rt) fore-limbs, not given Venalot	32.195	
Arithmetic mean of the length of a lymphangion in mm from 28 right fore-limbs, not given Venalot		1.150
Total	114.656 (: 100 =	1.147)

with a frequency of 30/min. with a dilation of the lymphatics and a consequent increase in the lymph flow. Under similar experimental conditions, but with preparations stored in the refrigerator for up to five days at 4 °C, after 4–5 hours at room temperature, active pulsation of the individual lymphangions occurred after injection of patent blue violet. After moistening the specimen with Venalot before the injection of the dye, an active pulse could be observed after only three hours, with removal of the dyestuff. Similar findings have already been reported by *Fabian* (14) in the mesojejunum of the cat. It should also be mentioned here that this drug has a three-fold myotropic effect, so that the pulse rate, the size of the vessels and also the tone of the lymphatics are all considerably increased (28). Also in these observations which always involved ten lymphangions with eleven valves from each fore-limb, bicuspid valves definitely predominated.

In the complete review of all the valves within the lengths of lymphatics, which comprised a total number of 1100 lymphatic valves, only three valves with four cusps could be found, in three different fore-limbs (Fig.-). The valves could only be demonstrated successfully after clearing in oil of wintergreen. However, even with careful preparation in this fluid medium, it is extremely difficult, and with the four-cusped valves impossible, to take a photograph from the outside to show the state of affairs present inside.

Discussion

The lymphatics are described as a drainage system of the connective tissue (24), in which the lymph pathways are divided into three sectors, the lymphatic capillaries, the connecting vessels and the transporting vessels. According to *Mislin* (29) there are very thin-walled lymph vessels which lead away from so-called lymphatic capillary network, and any retrograde flow from these is prevented by valves which are either infundibular or semilunar (pocket-like). The actual lymphatic vessel is divided up into numerous intervalvular segments, whose valves appear at regular intervals as folds or duplications of the endothelium. On account of this segmentation the lymph vessel has a beaded appearance. Each intervalvular section (valve segment) is called by *Mislin* (26, 27, 29) a lymphangion. From this he deduces that there are two types of lymphatics, firstly those vessels where extramural forces play the main part

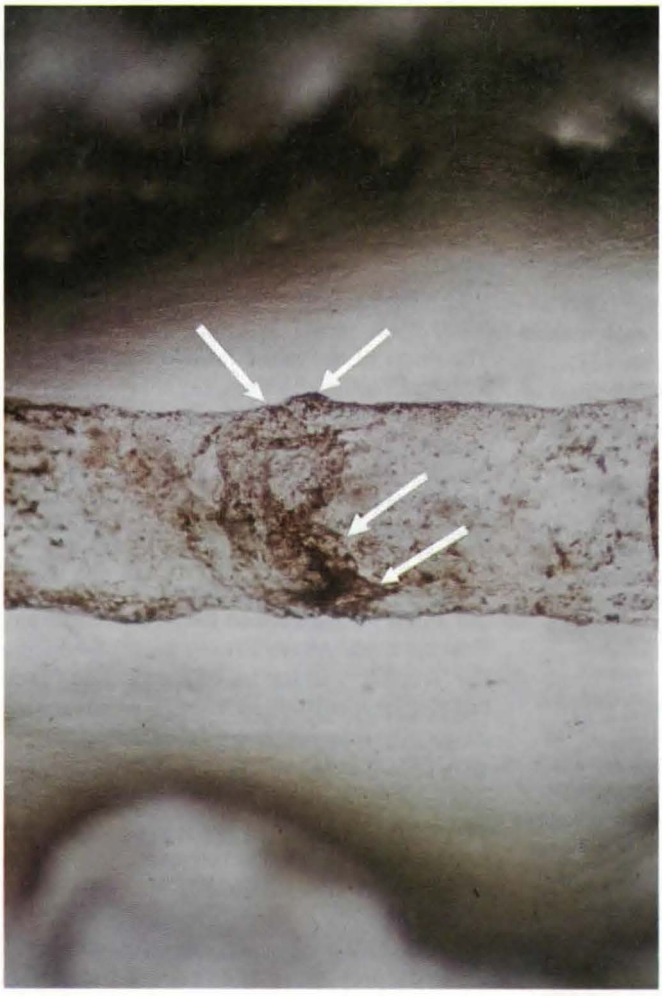

Figure: Valve with four cusps (the arrow indicates the individual four flaps) demonstrated by late injection of the lymphatic vessels in the fore-limb of the pig, with Japanese ink. Cleared in oil of wintergreen. Scale of illustration: 51 : 1

in lymph transport, which are therefore purely conducting vessels and secondly, those which show spontaneous rhythmic contractions and can be regarded as specialized transport vessels.

As regards the length of a lymphangion in relation to the left or the rhigt fore-limb, no opinion can be expressed, only insofar as no difference was noted. The pig's fore-limbs came from animals aged about five months. In this way there was a uniformity of the material, in contrast with man, where this leads to great difficulties (4). As regards the statement about the fore-limbs treated with Venalot, the calculation of the arithmetic mean shows there is only a very minimal rise to record. The calculated arithmetic mean of all 100 measurements of the length of a lymphangion amounted to 1.147 mm and lay within the tolerance of the figures of *Delamare* (8) and *Fabian* (9).

Some attempt at an interpretation of the other figures may be attempted, although on the basis of the measurements and the subsequent conditional conversions, these merely provide some insight into the variability of such investigations.

As regards the flow of lymph in the lymphatics, this in ensured by several factors. Among these, *Watzka* (55) has mentioned (1) the vis a tergo from the continual onward flow, (2) pressure from the immediate surroundlings, whereby the numerous valves only allow a forward flow, (3) the contraction of the vessel wall and (4) the suction action from the main vessels where they drain into the venous system.

The musculature of the lymph segments has a very rich nerve supply and as a rule each muscle fibre is supplied by a single nerve fibre (28). *Kubik and Szabó* (22) found sensory endings of the *Vater-Pacinian* type, adjacent to and closely surrounding the lymphatics.

The lymph capillaries are not considered here. According to the literature they are not seen in routine sections because of the collapse of their walls (3) but they are familiar to pathologists in other situations, through the filling of their lumen with masses of proliferating carcinoma cells (1).

However, by retrograde injection with Japanese ink, using the lower limb of the rat, it was possible to show that lymph capillaries in the subcutaneous layer can fill via their so-called blind ends (or blind origins) which have a calibre of 6 μ – 1 μ (9), and consequently this appears to involve a "cisterna initialis" which is connected with all the precisternal capillary lymph vessels or precapillary canaliculi (36). In the same way, the adjoining proximal section of capillary would then be termed a post-cisternal lymphatic capillary. These "dilated blind ends" were also described in the skin and mucous membranes by *Rauber* and *Kopsch* (37). As in other parts of the body, there are superficial and deep lymphatics in the fore-limb (3, 58) both of which carry the dye proximally, after an injection of patent blue violet into the distal part of the limb. However, in this investigation we are only concerned with the lymphatics in the subcutaneous tissues.

Similarly, in the literature there is general agreement that, in contrast to the lymph capillaries, the lymphatics are similar in structure to the veins (5, 53, 54) and are equipped with valves (1, 3, 8, 17, 18, 19, 23, 24, 29, 38, 41, 42, 43, 44, 45, 54, 55, 57). However, in the skin on the dorsum of the human foot valves can occasionally be detected, in pathologically dilated lymph capillaries.

These are merely endothelial duplications but are mostly adequate (34, 35). In the small post-capillary lymphatics, the valves consist solely of endothelium, which often has a uniform thin connective tissue membrane between the two layers of endothelium. In the larger lymphatics the valves have several layers or contain elastic fibres (41).

The shape of the valves varies enormously. Besides "sail"-shaped valves, funnel (infundibular) valves are found (1). Other authors speak about pocket-valves (9, 24, 43, 44), and also about "sail"-valves (45). In general the valves in the lymphatics are bicuspid (5, 9, 16, 17, 32, 33). Occasionally only a single flap was described (19, 41), a finding also confirmed in the present investigation. This flap is usually very drawn out and is folded back in the middle of its length. Tricuspid valves have already been described in the lymphatics in the leg of the chicken (9) and can also be identified here, at all events more frequently than just one valve (flap).

With regard to the distance between the successive valves in the lymphatics it may be noted that there are definitely more valves (57) and they succeed each other in greater numbers than in veins of comparable size (1, 56). This came out very clearly in these investigations where the intervalvular distance veins: lymphatics showed a proportion of 1:2 or even more. The valves in the lymphatics succeed each other at more or less regular intervals, but it should be mentioned here that according to *Bartels* (2) the valves are more infrequent or even absent in the lymphatics of the organs, while outside the organs these relationships are however, reversed. Figures are given by *Sappey* (42) who counted 60 – 80 valves in one lymphatic vessel along the whole length of the human upper limb, and in the lower limb 80 – 100, approximately one valve per cm. For comparison, figures are also given by *Delamare* (8) who found in very small vessels that the valves were at intervals of 2 – 3 mm, but in larger vessels the distance increased to 6 – 12 mm. In the upper and lower limbs of the chicken the distance between two valves amounted to 1.23 – 3.22 mm (9).

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ANNOUNCEMENTS

Xeroradiography of the Lymphatic System

A monograph on xeroradiography of the lymphatic system authored by Dr. *Josef Bruna* has been published recently in Acta Univ. Carol. Med. Prague, CSSR (in English). For further information please contact Dr. Bruna, Clinic of Radiology, Medical Faculty of Hygiene, Charles University, 100 34 Praha 10, Srobárova 50/CSSR.