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The Relationship between Tissue Fluid and Lymph

G. Szabó, Zs. Magyar, E. Pósch

National Institute of Traumatology and Department of Pathophysiology
Semmelweis University Medical School, Budapest, Hungary

Summary

Protein concentration in the fluid aspirated from subcutaneously implanted capsules is higher both in dogs and in rabbits than in the lymph collected from the same area. Besides the quantitative differences there are also qualitative differences in the composition of the two fluids. It is concluded, that the capsular fluid is not identical with normal tissue fluid and that from its composition no informations can be gained about the origin of lymph or the mechanism of its formation.

The blockage of the lymphatics of an organ leads to a progressive accumulation of oedema fluid with a very high protein content (3), and it is therefore generally accepted that the most important function of the lymphatics is returning the extravascular protein to the blood stream. It was nevertheless assumed that lymph and tissue fluid are identical and that the lymph actually represents a cross section of tissue fluid contained in the area concerned (2). Other investigators, however, did not accept this view. A concentrating mechanism was postulated in the lymphatic vessels causing the lymphatic protein concentration to be higher than that in true tissue fluid (1, 8).

Until recently no direct evidence was available to prove either of the above assumptions. *Taylor* and *Gibson* (12), however, analysed fluid from implanted capsules for plasma proteins and compared with fluid samples from lymphatics which drained the regions of the implanted capsules. They found, that the plasma protein concentration of capsular fluid and lymph are not statistically different. While the authors do not contest the possibility for a concentrating mechanism in large lymphatic vessels, they conclude from the data obtained that the lymphatic vessels contain plasma protein in concentrations that are fairly representative of the tissue fluid of the region. If this view is accepted, it would be difficult to see how the composition of tissue fluid could be kept constant through the function of the lymphatic system. The whole argumentation of the authors hinges in the identity of the composition of capsular fluid and lymph. It was decided, therefore, to reinvestigate this problem.

Material and Methods

The investigations were made on 10 dogs and 19 rabbits in pentobarbital general anaesthesia. Small perforated capsules (4) were implanted subcutaneously in one or both tighs of the animals. Capsular fluid was withdrawn and lymph collected from a femoral lymph vessel in the dogs 4 to 11 (avg. 7) weeks, in the rabbits 1 week or 6 weeks after the implantation. In 5 dogs, after lymph and capsular fluid collection in one leg the inferior vena cava was constricted above the diaphragm (11). Two to three weeks later, when massive ascites and leg oedema have already appeared, the lymph and capsular fluid collections were repeated on the other leg.

In the dogs the collected fluids and plasma samples were analysed for total protein content (5), haptoglobin (7) and coeruloplasmin (10) concentration. In the experiments made in rabbits the protein fractions in the biological fluids were separated by zone electrophoresis in agarose gel (9) and by disc electrophoresis in polyacrylamide gel (6). The percentage of the protein fractions

was determined in the agarose gel slides with a Zeiss ERI densitometer. The acrylamide columns were scanned with a Kipp and Zonen densitometer. Due to the well known difficulties in the identification of the individual protein fractions the densitometer readings of the disc electrophoresis are not reported here. The polyacrylamide gel columns had been evaluated only by comparing their general aspects and by constating the presence or absence of the individual fractions.

Results

Capsular "tissue fluid pressure" in 10 dogs was -5.0 ± 1.0 mm Hg. In the femoral lymph vessel the pressure was about atmospherical ($+0.33 \pm 0.27$ mm Hg). Caval constriction increased femoral venous pressure from 4.7 ± 0.3 to 14.6 ± 1.4 mm Hg and in the same time the subatmospheric pressure of the capsular fluid disappeared.

Total protein concentration in capsular fluid of the normal dogs (2.38 g%) was invariably higher than in the lymph (1.27 g%). These values were 42.5 and 22.5 per cent of the corresponding plasma total protein concentrations ($p < 0.001$). In venous congestion the protein concentration decreased both in capsular fluid, to 1.69 g% and in lymph, to 0.67 g% or 41.5 and 16.7 per cent of the corresponding plasma concentrations. The difference between the pre- and postcongestion protein concentrations in the same animals was significant (with the paired "t"-test $p < 0.05$).

The haptoglobin (mol. weight 85.000) concentrations behaved essentially in the same manner as the total protein contents. They were in the normal animals in the capsular fluid 56 per cent and in the lymph 36 per cent of the corresponding plasma concentration, and in the caval constricted dogs 49 per cent and 16 per cent respectively (Table 1). The lymphatic concentration of coeruloplasmin (mol. weight 165.000) was in normal dogs 33.4 and in cava constricted dog 32.5 per cent of the plasma concentration. In capsular fluid the coeruloplasmin concentrations were, however, disproportionately high: 72 per cent in normals and 106 per cent in caval vein constricted dogs.

In the rabbits again the capsular fluid contained significantly more protein than the lymph. The lymphatic total protein concentrations was 6 weeks after the implantations of the capsula the same as after 1 week. The protein content of capsular fluid was, however, lower in the samples collected after a longer lapse of time. The higher protein content after the shorter interval may be due to an inflammatory reaction. The cell content of the capsular fluid was much higher after 1 week (1380 ± 340 per cumm as compared with the lymphatic cell content of 650 ± 130 per cumm) than after 6 weeks (760 ± 180 per cumm in capsular fluid and 626 ± 115 per cumm in the lymph).

Table 1 Total protein, haptoglobin and coeruloplasmin concentrations in dogs

	Total protein g%			*Haptoglobin* mg%					*Coeruloplasmin* mg%				
	Plasma	Capsular fluid	Lymph	Plasma	Capsular fluid	Lymph	C/P	L/P	Plasma	Capsular fluid	Lymph	C/P	L/P
Normal dogs (n = 10)	5.63 ± 0.28	2.38 ± 0.27	1.27 ± 0.13	131 ± 16	77 ± 15	48 ± 6	56 ± 5	48 ± 4	12.2 ± 1.4	8.4 ± 1.7	4.3 ± 1.2	72 ± 6	33 ± 6
Venous congestion (n = 5)	4.08 ± 0.18	1.69 ± 0.22	0.67 ± 0.11	156 ± 8	77 ± 20	26 ± 4	49 ± 13	16 ± 2	12.5 ± 1.5	13.2 ± 3.0	3.7 ± 0.6	106 ± 12	32 ± 8

Table 2 Total protein concentration and protein fractions in rabbits 1 and 6 weeks after implantation of the capsule

	1 week (n = 11)			6 weeks (n = 8)		
	Plasma	Capsular fluid	Lymph	Plasma	Capsular fluid	Lymph
Total protein	5.61 ± 0.15	3.79 ± 0.18	2.72 ± 0.13	6.04 ± 0.24	3.23 ± 0.22	2.72 ± 0.15
Albumin	51.1 ± 5.7	51.3 ± 2.7	52.1 ± 8.3	53.0 ± 5.2	61.2 ± 6.4	52.8 ± 4.3
$Alpha_1$	3.5 ± 1.1	2.9 ± 0.9	2.8 ± 0.9	3.7 ± 0.8	3.7 ± 0.7	4.1 ± 0.1
$Alpha_2$	10.7 ± 3.5	13.6 ± 1.1	13.5 ± 2.8	10.6 ± 1.5	9.6 ± 2.5	11.3 ± 2.0
Beta	15.6 ± 2.2	16.3 ± 1.8	16.7 ± 3.8	12.7 ± 2.3	12.1 ± 3.5	16.6 ± 6.0
X($alpha_2$ Mg)	11.7 ± 2.7		8.1 ± 3.0	10.8 ± 1.6	1.1 ± 2.9	7.3 ± 2.5
Gamma	7.5 ± 2.3	15.9 ± 3.5	6.7 ± 1.9	9.3 ± 4.0	12.2 ± 1.2	7.8 ± 3.0

As revealed by zone electrophoresis (Fig. 1) the capsular fluid contains significantly more albumin and less alpha, beta and gamma globulin after six weeks than after 1 week. The albumin concentration in this fluid is also significantly higher than in the simultaneously collected lymph ($p < 0.01$). Besides these quantitative differences a marked qualitative difference was detected between capsular fluid and lymph or blood plasma. After electrophoresis of the rabbit lymph and plasma samples in agarose gel a distinct protein fraction could be seen between the beta and gamma globulin bands. On acrylamide gel columns this fraction could be identified with great probability as the $alpha_2$ macroglobulin fraction (Fig. 2). The above fraction was absent in the capsular fluid after separation both in agarose and in acrylamide gel. (In the 19 animals studied it was detected only on a single agarose gel plate.)

Separated in acrylamide gel columns the electrophoretical patterns were, the absence of the $alpha_2$ macroglobulin fraction in the capsular fluid exepted, identical in the three biological fluids.

Discussion

The present investigations revealed both quantitative and qualitative differences between the fluid aspirated from a subcutaneously implanted capsule and the lymph collected from the same territory. A more prolonged interval between the implantation and the collection of the fluid had

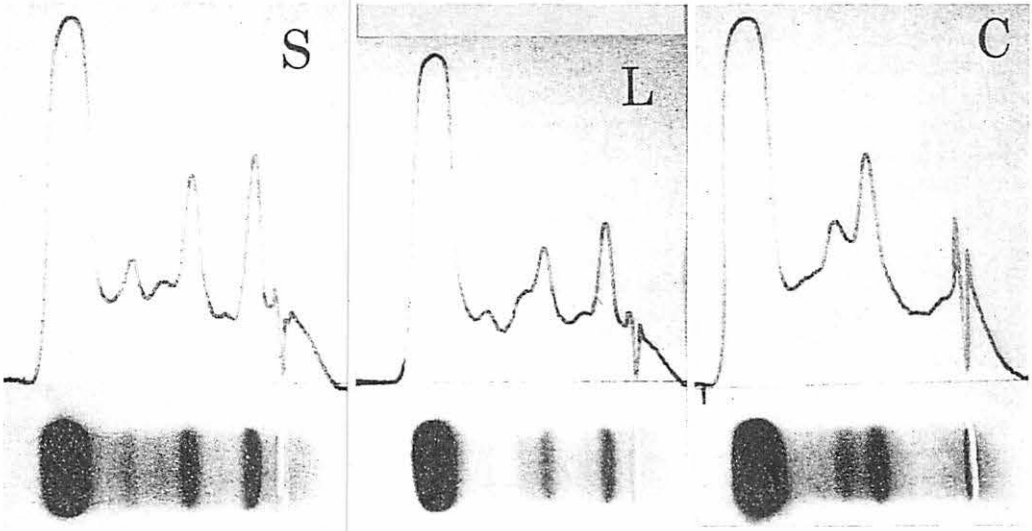

Fig. 1 Zone electrophoresis in agarose gel of rabbit serum (S), lymph (L) and capsular fluid (C).

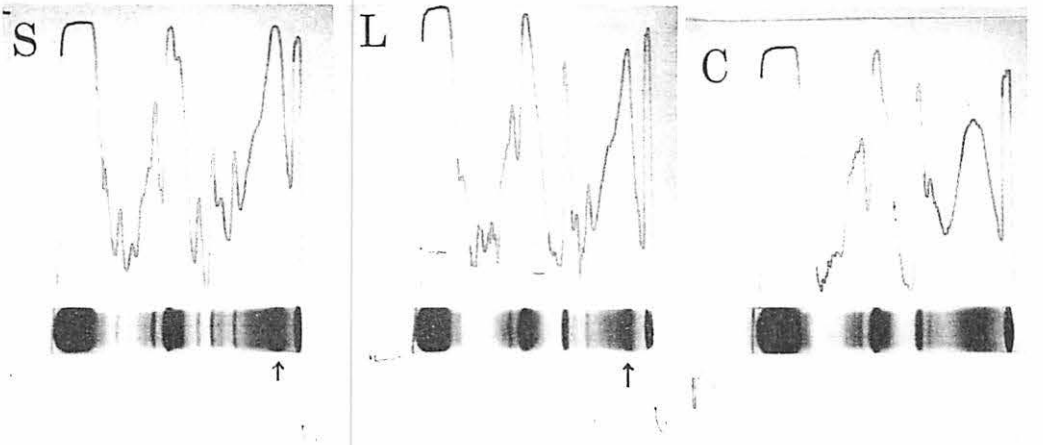

Fig. 2 Disc electrophoresis in polyacrylamide gel of rabbit blood serum (S), lymph (L) and capsular fluid (C). The arrows show the protein fraction (presumably alpha$_2$ macroglobulin) present in serum and lymph but absent in capsular fluid.

a significant influence on the composition of the capsular fluid, but its protein concentration remained still significantly higher than the protein content of the lymph. This difference was present also when fluid filtration in the extremity was increased in consequence of venous congestion.

Still more important are the qualitative differences: in the dogs the disproportionately high coeruloplasmin concentration and in the rabbits the absence of a protein fraction (presumably alpha$_2$ macroglobulin) in the capsular fluid. These differences make it rather improbable that the lymph and the capsular content are the same fluids and both identical with the tissue fluid. Of course, the higher protein content of the capsular fluid does by no means prove, that the lymph is formed by some concentration process from the interstitial fluid, but it makes it also highly improbable, that the fluid aspirated from the capsula is identical with normal tissue fluid.

In view of its high protein and cell content in the early stage after the implantation the capsular fluid might be partly the product of an inflammatory reaction. Later on, it is probably a filtrate of the newly formed capillaries in the granulomatous or fibrous tissue lining the capsular wall. Since the relative albumine concentration is high and the big alpha$_2$ macroglobulin molecules are absent from their filtrate, the permeability of these capillaries seems to differ from that of the normal connective tissue capillaries.

The high protein content of the capsular fluid might be the result of its stagnancy, i.e. of the fact that it is encapsulated and consequently it has more time for the equilibration with blood plasma. Repeated withdrawals of capsular fluid, as practized by *Taylor* and *Gibson* (12) might therefore decrease the protein concentration in the capsular fluid. A further reason of the discrepancies in the observations may be a difference in the implantation and lymph collection sites. *Taylor* and *Gibson* (12) have implanted the capsule in the paw region of the animals and cannulated the lymphatics below the popliteal node. In the present study the lymph collections were made nearer to the implantation site. The capsules were implanted on the tigh and the lymph was collected from femoral subcutaneous lymphatics.

The final conclusion of the present study is, that from the composition of the fluid inside an implanted capsule no conclusion can be drawn on the origin of the lymph or the mechanism of its formation. The question, whether the lymph is identical with the interstitial fluid of the tissue of its origin remains open to discussion.

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György Szabo, M.D., D.S.M., National Institute of Traumatology, H-1430 Budapest, Hungary