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Immune Proteins, Enzymes and Electrolytes in Human Peripheral Lymph

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Summary

Values of various biochemical constituents of leg lymph in 27 normal men have been presented. Concentration of immunoglobulins, complement proteins, acute phase reactants, enzymes, electrolytes and other small molecular weight substances were measured.

Lymph forms part of the interstitial fluid with a chemical composition resembling that of blood plasma. It seems to be almost identical with the mobile tissue fluid. The concentration of macromolecules in tissue fluid and lymph depends on the ultrastructure of blood capillaries and physical forces governing transport of substances across the capillary wall, mostly hydrostatic and osmotic pressure gradients. The capillary wall acts as a molecular filter restricting free flow of proteins from blood to the tissue space. Thus, the amount of protein in the extravascular fluid has to be lower than plasma. Under physiological conditions the amount of protein present in the tissue space is sufficient for the tissue processes. However, in pathological conditions as inflammation or tumor growth larger volumes of protein are needed for the local immune defence. The vasoactive substances released by the local and incoming inflammatory cells increase the permeability of the capillary wall causing a higher flux of proteins including those of high molecular mass. However, the tumor cells do not evoke any local inflammatory reaction, thus, the concentration of immune proteins at the site of their spread may remain unaltered. Efficient treatment of tumors may then depend on our abilities of augmenting the flux of specific proteins or protein-bound drugs to the tumor tissue by e.g. enhancing the local capillary wall permeability. To do this, a better knowledge of kinetics of transport of protein and other plasma constituents to the tissue space of normal humans seems to be necessary.

In the present paper we summarize the results of our studies on concentration of various proteins, among them of immunoglobulins, complement, and acute phase reactants, as well as enzymes and electrolytes in the leg prenodal lymph in 27 men, during normal daily activities and experiments increasing the capillary filtration rate. We also analyze factors which cause variations in the level of biochemical constituents of tissue fluid and lymph.

Materials and methods

Studies were carried out on 27 healthy male volunteers age 19–27.

Cannulation of lymphatics. Lymph was collected from a leg superficial lymph vessel, draining skin, subcutaneous tissue and perimascular fascia of the foot. The technique of lymphatic cannulation was described previously (1, 2). Altogether lymph vessels of 37 legs were cannulated. The collection of lymph lasted for periods from 3 to 7 days. The volunteers carried out their normal daily activities (3) and in some cases took part in experiments increasing capillary filtration like ergometer cycling, leg venous congestion or leg warm water bath (4).

Lymph and blood collection. Lymph samples were collected over different periods of time depending on the type of activity, on the average over 3–4 hours. No anticoagulants were used. Samples in sterile plastic sealed syringes were frozen at – 70 °C and kept in the frozen state until immediately prior to as-

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Tab. 1 Concentration of immunoglobulins in leg lymph and serum of normal men. Mean values ± 1 S.E.

Immunoglobulin	Mol. wt. x 10^3	Number of samples	Serum mg %	Lymph mg %	L/S ratio
G	150	165	1265 ± 29	355 ± 9.3	0.28 ± 0.012
A	160	20	180 ± 19	28.6 ± 2.5	0.16 ± 0.009
M	900	156	122.6 ± 5.4	23.6 ± 1.3	0.20 ± 0.008
D/units/ml	170	12	79.3 ± 9.9	8 ± 0.71	0.10 ± 0.01

Tab. 2 Concentration of complement proteins in leg lymph and serum of normal men. Mean values ± 1 S.E.

Component	Mol. wt. x 10^3	Number of samples	Serum mg %	Lymph mg %	L/S ratio
C1q	400	34	25.5 ± 3.2	3.79 ± 0.38	0.15 ± 0.012
C1s	86	28	2.51 ± 0.13	0.48 ± 0.04	0.19 ± 0.03
C4	230	22	22.1 ± 1.0	6.18 ± 0.72	0.28 ± 0.07
C3	185	22	90.0 ± 10.4	19.1 ± 1.14	0.21 ± 0.01
C3PA	80	57	19.0 ± 15.2	3.5 ± 0.15	0.18 ± 0.01
C9	179	42	1.16 ± 0.07	0.25 ± 0.01	0.22 ± 0.007
CH50		26	45.0 ± 5.8	6.91 ± 0.62	0.17 ± 0.02

say. At the end of the lymph collection period 5 ml blood samples were obtained from the cubital vein. Samples were allowed to clot at room temperature for 1 hr prior to centrifugation. Serum was stored in ampoules in 1 ml aliquots at −70 °C.

Total protein. Total protein concentration of blood serum and lymph was measured using the biuret method (5), and globulin concentration with paper electrophoresis.

Immunoglobulins. Immunoglobulin G, A, and M concentration was determined in lymph and serum by radial immunodiffusion method (6) using TRI-Partigen plates (Hoechst).

Complement proteins. The levels of C1q, C1s, C4, C3, C9, and C3PA (properdin factor B) were measured by radial immunodiffusion method (6).

Acute phase reactants and other proteins. Alpha-1-glycoprotein, albumin, transferin, plasminogen, haptoglobin, alpha-1-antitrypsin, alpha-1-anti-chymotrypsin, inter-alpha-1-trypsin inhibitor, alpha-2-macroglobulin, beta-lipoprotein, retinol, prealbumin, alpha 2-HS-glycoprotein, and beta-2-glycoprotein were measured by radial immunodiffusion method (6) using M-Partigen plates (Hoechst).

Enzymes. SGOT, SGPT and acid phospatase were measured using Biochemica Test Combination (Boehringer), alkaline phosphatase with Bio-Test (Lachema), LDH with Fermognost LDH-Test.

Other biochemical constituents. The concentration of glucose was determined with Bio-Test (Lachema), creatinine with Jaffe test (9), creatine with the method of *Abelin* and *Raaflaub* (10), urea with Biochemica Test Combination (Boehringer), total cholesterol with Bio-Test (Lachema).

Electrolytes. The concentration of Na^+, K^+, Cl^- and Ca^{++} was determined with flame photometry.

Statistical evaluation. The values of concentration of lymph serum constituents were presented as means ± 1 S.E. and also as lymph/serum ratio ± 1 S.E. Different number of measurements of various constituents is due to different protocols of the experimental groups (3, 4, 5, 6).

Results

Concentration of lymph biochemical constituents.

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Tab. 3 Concentration of acute phase reactants in leg lymph and serum of normal men. Mean values ± 1 S.E.

Protein	Mol. wt x 10^3	Number of samples	Serum mg %	Lymph mg %	L/S ratio
Alpha-1-glycoprotein	44	162	51.5 ± 3.9	22.1 ± 0.6	0.45 ± 0.02
Albumin	69	150	4366 ± 147	1678 ± 70	0.48 ± 0.02
Transferrin	80	9	453 ± 23	234 ± 31	0.55 ± 0.12
Plasminogen	81	82	13.3 ± 0.4	2.65 ± 0.17	0.22 ± 0.01
Haptoglobin	100–400	26	86.5 ± 18.1	14.7 ± 3.6	0.17 ± 0.04
Alpha-1-antitrypsin	54	65	301.3 ± 24	69.5 ± 2.8	0.23 ± 0.012
Alpha-1-anti-chymotrypsin	68	8	49 ± 7.8	12.3 ± 1.7	0.25 ± 0.03
Inter-alpha-1-trypsin inhibitor	160	8	60.2 ± 5.9	6.5 ± 0.7	0.11 ± 0.02
Alpha-2-macro-globulin	820	66	331 ± 9.0	647 ± 7.5	0.18 ± 0.02
Beta-lipoprotein	2400	60	526 ± 11.0	111 ± 8.1	0.20 ± 0.015

Tab. 4 Concentration of proteins of less known biological activity in leg lymph and serum in normal men. Mean values ± SE.

Protein	Mol. wt x 10^3	Number of samples	Serum mg %	Lymph mg %	L/S ratio
Retinol	21	33	4.22 ± 0.25	0.7 ± 0.09	0.11 ± 0.04
Prealbumin	50	8	29.7 ± 6	11.9 ± 1.2	0.35 ± 0.05
Alpha-2-HS-glycoprotein	49	60	53.3 ± 1.3	18.7 ± 0.9	0.35 ± 0.016
Beta-2-glyco-protein	40	8	18.5 ± 2.6	7.4 ± 2.6	0.4 ± 0.03

Immunoglobulins and complement proteins. The mean levels of immunoglobulins have been presented in Table 1 and of complement in Table 2. The immunoglobulins were present in lymph at concentrations between 10 to 28% of those of plasma. The level of complement proteins ranged between 15 to 28% of that of plasma, with the high molecular C1q represented at lowest concentrations. Only the main complement components of the classical activation pathway were determined, starting with C1q through C4 and C3 to the final C9. The initial complement component of the alternate activation pathway was represented by C3PA. The low amount of immunoglobulins and complement proteins in lymph has been due to low concentration of total protein, as well as to the differential transport of macromolecules of different size through the capillary wall.

Acute phase reactants and other proteins.

Results have been summarized in Table 3. Alpha-1-glycoprotein was found in lymph in around 45% of the plasma level, albumin in 48%, transferin in 55%, plasminogen in 22%, haptoglobin in 17%. The inhibitors of proteases had the following concentrations: alpha-1-antytrypsin 23% of that of plasma, alpha-1-antichymotrypsin in 25% and the inter-alpha-1-trypsin inhibitor 11%. One of the most potent protease inhibitor alpha-2-macroglobulin had the concentration of 18%.

The low molecular weight proteins of less defined biological properties as retinol had the concentration of 11% of that of plasma, prealbumin 35%, alpha-2-HS-glycoprotein 35% and beta-2-glycoprotein 40% (Table 4).

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Tab. 5 Concentration of lymph biochemical constituents in legs of 13 normal men. Mean values of 21 samples ± 1 S.E.

	Lymph	L/S ratio
Total protein (mg %)	2.39 ± 0.04	0.39 ± 0.013
SGOT (μ/l)	2.6 ± 0.27	0.43 ± 0.031
SGPT (μ/l)	2.0 ± 0.1	0.64 ± 0.01
Acid phosphatase (μ/l)	3.69 ± 0.84	0.57 ± 0.02
Alkaline phosphatase (μ/l)	2.76 ± 0.5	0.43 ± 0.06
LDH (μ/l)	22.2 ± 2.0	0.26 ± 0.053
Urea (mg %)	39.3 ± 3.9	0.68 ± 0.08
Creatinine (mg %)	0.9 ± 0.1	1.0 ± 0.01
Creatine (mg %)	1.43 ± 0.14	1.06 ± 0.05
Glucose (mg %)	65.7 ± 4.5	0.84 ± 0.02
Total cholesterol (mg %)	21.7 ± 1.5	0.14 ± 0.024
Ca^{++} (mEg/l)	6.2 ± 0.31	0.75 ± 0.03
K^{+} (mEg/l)	3.41 ± 0.1	0.98 ± 0.014
Na^{+} (mEg/l)	136.5 ± 3.0	1.02 ± 0.04
Cl^{-}(mEg/l)	107.0 ± 2.5	0.97 ± 0.02

Enzymes electrolytes and other chemical constituents.

Lymph SGOT and SGPT activity was 39% and 43% of that of plasma, of acid phosphatase 57%, whereas of alkaline phosphatase of 43%. LDH was represented at a concentration of 26% of that of plasma (Table 5).

Electrolytes as Na^{+}, K^{+} and Ca^{++} were represented in lymph in the same concentration as in plasma. Calcium, determined jointly as free and protein-bound, had the concentration of 75%.

Lymph urea concentration was 68%, creatinine 100%, creatine 106%, glucose 84% and total cholesterol 14% (Table 5).

Physiological variations of concentration of biochemical constituents of leg lymph.
The effect of changes of body position on lymph protein level was studied in 3 healthy volunteers. Concentration of eight proteins of different molecular weight were determined. These were: alpha-1-glycoprotein, alpha-2-HS-glycoprotein, albumin, plasminogen, IgG, IgM, alpha-2-macroglobulin and beta-lipoprotein. Lymph was collected continuously with the collecting syringes changes every 3 rd hour. The volunteers were walking for 9 hours, lying for another 9 hours, then sitting for 12 hours without moving their legs. Then they were allowed to walk for 3 hours, what was followed by lying for 9 hours, and walking for 3 hours (Fig. 1). Results have been presented on Fig. 1a and b. Walking was accompanied by a continuous decrease in concentration of all proteins, whereas resting in the horizontal position by considerable increase in concentration.

Sitting with dependent legs was characterized by a gradual drop of concentration of all proteins.

In order to document the differences in the rate of changes of concentration between proteins of various molecular mass, the L/S values for each protein have been calculated in percent of the 2nd night value for that protein (Fig.2). It was found that large molecular proteins like alpha-2-macroglobulin and IgM had a faster decrease in concentration than the low molecular weight proteins. However, after some hours in a horizontal position concentrations of both high and low molecular weight proteins tended to return to high and rather close levels.

Discussion

Lymph represents the mobile fraction of tissue fluid. Its biochemical composition in most tissues, among them in the skin, is almost identical with that of the tissue fluid (11, 12). Thus, measuring concentration of substances in lymph gives an insight into their concentration in the interstitial fluid.

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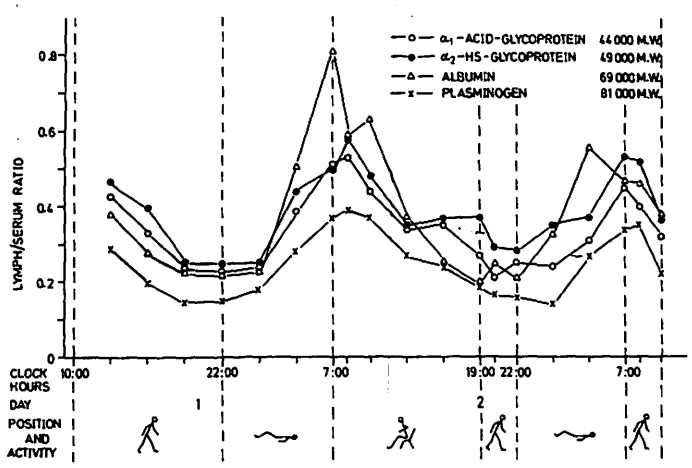

Fig. 1 A, B. Variations of lymph/serum ratio of proteins of different molecular weight during walking, lying and sitting. Mean values from 3 legs of 3 normal men.

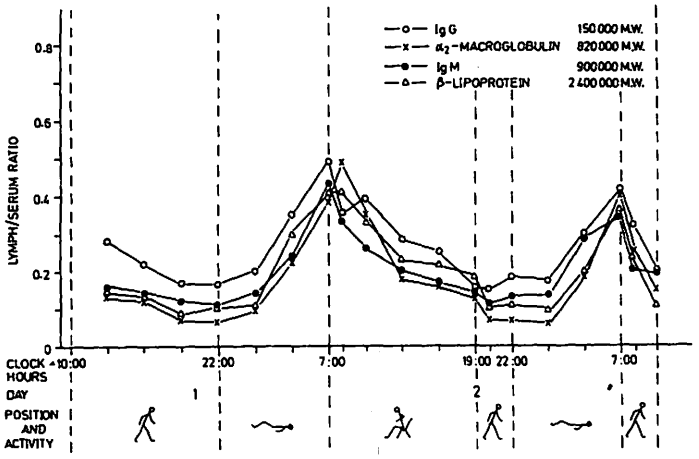

Fig. 1 B

Macromolecular substances found in the peripheral lymph originate from three sources: (a) plasma, (b) tissues from which lymph is drained, and (c) free floating lymph cells. The prevalent mass comes from plasma through the capillary wall by diffusion, filtration and vesicular transport. However, local contribution to the macromolecules of capillary filtrate by the parenchymal cells and cells of the lymphatic series present in tissues of the drained region, should always be taken into consideration.

Low concentration of immunoglobulins which we found in human leg lymph is in agreement with our previous studies (13), and is also observed in the afferent lymph of the sheep leg (14, 15), and in the skin of the guinea pig (16). Low IgG levels were also found in the samples of human leg interstitial fluid (17) sampled by the wick method. The reported levels were, however, twice as high as those reported here, this might be explained by injury to blood capillaries during insertion of the wick into tissues.

The low total complement activity and low complement protein concentration which we found in human leg lymph are interrelated phenomena and may depend on at least three factors: (a) physiologically restricted transport of protein through the capillary wall, (b) partial consumption of complement in the tissues, and (c) inactivation of complement in the tis-

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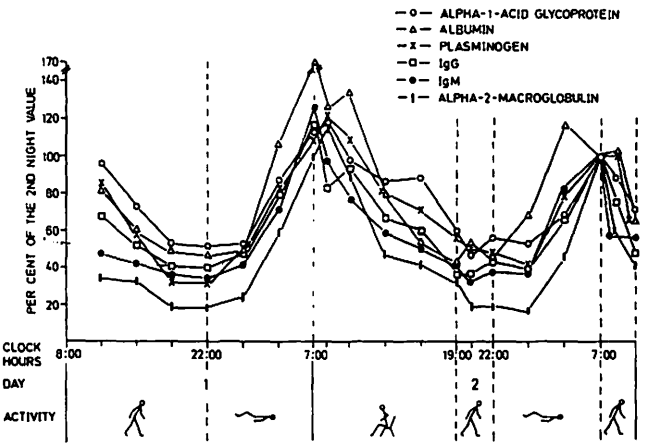

Fig. 2

sues (18). The collection and storage procedures do not seem to have affected our results. Even a 24 hour storage at room temperature and bacterial contamination do not alter complement stability (19). Low total complement haemolytic activity was observed in the peripheral lymph in dogs (20) and rats (21).

Acute phase reacting proteins are those participating in the inflammatory processes. Their blood concentration is altered after trauma with an increase of fibrinogen, haptoglobin, alpha-1-glycoprotein, C-reactive protein, alpha-1-antitrypsin, inter-alpha-globulin and a decrease of beta-lypoprotein, transferrin and albumin (22). In our studies the lymph concentration of all acute phase reacting proteins was low ranging between 11% and 55% of the plasma level. Determinations were performed in normal men without any clinically detectable inflammatory processes, however, in some of them Patent Blue Violet was injected into the skin to visualize the lymph vessel for cannulation. Then, it is possible that the dye may have evoked a minor local inflammatory reaction, although no reaction was observed clinically. Thus, no major changes in protein transport and their local consumption were expected.

Alpha-1-acid-glycoprotein was found in lymph in 45% of the plasma level. This protein is a constituent of normal serum whose increase parallels the severity of an inflammatory response. It was found by others (23) to markedly inhibit the proliferative response of human peripheral blood lymphocytes to PHA and to a lesser extent by ConA and PWM.

Transferrin was present in peripheral lymph at a concentration of 55% of that of plasma. Besides its biological function of transportation of iron it plays a role in defence against certain infections.

Plasminogen was found at a concentration of 22%. This relatively low level, despite of the low molecular weight, indicates the possibility of partial consumption of plasminogen in the lymphatic cannulas, where minor clots could be found.

The concentration of haptoglobin which is another acute phace reactant and also binds hemoglobin, was low. This might be due to its large molecule and differences in genetic type of samples.

Alpha-1-antitrypsin was present in lymph in a concentration of 23% of the plasma level. Its molecular mass is small and rather higher lymph concentrations were expected. It can not be excluded that some of alpha-1-antitrypsin was used up in tissue metabolic processes. The biological properties of alpha-1-antitrypsin are numerous. It inhibits trypsin and chymotryp-

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sin and is an acute phase reactant. Intravenously administered labelled alpha-1-antitrypsin accumulates in extravascular space of the granuloma or sarcoma tissues. It could play a role in regulation of inflammatory processes or in controlling the proliferation of a tumor (24).

Various reports cite elevated levels of chymotrypsin, trypsin, and catepsin-like enzymes in transformed cells. The proteolytic activity is believed to be responsible for uncontrolled proliferation and increased migration. Hydrolazes are also believed to play a role in metastases by decreasing cohesiveness between cells in the primary tumor and in metastases. Naturally occurring inhibitors of proteolytic activity are observed in higher amounts in neoplastic tissues. They may arrest cell growth of tumor cells. Alpha-1-antitrypsin has been found in the 90000 g supernatant fraction of malignant and adjacent normal human breast, stomach, ileum, colon, lung, and anal tissue (25).

The two other protease inhibitors in our study, alpha-1-antichymotrypsin and inter-alpha-1-trypsin inhibitor,were found in lymph in low concentrations, what may partly be explained by their possible involvement into normal tissue processes.

Alpha-2-macroglobulin had a lymph concentration of 18%. Its high molecular mass restricts the rate of transport across the capillary wall. It is the most potent inhibitor of plasmin. Sera of patients with cancer contain an immunosuppressive factor which is an alpha-macroglobulin and is believed to be a prostaglandin antagonist (26). Reports indicate an association between alpha-2-macroglobulin and the development and function of lymphocytes (27). Alpha-2-macroglobulin was found on the surface of a subpobulation of lymphocytes (28) and may be synthesized by peripheral blood lymphocytes (29). In youth the alpha-2-macroglobulin levels are high, reach their minimum in middle age, and gradually increase with old age. In CLL the level of alpha-2-microglobulin might be below the normal mean (30).

The biological functions of beta-lipoprotein are transport of glycerides, cholesterol, phospholipids, lipid soluble vitamins and hormones and numerous-enzymatic activities. It has a high molecular mass (2.400.000), and despite of this was found in lymph in relatively high concentration. This might be explained by other pathways of transport than of ordinary protein, namely through the cell cytoplasm.

The enzyme concentration in lymph in general is lower than in the plasma and the concentration runs parallel with the concentration of protein. In the lymph draining a tissue in which the enzyme is produced in the concentration of that enzyme may be higher than in the plasma. In the lymph collected from skin and subcutaneous tissue only a small admixture of locally released enzymes could be expected. The concentration of investigated enzymes in our group of studies ranged between 26% and 64% of the plasma level.

The urea, creatine and glucose levels were all approximately the same both in plasma and lymph.

The ionic pattern of lymph should not be essentially different from that of plasma. Total cations are usually slightly lower, wheras anions are higher in lymph than in plasma (due to the effect of Donnan equilibrium) (12). In our studies the concentration of Na^+, K^+ and Cl^- equaled that of serum. Lower lymph level of Ca^{++} should be explained by the restricted capillary transport of the part of calcium bound to protein.

The literature concerning local production of proteins by the resident and incoming cells of skin and subcutaneous tissue is scanty. It has been shown recently that free-floating cells in lymph can produce IgG and IgM upon antigenic stimulation (31), but this applies mostly to efferent and not afferent lymph. Also skin macrophages can produce, when studied in vitro, C3 component (32), but the number of these cells in afferent lymph is extremely small. Cultured human fibroblasts synthetize and secrete alpha-2-macroglobulin (33). Also cultured human monocytes synthetize and secrete

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alpha-2-macroglobulin. This substance has also been identified on the luminal surface of endothelial cells in sections of normal human lymphatics by the indirect immunofluorescent technique (34).

The values of lymph concentration of macromolecules presented in this paper are means from samples collected during various activities and positions of the body, and give only an idea about the possible physiological ranges. It has been shown by us previously (3, 4, 5, 6) that major differences can be observed when the men were walking, sitting or resting for several hours. The lymph/serum concentration ratios undergo considerable physiological diurnal changes and the range of these changes is particularly great for proteins of high molecular mass. When interpreting the data on lymph concentration of macromolecules one should take into consideration factors influencing at the moment capillary transport in the tissue from where lymph is derived.

Acknowledgements

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