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On the Transport of Various Endogenous Plasma Proteins from Blood to Peripheral Lymph in Man

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Summary

A method to prepare peripheral lymph out of the foot of clinically healthy patients with respect to their blood capillaries and lymph vessels is presented.

Following electrophoresis in molecular sieving polyacrylamide gel the lymph/plasma quotients of precentages of identical protein fractions in both body fluids as well as of their concentrations were graphically displayed dependent on their migration velocity within the gel. As the high or low lymph-plasma-relations are necessarily to be interpreted in the sense of a relatively high or low blood-lymph permeation the findings are speaking against an exclusive effect of the molecular sieving principle at the blood-tissue-barrier. An explanation for special lymph-plasma-relations would be provided by the assumption of a gel filtration effect in the extravascular circuit of plasma-proteins. The results affirm the findings of former experiments on animals in an analogue model.

The method as well as simplified variants might be useful for the investigation of peripheral nutritive disorders as well as of clinicopharmacological questionings.

A great part of the plasma proteins are circulating permanently from the peripheral blood vessel system across the capillary wall through the interstitial space into the lymphatic vessel system and from hence back into the venous part of the blood vessel system (*Drinker and Field* 1933). It must be regarded, however, that this extravascular circulation does not take place for all plasma proteins to the same extent. According to several authors (*Courtice* 1972, *Mayerson* 1963, *Rusznyak* et al. 1967, *Renkin* 1970, 1974 *Reichel* 1970, 1971, 1974) it depends in the first place on the molecular size. Besides it is considerably determined by the differences between the types of capillaries in the various organs.

For the assessment of the functional state of the blood capillaries – the decisive selective (molecular sieving) link – a method can be provided by the investigation of the above mentioned differences between the transport of special proteins. Well known as yet is the relative higher concentration with respect to the blood plasma of albumin in the interstitial fluid and lymph (see above authors, also *Schultze* and *Heremans* 1966). Much less investigated, however, are the concentrations of other endogenous plasma proteins in the peripheral lymp. A spectrum of the capillary permeabilities for different molecular sizes may be described according to *Renkin* (1972, 1974) by concentration ratios in lymph and plasma under steady state conditions (C_L/C_P).

Preceding investigations of blood and lymph in frog, especially by means of electrophoresis in a molecular sieving medium (polyacrylamide gel) provided a survey on the quality and practicability of a model which moreover enables a comparison between the migration of protein fractions within an artificial molecular sieve on the one hand and their natural permeation across the blood-lymph barrier on the other hand (*Reichel* 1970, 1971, *Reichel* et al. 1971, *Reichel* et al. in prep.). Investigations in human patients thereupon seemed to promise success, the decisive problem lying in the development and testing of a procedure of peripheral lymph puncture. We are basing on the method of foot lymphography according to *Kinmonth* (1952) and an apparatus which has been successfully used for the infusion of contrast media into the foot lymph vessel. However, for our purposes the apparatus must be equipped with a reversal gear which enables very slow puncture of lymph fluid. Moreover the puncture needle was inserted into the lymph vessel in the reverse direction, i.e. from central to peripheral to enable lymph sampling according to the physiologic lymph flux (for details see *Reichel* et al. 1975, also *Engeset* 1975).

Patients; Material and Methods

Investigations were performed on 20 patients in a generally good physiologic state and particularly no signs of illness of the vascular system, the average age of them being 28 years. In all cases an indication for lymphography was clinically prescribed with respect to diagnostic purposes. Essential alterations of the blood protein did not exist. Besides, this would have been without major relevance for our questioning because only identical fractions in blood plasma and lymph were to be compared with each other.

Separation of proteins was performed by means of disc-electrophoresis. Prior to this the total protein concentration was determined according to *Lowry* et al. (1951). The percentages of identical fractions in lymph and plasma (L% or P%) was evaluated directly from the densitographs.

Results

The mean value and standard deviation of protein concentration was 1.77 ± 0.40 g/100 ml in lymph and 7.04 ± 0.60 g/100 ml in plasma, respectively. Fig. 1 shows typical densitographs following electrophoresis of plasma and lymph. It also demonstrates the uniform mode by which the protein fractions were marked and assessed: The beneath scale is divided in 100 units of relative mobility (m_r), m_r = 0 being marked by the boundary between the upper and the lower gel, m_r = 100 by the midst of the albumin peak. The numbers 1 to 11 characterise the quantitatively determined fractions, the boundaries of which were exactly fixed in accordance with *Ornstein* (1964), *Davis* (1964), and *Felgenhauer* (1967). Thus evaluated fractions (in fig. 1 from left to right) were proteins within the macroglobulin area (1), haptoglobin area (2), area of so called "slow globulins" (3), posttransferrinarea (4), transferrin area (5), 4th postalbumin (6), 2nd/3rd postalbumin (7), 1st postalbumin (8), an unidentified fraction (9), albumin (10), and praealbumin (11). The arrows point to some special proteins in the postalbumin area. A description of further methodical questions and details of protein profiles as well as statistical proofs are given elsewhere (*Reichel* et al. 1975).

Fig. 2 demonstrates the lymph/plasma ratios of the percentages (L%/P%; mean value and medium error of the mean value) as well as the ratios of the protein concentrations (C_L/C_P) of the protein fractions in dependence on their migration in polyacrylamide gel electrophoresis.

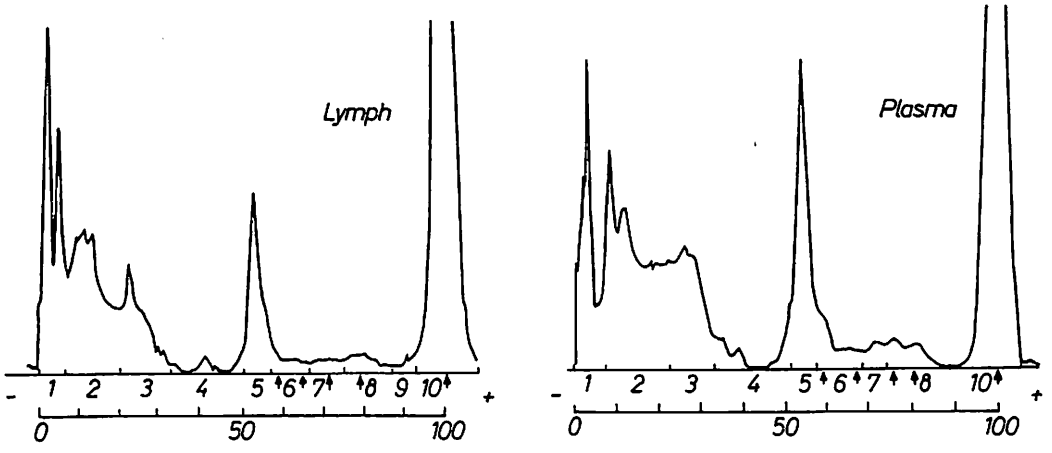

Fig. 1 Densitographs of human lymph and plasma following disc-electrophoresis in polyacrylamide gel and Coomassie-blue R staining. For details see text!

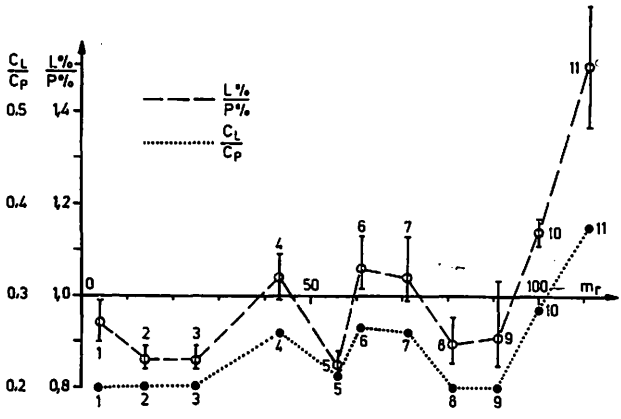

Fig. 2 Lymph/plasma ratios of the percentages (L%/P%) and of the concentrations (C_L/C_P) of the protein fractions in dependence on their migration in polyacrylamide gel. n = 20.

Discussion

As it was to be expected the lymph/plasma ratios within the albumin-praealbumin-area were relatively high due to relatively small molecular weights of their proteins. In contrast the slowly migrating high molecular weight proteins displayed relatively low lymph/plasma ratios. Unexpected, however, were relatively low ratios of two postalbumin fractions (Nrs. 8 and 9) as well as the relations of some other fractions. As the high or low lymph/plasma-relations are necessarily to be interpreted in the sense of a relatively high or low blood-lymph permeation the findings are speaking against an exclusive effect of the molecular sieving principle at the blood-tissue-barrier. An explanation for special lymph-plasma-relations (especially of postalbumins) may be provided by the assumption of a gel filtration effect in the extravascular circuit of plasma-proteins. The results affirm the findings of former experiments on animals in an analogue model (*Reichel* 1971, 1974, *Reichel* et al. 1971, 1975, in prep.) as well as in a similar model basing on protein bound dye concentrations (*Csako* et al. 1975).

Thus a far-reaching transferability of such findings on men has been secured. Another method used by *Ainson* (1972) in different animals and *Reichel* et al. (1975) in man seemed faster and easier to bring about. It consists in calculating the quotient of the C_L/C_P or L%/P% of a low molecular and a high molecular weight fraction, e.g. $\frac{\text{L\%/P\% albumin}}{\text{L\%/P\% macroglobulin}}$ which was, by the way, 1.32 ± 0.64 in our material. The calculation of L%/P% is practical and suitable only in connection with electrophoretical methods and densitometrical evaluation of protein bound dye. For rapid and direct determination of C_L/C_P of specified proteins quantitative immunological assays (e.g. the Partigen test [Behring-Werke]) may be useful. The functional state of the capillary wall can be expressed then by a quotient of the above kind, or even better and more meaningful by using always C_L/C_P for albumin as a reference, i.e.

$$\frac{C_L/C_P \text{ (for any molecule)}}{C_L/C_P \text{ (albumin)}}.$$

Possibilities for the application of the method as well as simplified variants for the examination of pathogenetic mechanisms in hypertonia and in diagnostics of nutritive disorders in vascular peri phery or in clinicopharmacological problems (*Reichel* et al., in prep.) might be discussed.

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References

1 *Ainson, H.:* Physiological properties of the selective permeability of the capillaries and the possibilities of affecting them (russ.) Izwest. A N Est. SSR, Biol. Tallinn 21 (1972), 295–299

2 *Courtice, F.C.:* The Chemistry of Lymph. In: Handbuch der allgemeinen Pathologie, Vol. 3 Lymphgefäßsystem, (1972) 309–362. Ed. H. Meesen. Springer-Verlag, Berlin, Heidelberg, New York

3 *Csako, G., A. Reichel, H. Csernyanszky, U. Reichel:* Effect of Various Lysosomes and Endotoxin on Vascular Permeability in Frogs and Mice. Pflügers Arch. (Europ. J. Physiol.) 353 (1975) 151–157

4 *Davis, B.J.:* Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121 (1964) 404–427

5 *Drinker, C.K., M.E. Field:* The protein content of mammalian lymph and the relation of lymph to tissue fluid. Am. J. Physiol. 97 (1931) 32–39

6 *Engeset, A.:* Paper, read at the V. Internat. Congress of Lymphology 1975

7 *Felgenhauer, K., S. Bach, A. Stammler:* Elektrophorese von Serum und Liquor cerebrospinalis in Polyacrylamid-Gel. Klin. Wschr. 15 (1967) 371–377

8 *Kinmonth, J.B.:* Lymphography in man. Clin. Sci. 11 (1952) 13–19

9 *Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall:* Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1951) 265–275

10 *Mayerson, H.S.:* The physiologic importance of lymph. In: Hamilton, W.F., Dow, P. (Eds): Handbook of Physiology, American Physiological Society, Washington, Sect. 2: Vol. 2 (1963) 1035

11 *Ornstein, L.:* Disc electrophoresis – I. Background and theory. Ann. N.Y. Acad. Sci. 121 (1964) 321–349

12 Reichel, A.: Comparative investigations of plasma and lymph in the frog. A qualitative approach to the blood-lymph transfer of proteins. Acta physiol. Acad. Sci. hung. 37 (1970) 1–18

13 *Reichel, A.:* Vergleichende Untersuchung zwischen der Migration von Plasmaprotein-Fraktionen in künstlichen molekularsiebenden Gelen und ihrem Transfer durch die Blut-Lymph-Schranke. Pflügers Arch. (Europ. J. Physiol.) 323 (1971) 310–314

14 *Reichel, A.:* Permeationsvorgänge an der Blut-Gewebe-Schranke II. Vergleichende Untersuchungen von Blut und Körperflüssigkeiten jenseits der Blut-Gewebe-Schranke. Z. inn. Med. (Leipzig) 29 (1974) 909–916

15 *Reichel, A., K. Brachmann, S. Krantz, H. Zühlke:* Vergleichende Betrachtungen zum Blut-Lymph-Transfer von Plasmaproteinen und ihre Wanderung in künstlichen molekularsiebenden Gelen. Acta biol. med. germ. 26 (1971) 747–759

16 *Reichel, A., U. Rother, J. Werner, U. Reichel:* Vergleichende Untersuchungen zwischen den Proteinen der peripheren Lymphe und des Blutplasmas beim Menschen – Zur Frage einer selektiven Proteinpermeation an der Kapillarwand. Dtsch. Ges. wesen 30 (1975) 640–647

17 *Reichel, A., G. Csako, A.K. Riehtling, U. Reichel:* Effects of Various Vasoactive Substances of Endogenous Plasma Protein Fractions and Protein Bound Dye – Some Test Models in the Frog. In preparation

18 *Renkin, E.M.:* Permeability and molecular size in "peripheral" and glomerular capillaries. In "Capillary Permeability" (Alfred Benzon Symposium II, C. Crone and N.A. Lassen, Eds), 544–547. Munksgaard, Copenhagen 1970

19 *Renkin, E.M.:* Fate of Large Molecules on their Journey from Capillaries to Lymph. In: Capillary Exchange and the Interstitial Space. (Eds.: O.H. Gauer, Ch. Crone, A.C. Guyton, F. Hammersen, T.C. Laurent, B.W. Zweifach) Pflügers Arch. (Europ. J. Physiol.) 336 (1972) Suppl. 72

20 *Renkin, E.M., R.D. Carter, W.L. Joyner:* Mechanisms of the sustained action of histamine and bradykinin on transport of large molecules across capillary walls in the dog paw. Microvasc. Res. 7 (1974) 49–60

21 *Rusznyak, I., M. Földi, G. Szabo:* Lymphatics and Lymph Circulation, Physiology and Pathology. 2nd edition (edited by L. Youlten). Pergamon Press, Oxford 1967

22 *Schultze, H.E., J.F. Heremans:* The proteins of interstitial fluids and effusions. Molecular Biology of Human Proteins, Vol. I, Sect. IV, Chapt. 1. Elsevier Publ. Co., Amsterdam 1966

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