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The Determination of Lipids and Proteins in Suction Blister Fluid

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The concentration of 5 different proteins in suction blister fluid and serum was determined by immunotechniques. These proteins, varying in size and molecular weight (6,600–2,300,000) were insulin, albumin, high density lipoprotein determined as apoprotein A-I, α_2-macroglobulin and low density lipoprotein measured as apoprotein B.

The difference in the blister fluid/serum concentration ratio of the proteins was dependent on the molecular weight and followed mainly the law of diffusion. Moreover, the amounts of insulin, albumin and apoproteins A-I and B in suction blister fluid were the same as those reported in peripheral lymph.

The results indicate that the sieve function of the capillary basement membrane remains intact during the formation of the suction blisters. Suction blister fluid might therefore be regarded as representative of interstitial fluid.

The concentrations of 4 different lipids (cholesterol, cholesterolesters, triglycerides and phospholipids) were also determined and their blister fluid/serum concentration ratio proved to have a fairly constant value of 0.25.

Interactions between cells (e.g. smooth muscle cells, macrophages) and lipoproteins seem to play an important role in the development of atherosclerosis and xanthomatosis [1,4]. It is important to keep in mind that only endothelial cells are in direct contact with the blood; the smooth muscle cells in the vascular wall and cells in the dermis are surrounded by interstitial fluid.

It has been suggested that blister fluid obtained by mild (−200 mm Hg) suction represents interstitial fluid. So far, only a study by Kiistala [5] has demonstrated an inverse relationship between molecular weight and blister-to-serum concentration ratios of proteins, thus indicating that the sieve function of the vascular wall remained intact. We have determined the blister/serum concentration ratios of 5 proteins ranging in molecular weight from 6,600 (insulin) to 2,300,000 (low density lipoprotein), to find out whether the aforementioned suggestion is also valid for these proteins.

We assumed that the concentrations of apoprotein B and apoprotein A-I were representative for the concentrations of low density lipoprotein (LDL) and high density liproprotein (HDL). Separately, we also determined the concentration in the blister fluid of various lipid substances which are present in the lipoproteins.

MATERIALS AND METHODS

Suction blister fluid (0.5–0.8 ml) was collected between 9.00 AM and 11.00 AM from 6 fasting volunteers by mild suction (−200 mm Hg) with the aid of the suction blister device described by Kiistala [6]. The blister fluid (0.5–0.8 ml) obtained from 7 blisters per person was checked for the presence of cells in order to exclude damage to the vascular wall in individuals with a high susceptibility for this type of suction. Only when no cells were found the fluid was used for analyses. Blood was collected from the fasting subjects at 11.00 AM by venapuncture of the cubital vein and allowed to clot.

The $\frac{\text{concentration blister fluid}}{\text{concentration serum}}$ = CB/CS of α_2-macroglobulin and apoprotein A-I (the major protein component of HDL) was determined by radial immunodiffusion according to Mancini, Carbonara, and Heremans [7] using monospecific antisera prepared in our laboratory [8,9]. These antisera were tested for monospecificity by means of immunoelectrophoresis and Ouchterlony double diffusion. The CB/CS of apoprotein B (the major component of LDL) and albumin were determined on commercially available immunodiffusion plates (Partigen-Behringwerke A.G., Marburg am Lahn, W. Germany). Samples of blister fluid and serum were applied to the same Mancini plates. The 2 immunoprecipitate areas were measured and used to calculate the CB/CS ratio. The plates were read by a measuring projector for immuno-analysis (Behring Institute A.G., Marburg am Lahn, W. Germany). Insulin was determined by a radioimmunoassay according to Berson and Yalow [10].

Lipid composition was determined by densitometry after thin-layer chromatography and charring with sulfuric acid, as previously described [11]. Total lipids were determined after extraction [12] and by weighing [13].

RESULTS

The blister fluid/serum concentration ratio (CB/CS) of the proteins are shown in the Figure and the Table and are plotted as a function of the logarithm of their molecular weight (triangles). It is evident that the CB/CS for LDL with a molecular weight (MW) of circa 2,300,000 measured as apo B, as well as the CB/CS for α_2-macroglobulin (MW 800,000) is considerably lower than that of HDL (MW circa 250,000) and albumin (MW 69,000). Furthermore, the CB/CS ratio for insulin (MW 6,600) is 0.90 ± 0.01.

Lipid analysis showed that all major classes of lipids present in plasma (triglycerides, cholesterol, cholesterolesters and phospholipids) are also present in blister fluid. The concentration ratio of these lipids in blister fluid was 0.20–0.27 (see the Table).

The movement of a macromolecule is expressed in terms of its diffusion coefficient (D), a parameter which is inversely related to its molecular weight. We assumed that a diffusion process would be responsible for the differences in concentrations of the various proteins measured in blister fluid. Therefore, the known [14] D of 9 globular proteins are plotted against the logarithm of their molecular weight (Figure; *closed circles*). The 9 proteins were chosen in such a way that a large range of molecular weights was covered. Moreover, the diffusion coefficients of the 5 proteins of which the CB/CS was measured were included.

DISCUSSION

As shown in the Figure, the blister fluid/serum concentration ratio of proteins is dependent on the logarithm of their molecular weight, and this ratio drops sharply from 0.90 to 0.29 for substances with a molecular weight lying between 6,600 (insulin) and 60,000 (albumin). These findings are in accordance with the rapid decrease of lymph/serum concentration ratios for dextran molecules with molecular weights between 6,000 and 30,000 observed by Grotte [15].

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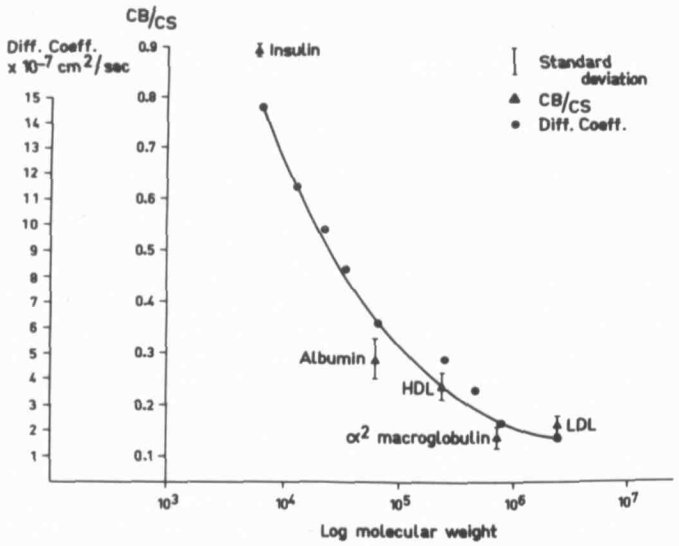
Abbreviations:
D: diffusion coefficient
HDL: high density Lipoprotein
LDL: low density Lipoprotein



The blister fluid/serum concentration ratio (CB/CS) of 5 different proteins ▲——▲, and the diffusion coefficients (*Diff. Coeff.*) ($D = 10^{-7}$ cm^2/sec), at 38°C of 9 globular proteins ●——●, plotted as a function of the logarithm of their molecular weight. The 9 globular proteins ascending in molecular weight were respectively: (1) insulin (6,600); (2) cytochrome C (13,300); (3) trypsinogen (23,560); (4) carboxypeptidase (34,280); (5) albumin (68,460); (6) HDL (250,000); (7) apoferritin (466,900); (8) α_2-macroglobulin (775,500); (9) LDL (2,300,000). See reference 14.

The blister fluid/serum concentration ratio (CB/CS) of 5 proteins and 4 lipids

Proteins	CB/CS	Average SD ± 1 (n = 6)
Insulin	0.90	± 0.01
Albumin	0.29	± 0.04
Apo A-I	0.24	± 0.02
α_2-macroglobulin	0.14	± 0.01
Apo B	0.16	± 0.02
Lipids	**CB/CS**	**Average SD ± 1 (n = 6)**
Sterol	0.27	± 0.04
Sterolesters	0.27	± 0.04
Phospholipids	0.20	± 0.01
Triglycerides	0.21	± 0.05
Total lipids	0.25	± 0.04

Moreover, the further slow decrease of the blister fluid/serum concentration ratio for molecules exceeding molecular weights of 60,000 was also found by Garlick and Renkin [16] in investigations on the lymph/serum concentration ratios for dextran molecules with molecular weights ranging between 60,000 and 500,000.

From these observations it can be concluded that the capillary pore system with a diameter of 45 nm and the system responsible for transport of large molecules remain intact under the conditions of our experiment [17]. Furthermore, the line drawn through the diffusion coefficients of globular proteins (Figure) could be superimposed on the measured data shown in the Figure. The concentration of high molecular weight substances in blister fluid therefore seems to be determined mainly by passive diffusion, which is in agreement with the findings of Garlick and Renkin [16] for peripheral lymph.

In addition, it was found by Herfst and van Rees [18] that the flow of low molecular weight substances (<6,000) into the blister fluid also obeyed the law of diffusion.

If we consider peripheral lymph to represent the interstitial fluid, we must keep in mind the fact that the protein content in peripheral lymph is variable and is dependent on the lymph flow [19]. The blister fluid/serum concentration ratio we found for albumin, i.e., 0.3, is in accordance with the findings of Kiistala [5]. This ratio was also found for peripheral lymph at a lymph flow which is maximal during exercise [19].

Comparison of the concentration ratio of 0.16 (±0.02) for Apo B and of 0.24 (±0.02) for Apo A-I with those given by Reichl and Myant [20] for peripheral lymph, shows that their values are somewhat lower than ours (Apo B = 0.10; Apo A-I = 0.20) but the difference is very small. Moreover, Reichl et al [21] showed that on the basis of biological activity, the concentration of LDL is approximately 10% of the LDL concentration in serum.

The concentration ratio (CB/CS) of various lipids in blister fluid were in agreement with the values obtained for peripheral lymph of rabbits [22] and sheep [23]. Reichl's [24] values for total cholesterol and triglycerides in human peripheral lymph are 5–10% of those in plasma. Whether this difference must be attributed to variations in the composition of peripheral lymph under various conditions or to differences in the method used for lipid determination remains to be answered. By determining 4 different classes of lipid, we have shown that there is no selectivity for the lipid classes in the blister fluid.

The lipid and apoprotein levels found in blister fluid suggest that the composition of the lipoproteins in interstitial fluid differs from that of the lipoproteins in blood. This assumption is supported by the difference in the electrophoretic mobility of apoprotein B-containing lipoproteins in peripheral lymph found by Reichl et al [25].

The pathogenesis of atherosclerosis and xanthomatosis is studied extensively using cultured cells exposed to different lipoproteins [26]. However, the incubation of the cells with the various lipoproteins should resemble as closely as possible the *in vivo* situation. The measurement of the different lipoprotein concentrations in suction blister fluid is of importance to determine the correct incubation conditions for cultured cells, which are *in vivo* surrounded by interstitial fluid.

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CORRECTIONS

In the article Aging of Melanocytes, (73:70–79, 1979) on page 70 the first sentence of the first paragraph of the text should read:

"Diploid human fibroblasts in culture change from rapidly replicating (phase II), to slowly replicating (phase III), to nonreplicating (phase III) cells [1]."

In the abstract Stages of Connective Tissue Alteration and Tumor Development under UVA Radiation in Mice Skin by G. Mahrle, H. Berger, and K. Kölmel (72:272, 1979) instead of 4 % UVB (at the beginning of the third line) it must be 4 0.4% (4 per thousand) UVB.