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NOTE

Hydrodynamic effects on the solute transport across endothelial pores and hepatocyte membranes

Dumitru Popescu†‡, Liviu Movileanu‡§¶, Stelian Ion‖ and Maria-Luiza Flonta‡

† Membrane Biophysics Laboratory, Institute of Biology, Splaiul Independentei 296, PO Box 56-53, Bucuresti R-79651, Romania
‡ Department of Animal Physiology and Biophysics, University of Bucharest, Faculty of Biology, Splaiul Independentei 91-95, Bucharest R-76201, Romania
§ Medical Biochemistry and Genetics, Texas A&M Health Science Center, 440 Reynolds Medical Building, College Station, TX 77843-1114, USA
‖ Institute of Applied Mathematics, Calea 13 Septembrie 13, PO Box 1-24, Bucharest, Romania

E-mail: movileanu@medicine.tamu.edu

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Abstract. In this short note we propose a simple and rapid procedure to calculate the net quantity of metabolites absorbed by hepatocytes from blood plasma. The blood movement through sinusoids determines an opposed circulation of plasma through the space of Disse. Hydrodynamic considerations lead to the conclusion that hepatocytes absorb for their own synthesis processes a quantity of metabolites in a volume flow of the order of 10^{-12} nl s^{-1} through a sieve plate surface with an area of 1 μm^2. At pathological temperature (40 °C), the excess of the net absorbed volume flow for the entire sinusoidal surface of the mammalian liver may be as high as 1.9 nl s^{-1}. Some observations on the effect of red and white blood cells on the chylomicron traffic through endothelial pores are made.

1. Introduction

There have been a tremendous number of theoretical and experimental studies on the appearance, existence and stability of pores through lipid membranes (Abidor *et al* 1979, Popescu *et al* 1991, Popescu and Victor 1991, Weaver and Chizmadzhev 1996, Movileanu *et al* 1997). An interesting case of pores genetically formed was found in the wall of sinusoid vessels from the mammalian liver. The sinusoids represent the main microcirculatory component of the liver. They lack portal venules and arterioles, the tributaries of the liver vein system (Wisse *et al* 1983). The liver sinusoidal wall is mainly made of endothelial and Kupffer cells (Smedsrød *et al* 1994). The endothelial cells have numerous sieve plates with diaphragmless pores (Wisse 1977, Wisse and Knook 1979). These pores of about 0.1 μm diameter enable a part of blood plasma and chylomicrons to pass from sinusoids into the space of Disse (Wisse *et al* 1983). Therefore, the endothelial pores (also called fenestrae) control the exchange of fluids, solutes and particles between the sinusoidal blood and the space of Disse. In other words, the pores constitute an open connection between the sinusoidal lumen and the space

¶ To whom correspondence should be addressed: Dr Liviu Movileanu, Medical Biochemistry and Genetics, Texas A&M Health Science Center, 440 Reynolds Medical Building, College Station, TX 77843-1114, USA.

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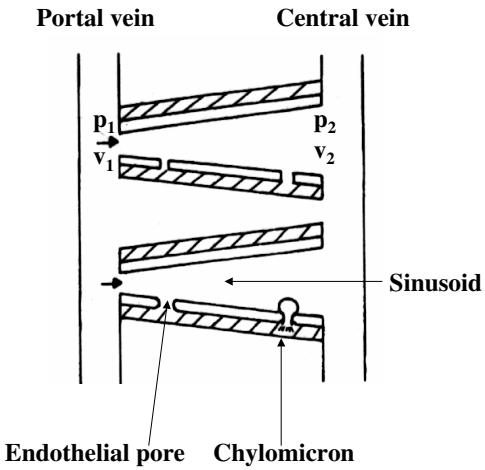

Figure 1. A simplified scheme of a sinusoid from the mammalian liver with the smaller diameter in the periportal vein region and the larger diameter in the central vein region. In this figure, the breaking of a chylomicron is illustrated.

of Disse containing the parenchymal cell surface. In normal liver the endothelial cells of sinusoids lack basal membrane (Wisse 1970).

The effects of haemodynamic alterations on hepatic uptake of substrates and drugs are not yet well understood. We still lack a rigorous experimental methodology to identify the local hepatic blood flows in the human liver. In this paper we analysed some possible blood microcirculation effects on the substances transport through endothelial pores by making some simple hydrodynamic considerations. The results of the present estimations are of fundamental importance in pharmaceutical practice, since the changes in hepatic vascular pressures and volume flows may result in alterations in hepatic uptake of metabolic substrates and drugs.

2. Computing strategy

In the atomic force and scanning electron microscopy, some fenestrae were observed in the endothelial cells from the sinusoidal wall (Montesano and Nicolescu 1978, Braet *et al* 1996). These fenestrae are arranged in clustered domains that were defined as 'sieve plates'. The diameter of pores follows a Gaussian distribution (Wisse *et al* 1985). Unlike the vessels in the peripheral circuit, the sinusoid diameter increases down the duct. This has a smaller diameter near the portal vein than near the central vein. A direct consequence of this fact would be a decrease in blood velocity along the sinusoid. Therefore, the largest value of the blood velocity is located at the portal vein. According to the Bernoulli law blood movement will produce a depression between the space of Disse and the interior of the sinusoid. This depression will promote suction of blood plasma from the space of Disse into the sinusoid through the pores located in the endothelial wall.

In order to evaluate the hydrodynamic effects of blood circulation in sinusoids a simplified sinusoid model with two orifices in the wall, near the portal vein and central vein junction, was considered (figure 1). Blood velocities are v_1 and v_2 at points '1' and '2', respectively (figure 1). p_1 is the blood pressure at point '1', near the sinusoidal domain of the portal vein, and p_2 is the pressure at the other junction ('2'), in the proximity of the central vein (figure 1).

If the volume flow is caused exclusively by the pressure difference, and also fluid weight does not play an important role in the local haemodynamics, then the volume flow expression is given by the Darçy law (Bear 1972, Massey 1998):

$$\vec{v} = -\frac{k}{\eta}\text{grad } p \tag{1}$$

where k has the dimension of area surface. η indicates a viscosity coefficient.

If the pressure difference between the porous layers is Δp and the length of the volume flow across an area surface A is l, then the volume flow is the following:

$$Q_v = \frac{K_v}{\eta}\frac{\Delta p}{l}A \tag{2}$$

where K_v is a constant.

In the case of cylinder pores with the same transversal diameter (with radius r), the Darçy law becomes the Hagen–Poisseuille law (Bear 1972, Massey 1998):

$$Q = \frac{\pi r^4}{8\eta l}\Delta p \tag{3}$$

where η is the blood plasma viscosity and Δp is the pressure gradient across the pore. l denotes the pore length. Applying the Bernoulli law at both points '1' and '2', we have:

$$\begin{aligned}\Delta p_1 &= \frac{\rho v_1^2}{2}\\ \Delta p_2 &= \frac{\rho v_2^2}{2}\end{aligned} \tag{4}$$

where ρ is the blood plasma density.

As $v_1 > v_2$ the pressure gradient $\Delta p_1 = p_1 - p_{\text{Disse}}$ along the first pore is bigger than the pressure gradient $\Delta p_2 = p_2 - p_{\text{Disse}}$ along the second pore. Here we assume that there is the same pressure, p_{Disse}, at the edge of the two pores, towards the space of Disse. The consequence on the blood plasma circulation will be that plasma will go out of the sinusoid through pore '2' and go into it through pore '1'. Therefore, the blood plasma circulation in the space of Disse is reversed to that inside the sinusoid.

The space of Disse is common for the two pores, so a coupling between them takes place, and $\Delta p_1 - \Delta p_2$ is a common pressure gradient which gives rise to a suction force from the sinusoid towards the space of Disse in pore '2' and vice versa in pore '1'. Taking into consideration, the factors mentioned above, the difference $\Delta Q = Q_2 - Q_1$, that is the quantity 'disappearing' due to absorption by hepatocytes, can be computed. From equations (3) and (4) the quantities Q_1 and Q_2 passing through pores '1' and '2' were derived:

$$\begin{aligned}Q_1 &= \frac{\pi\rho r_1^4\left(v_1^2 - v_2^2\right)}{16\eta l_1}\\ Q_2 &= \frac{\pi\rho r_2^4\left(v_1^2 - v_2^2\right)}{16\eta l_2}.\end{aligned} \tag{5}$$

Writing the continuity equation for points '1' and '2' (that is right before the first pore junction and right after the second pore junction) we have:

$$Q_1 + S_1 v_1 = Q_2 + S_2 v_2 \tag{6}$$

where S_1 and S_2 are the cross-sectional areas of the two openings.

By solving the system of equations (5), (6), v_2, Q_1 and Q_2 can be found, because the other unknowns may be determined experimentally.

Table 1. Input data. Experimentally determined data for sinusoid diameters (D), pore density (n) and diameters (d) in peripostal and centro-lobular region (in text region '1' and region '2', respectively) (from Wisse *et al* 1983, 1985). The data were derived from scanning electron microscopy measurements (Wisse *et al* 1983).

	Periportal region	Centro-lobular region
D (μm)	4.09 ± 0.06	5.67
n (pores μm^{-2})	9.08 ± 0.30	13.3 ± 0.5
d (nm)	110.7 ± 0.25	104.8 ± 0.22

3. Results and discussion

3.1. Net quantity of absorbed substance

The endothelial cells have a flat region which has almost no cytoplasm pierced by pores making up sieve plates. The diameters of these pores are in the range 50–300 nm. Most pores have a diameter around 100 nm (Wisse 1970, Wisse and Knook 1979, Smedsrød *et al* 1994). The diameter and density of endothelial pores as well as the cross-sectional area of the sinusoid at its ends are given in table 1 (Wisse *et al* 1983).

Adapting the system of equations (5), (6) to the case of two sieve plates in two regions '1' and '2' (see table 1) and considering a common length for all the pores, we have the expression of the net absorbed volume flow in the space of Disse:

$$\Delta Q = \frac{\pi}{4}\left(D_1^2 v_1 - D_2^2 v_2\right) \tag{7}$$

$$\Delta Q = \frac{\pi\rho}{256\eta l}\left(n_2 d_2^4 - n_1 d_1^4\right)\left(v_1^2 - v_2^2\right) \tag{8}$$

where η and ρ are the viscosity and density of blood plasma, respectively. D_1 and D_2 are the sinusoid diameters, respectively. The variables n_1, n_2 and d_1, d_2 represent the pore density (per μm^2) and diameter, respectively. The two regions are indicated by '1' and '2', respectively.

With the knowledge of the sinusoid diameters D_1 and D_2, the system can be solved, if the blood velocity (either v_1 or v_2) is known. The values for blood velocity at the entrance of the sinusoid are in the range $(27\text{–}41)\times 10^{-5}$ m s^{-1} (Greenway and Lautt 1989, Galloway 1991). The average pore length is 500 Å (Wisse, 1970). As red and white blood cells cannot pass through pores, the blood plasma viscosity is considered. This last parameter is generally given relative to the water viscosity and varies with the temperature. Its values are in a range $(1.10\text{–}1.23)\eta_{\text{water}}$ (Galloway 1991). The blood plasma density was used and not the blood density. We were focused to evaluate the impact of the fluctuations of the blood plasma viscosity induced by the changes of the blood temperature on the net volume flow absorption. The liver cells absorb nutrients from the blood plasma in the space of Disse, which are used subsequently for other metabolic processes. They also secrete anabolites in the space of Disse. Therefore, we can say that ΔQ would represent the difference between the substances absorbed from plasma and those eliminated in the space of Disse. Therefore, we can say that ΔQ represents the volume flow of substances absorbed from blood plasma and used for internal synthesis processes by hepatocytes.

An interesting comparison of the data is between the normal temperature (36 °C) and the pathological temperature (40 °C). Our results are listed in table 2.

The data from table 2 are computed for the extreme values of the blood plasma viscosity that corresponds to both temperatures and the extreme values of the blood velocity in the proximity of portal vein ($v_1 = 27 \times 10^{-5}$ and 41×10^{-5} m s^{-1}). As you can see from table 2, the blood velocity for the normal human temperature may have a serious effect on the net

Table 2. The calculation of values of v_2, Q_1, Q_2 and ΔQ at the human temperature ($t_1 = 36\,^{\circ}$C) and at pathological temperature ($t_2 = 40\,^{\circ}$C).

	$\eta \times 10^6$ (kg m^{-1} s^{-1})	$v_1 \times 10^5$ (m s^{-1})	$v_2 \times 10^5$ (m s^{-1})	$Q_1 \times 10^{15}$ (μl s^{-1})	$Q_2 \times 10^{15}$ (μl s^{-1})	$\Delta Q \times 10^{15}$ (μl s^{-1})
$t_1 = 36\,^{\circ}$C	$\eta_{min} = 13\,566$	41	14.049	1.344	1.582	0.237
		27	21.334	3.100	3.647	0.547
	$\eta_{max} = 16\,422$	41	14.049	1.111	1.307	0.196
		27	21.334	2.561	3.013	0.452
$t_2 = 40\,^{\circ}$C	$\eta_{min} = 12\,255$	41	14.049	1.488	1.751	0.263
		27	21.334	3.431	4.037	0.606
	$\eta_{max} = 14\,835$	41	14.049	1.229	1.446	0.217
		27	21.334	2.835	3.335	0.501

volume flow absorbed in the space of Disse. A decrease of the blood velocity from 41×10^{-5} to 27×10^{-5} m s^{-1} may cause an almost twofold and 2.5-fold increase, respectively, in the net quantity of absorbed substances for the minimum and maximum viscosities, respectively. If the blood plasma temperature is raised to the pathological value of 40 °C, then the relative volume flow absorption is higher. Thus, the largest value of ΔQ (0.606×10^{-15} μl s^{-1}) was computed for the minimum blood viscosity and the minimum blood velocity across the portal vein of 12 255 kg m^{-1} s^{-1} and 27×10^{-5} m s^{-1}, respectively, that correspond to the pathological temperature. This is because the influx of nutrients in the space of Disse is the largest one for these conditions (4.037×10^{-15} μl s^{-1}). By contrast, the lowest value of ΔQ (0.196×10^{-15} μl s^{-1}) was obtained for a maximum blood viscosity (16 422 kg m^{-1} s^{-1}) and maximum blood velocity (41×10^{-5} m s^{-1}) that correspond to the normal temperature.

3.2. Some considerations about the chylomicron transport through endothelial pores

Chylomicrons are supermolecular structures formed by the aggregation of fatty acids, proteins and cholesterol. They are either spherical or ellipsoidal. When the cholesterol quantity is small, the cholesterol molecules have their rigid carbon ring in the hydrophobic region. Under such circumstances, a disorder in the arrangement of hydrophobic chains is produced. Accordingly, an increased membrane fluidity of the lipid membrane takes place (Popescu and Rucareanu 1992). The micelle diameter is determined by hydrophobic chain length of lipids. The mechanical resistance of the micelles would be large, if they are made from the only one fatty acid type. Certainly, the chylomicrons in the blood plasma exhibit more than one fatty acid type. In addition, they contain cholesterol, so that a higher fluctuation in shape and size is expected. Moreover, the chylomicrons lose an important amount of triglycerides during circulation (Smedsrød *et al* 1994). As an immediate result, their diameters decrease. Therefore, there is a wide range of chylomicron sizes. In order to understand the passage of chylomicrons through endothelial pores we classify them into three categories according to their size. Also the pores are divided into two categories: outlet pores situated in centro-lobular region (pores though which plasma exits the sinusoid entering the space of Disse) and inlet pores situated in the periportal region (pores through which plasma exits the space of Disse entering the sinusoidal region). The only way the chylomicrons reach the space of Disse is through the outlet pores.

The chylomicrons in the first class have their diameter smaller than the pore diameter. They pass from the sinusoidal blood into the space of Disse, thus having no resistance to overcome. The second class of chylomicrons is characterized by a diameter equal to or

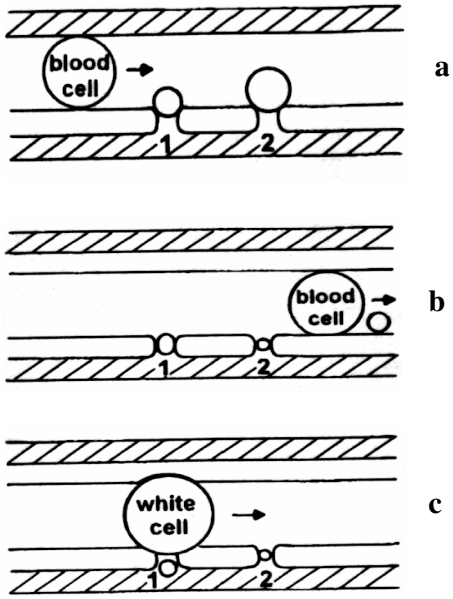

Figure 2. The representation of a centro-lobar region of a sinusoid: (a) two chylomicrons of different sizes are stuck at the entrance of pores via the suction forces, whereas a red blood cell (erythrocyte) is located just before the two pores; (b) after the passage of a red blood cell along the two pores, the chylomicron from the second class was pushed within the interior of pore '1', whereas the larger chylomicron (from the third class) has been broken in two smaller chylomicrons. One of them blocked pore '2'; the other can continue the diffusion and finally may be pushed in a subsequent pore like the chylomicron from the second class in pore '1'; (c) the passage of a white blood cell (leukocyte) along two blocked pores with chylomicrons. One of the chylomicrons is pushed into the space of Disse following the local deformation of the sinusoidal wall.

slightly bigger then the pore diameter. The chylomicrons in the third class have a diameter much larger than pore dimensions. The chylomicrons from the second class partially enter the pores due to the negative pressure. The erythrocytes circulating in the sinusoidal region will produce advancement of chylomicrons along the pores, pushing them deeply inside the sinusoid. Depending on the pushing effect of the erythrocytes and their own size, chylomicrons can pass through the pores or clog them (figure 2(a)). A strong contact interface is created between the pore membrane and the membrane of a chylomicron stuck in the pore. The chylomicrons stuck inside a pore can undergo one of the following two processes:

(a) First, the chylomicron can be disrupted due to the suction force and its content reaches the space of Disse. It is worth mentioning that the pore clogging by a chylomicron favours the breaking of the chylomicron because of the resulting great increase in the suction force. The following calculation demonstrates this statement. The suction force at outlet sieve plate level is given by the expression:

$$F_2 = \frac{2\rho\left(v_1^2 - v_2^2\right)}{\pi n_2 d_2^2}. \tag{9}$$

The obturation of $\mathrm{d}n_2$ pores in the centro-lobular region causes an increase in the suction force equal to

$$\mathrm{d}F_2 = -\frac{2\rho\left(v_1^2 - v_2^2\right)}{\pi n_2^2 d_2^2}\mathrm{d}n_2 \tag{10}$$

or in a more suggestive form:

$$\mathrm{d}F = -\frac{F}{n_2}\mathrm{d}n_2. \tag{11}$$

If we take into account that v_1 is in the range of $(27\text{–}41)\times10^{-5}$ m s^{-1}, then the values of F_2 are in the range 2.37×10^8–5.47×10^8 N. This means that the increase in force, subsequent to the pore clogging by a chylomicron, is in the range 1.78×10^7–4.12×10^7 N. This increase is a feed-back reaction caused by the clogging. That makes more likely the break-up of the chylomicron stuck in the pore and thus the pore unclogging.

(b) The second process consists in the cholesterol diffusion from the chylomicron membrane into the endothelial cell membrane. This process is facilitated by the close contact between the two membranes. The cholesterol diffusion may be partially facilitated by the drop in the packing pressure of one membrane layer by comparison with the other (Movileanu *et al* 1997). Consequent to this second process, the chylomicron may become smaller and is finally able to pass through the pore.

Chylomicrons in the third class are stuck at the outlet pore entrance. Such a chylomicron is divided into two unequal chylomicrons by passing red or white blood cells, if the suction force is not large enough to break it (figure 2(b)). One of the two resulting chylomicrons is inside the pore, whereas the other goes ahead down the sinusoidal region. Because the white cells are bigger than the red ones, they may deform the sinusoidal wall, thus pushing the chylomicron inside the endothelial pore further on into the space of Disse (figure 2(c)).

The above mentioned processes make all the chylomicrons circulating in liver sinusoids pass into the space of Disse. The model presented in figure 2 is known as 'force sieving' (Wisse *et al* 1985). An alternative to this model is known as 'endothelial massage' (Wisse *et al* 1985). In this case, especially for periportal domains, the passage of white blood cells will compress the endothelial lining. Accordingly, the fluid present in the space of Disse is pushed ahead. More fenestrations are encountered, greater fluid flow is pushed out of the space of Disse. Following the passage of white blood cells across sinusoids, a suction of fresh plasma fluid takes place in the space of Disse through the endothelial pores.

3.3. An evaluation of substance absorbed by rat liver

A very rough evaluation of the quantity absorbed by the whole liver is made below. We considered all the sinusoids in a lobule placed in parallel planes that are perpendicular to the central vein. We introduced in the present calculation an average number of six sinusoids in such a cross section plan. We took for all lobules a common length of 5 mm for their longitudinal axes represented by the central vein. We also assumed that the planes mentioned above, each containing six sinusoids normal to the lobule axis, are equidistantly crossing the central vein of the lobule. The distance between two such planes was taken as twice the average diameter of a sinusoid for which the value of 5 μm was used. The result of these assumptions is a number of 3000 sinusoids per lobule. The average lateral surface of one sinusoid was also estimated by multiplying 1 mm, the common length of our hypothetical sinusoids (namely the radius of the cross-sectional area containing the sinusoids), with the circumference of a sinusoid cross-section. The result of 15×10^3 μm^2 multiplied by 3000 gives an average sinusoidal surface of 45×10^6 μm^2 for a lobule. If the liver has 10^5 lobules, then the sinusoidal surface for the whole liver is 45×10^{11} μm^2.

At normal temperature the net quantity of absorbed volume flow is in the range $(0.19\text{–}0.55)\times10^{-12} \times 45 \times 10^{11} = (0.882\text{–}2.444)$ nl s^{-1}. Based on the present

calculations (table 2) and assumptions, at pathological temperature (40 °C) the excess of net absorbed volume flow for the entire sinusoidal surface of the mammalian liver may be up to 1.9 nl s^{-1}.

These estimations may be useful in pharmaceutical practice, if a particular drug permeability across the parenchymal cell surface is determined experimentally. Undoubtedly, the model, in its present form, is subject to some severe assumptions. For instance, we considered that the pressures at the edge of two endothelial pores toward the space of Disse are equal. With more information about the pressure distribution inside the space of Disse and with experimental data regarding fenestrae dynamics (pore density per sieve plate, distribution of sieve plates and distribution of fenestrae size), the model is applicable to a variety of concrete practical situations such as dynamics of distribution and size of fenestrae after chronic alcohol consumption (Mak and Lieber 1984) or microfilament disruption (Braet *et al* 1998).

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