HEPATIC ZONATION OF GLUCOSE METABOLISM

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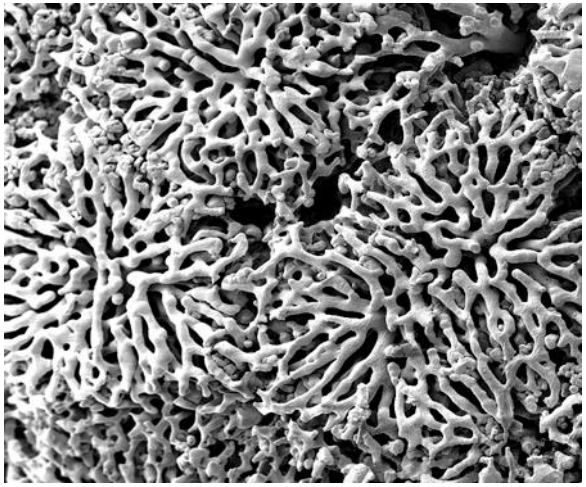
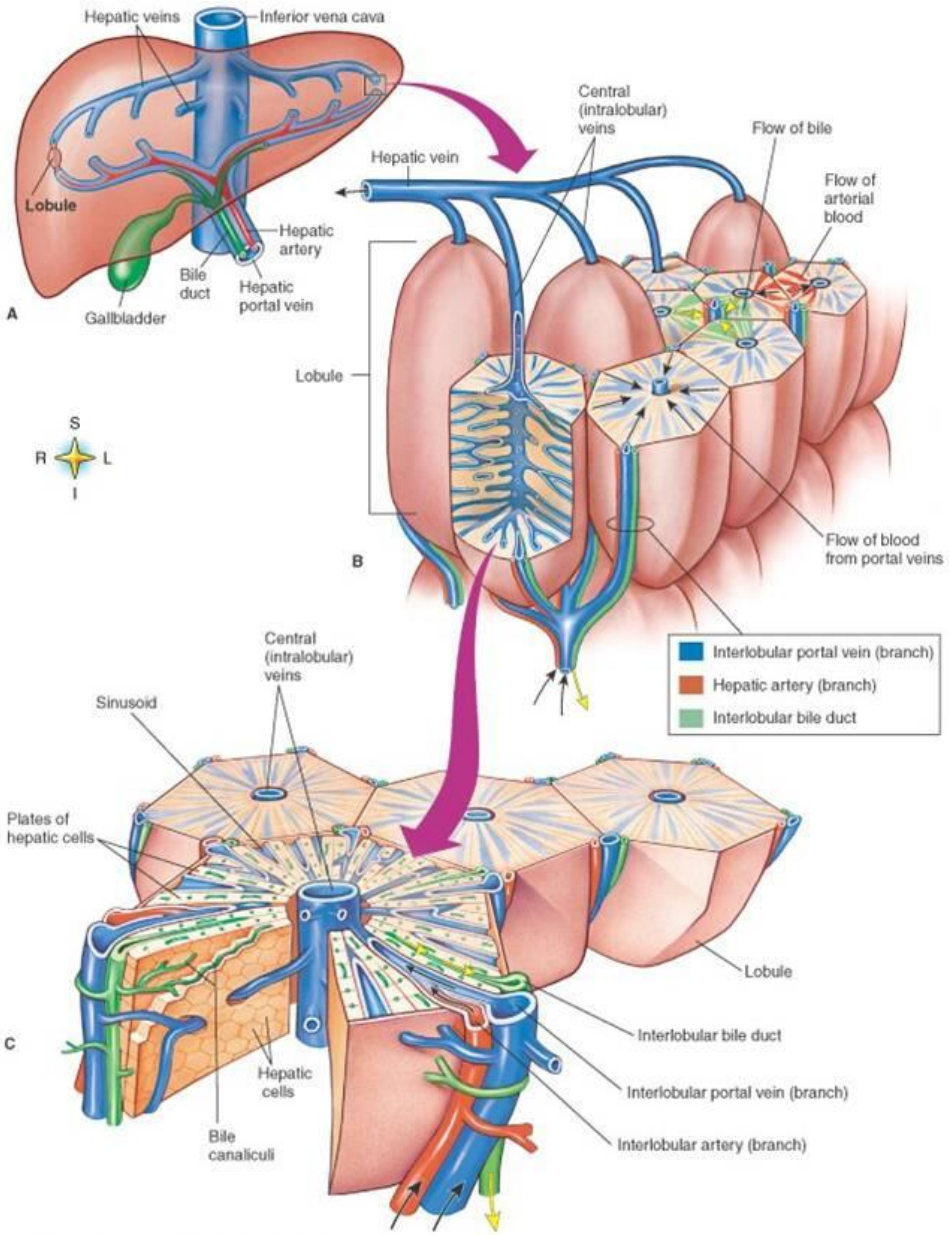
Berlin, 2013-03-11

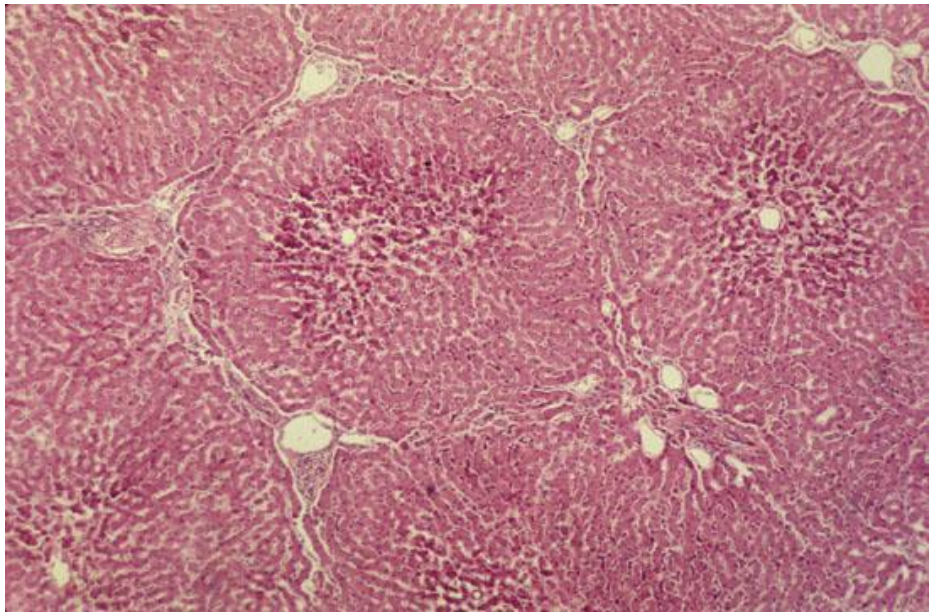


LIVER ARCHITECTURE ON A TISSUE-SCALE

LIVER LOBULE

- liver structured in identical subunits **liver lobule**
- dual blood supply
  - 80% poorly oxygenated venous blood via **portal vein**
  - 20% well oxygenated via **hepatic artery**
- blood flows via **sinusoids** towards **central vene**
  - **pp** → **pv**





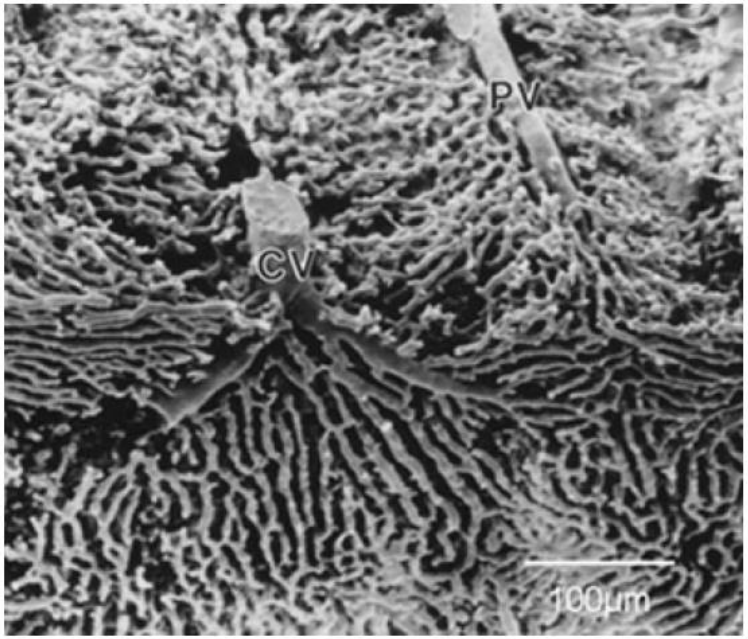


Fig. 2. Vascular cast of the hepatic microvasculature illustrating the tortuous, anastomotic sinusoids adjacent to the portal venule (PV) and the more parallel and larger sinusoids near the central venule (CV) (McCuskey, 1993).

SINUSOID

- principal vessels for exchange between blood and hepatocytes
- ~ 6-8µm diameter
- periportal sinusoids are narrower and more tortuous than the wider and straighter central ones
- Sinusoid network is heterogeneous
  - **near portal vene** arranged as **interconnecting polygonal networks**
  - farther away from portal vein organized as **parallel vessels terminating in the central vein**
  - short **intersinusoidal sinusoids** connect adjacent parallel sinusoids

Scanning electron micrograph showing fenestrated sinusoids and hepatocytes in a mouse liver.
http://www.easloffice.eu/jhep/contest/website/see_photos.html

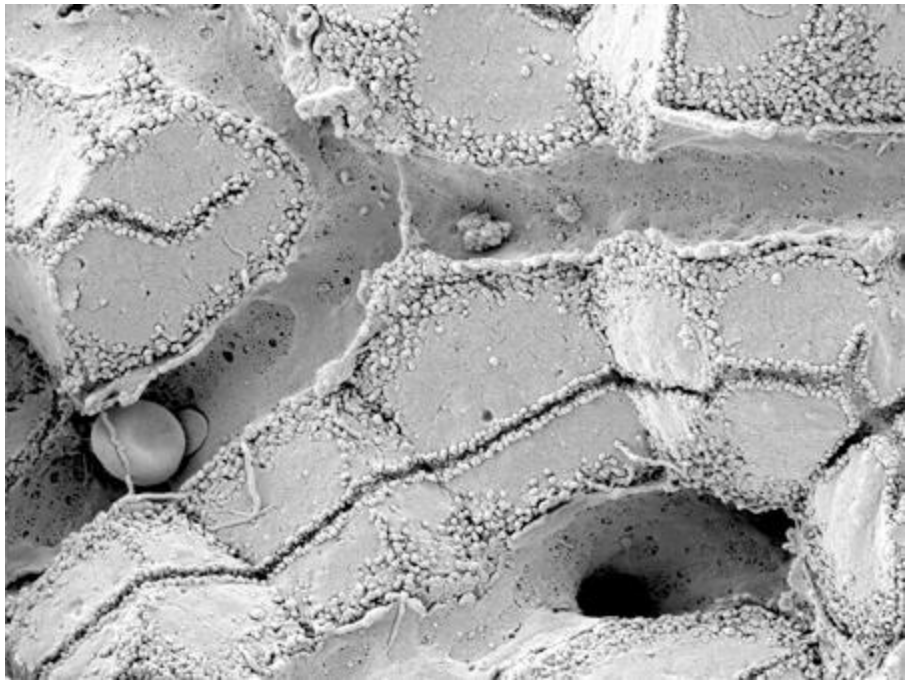


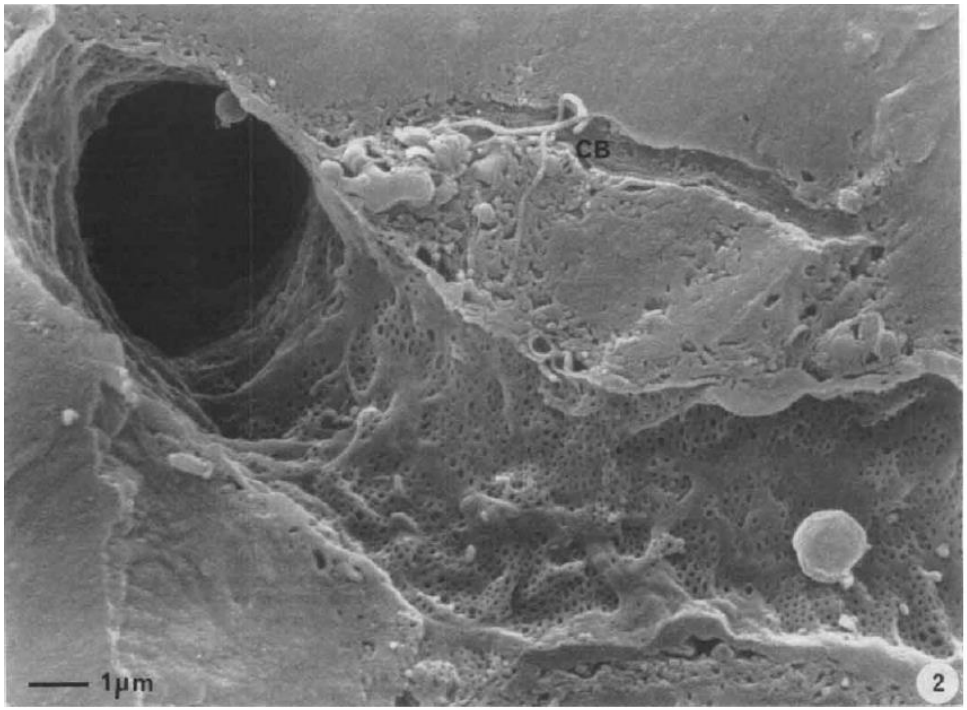
TABLE 1. COMPARISON OF MEASUREMENTS ON SINUSOIDS AND BLOOD CELLS IN MICRONS ± S.E.

	In vivo/LM[a]	In plastic/LM	After CPD/SEM
Portal sinusoid	5.9 ± 0.17 (n = 545, 6 rats)	6.42 ± 0.12 (n = 696, 2 rats)	4.09 ± 0.06 (n = 1, 452, 10 rats)
Central sinusoid	7.1 ± 0.29 (n = 498, 6 rats)	7.62 ± ND[b] (n = 696, 2 rats)	5.67 ± ND (n = 1, 452, 10 rats)

Scanning electron microscope observations on the structure of portal veins, sinusoids and central veins in rat liver. Wisse, E.; De Zanger, R. B.; Jacobs, R. & McCuskey, R. S.; *Scan Electron Microsc,* **1983**, 1441-1452
The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Wisse, E.; De Zanger, R. B.; Charels, K.; Van Der Smissen, P. & McCuskey, R. S. *Hepatology,* **1985**, *5*, 683-692

ENDOTHELIAL FENESTRAE

- liver sinusoids unique capillaries
  - **open pores (fenestrae)** in sinusoidal wall
  - **lack basal membrane** underneath endothelium
- fenestrae act as sieving barrier between blood and heptocytes
  - morphological & physiological evidance that fenestrae act as a **dynamic filter**
  - important role in lipid metabolism (namely **chylomycrons**)
- Liver sinusoidal endothelial cells (**LSEC**) constitute sinusoidal wall
  - numerous endocytotic vesicles
  - effective uptake of a wide variety of substances from blood via receptor-mediated endocytosis (Braet, 2004)
  - transcytosis transport across the endothelium to surrounding tissue
  - scavenger system



Contribution of high-resolution correlative imaging techniques in the study of the liver sieve in three-dimensions. Braet, F. et al; *Microsc Res Tech,* **2007***, 70*, 230-242
Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. *Comp Hepatol,* Braet, F. & Wisse, E.*,* **2002***, 1*, 1
The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Wisse, E.; De Zanger, R. B.; Charels, K.; Van Der Smissen, P. & McCuskey, R. S. *Hepatology,* **1985***, 5*, 683-692

FENESTRAE

- diameter ~**50-200nm**
- ultrastructure same across species
  - groups of fenestrae arranged in so called **sieve plates**
- differences periportal & perivenious
  - diameter decreases (110.7 to 104.8nm)
  - frequency increases (9 to 13 per μm^2)
  - increased porosity from pp to pv from 6 to 8%
- number and diameter may vary between individuals & within single indiviuum under various physiological and pharmacological circumstances

Scanning electron micrograph of hepatocyte microvilli protruding through the sinusoidal endothelial cell fenestrations.

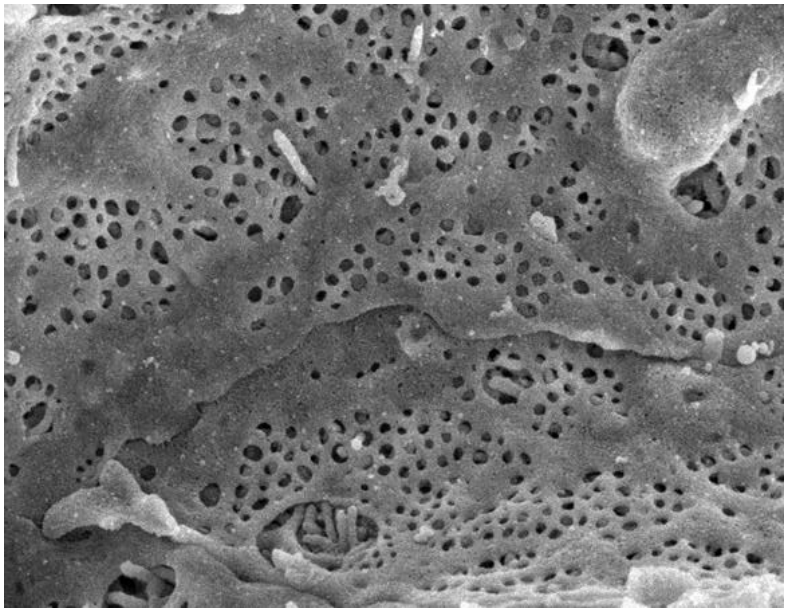
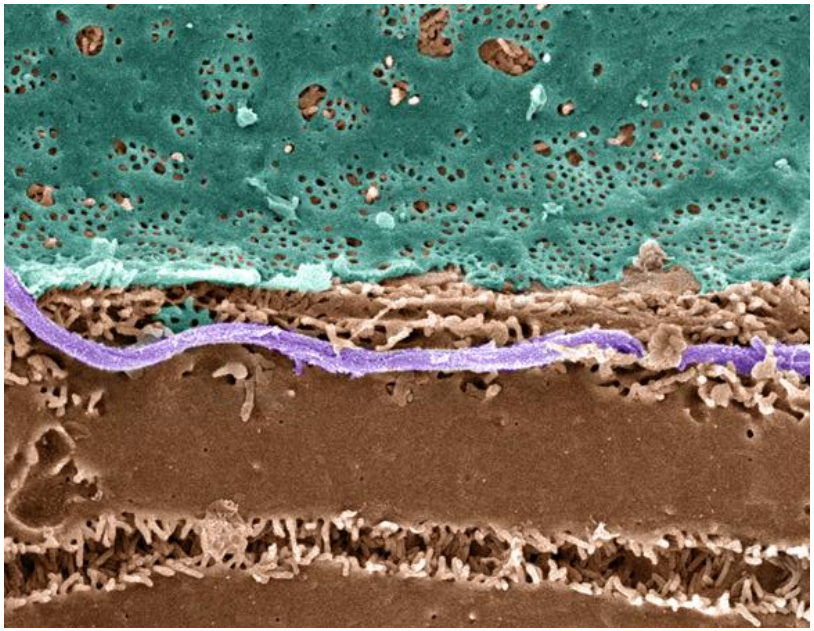


Table 1: Fenestration pattern in different species Brief overview of fenestrae characteristics of different species. Notice the large variations in diameter and number of fenestrae between the different species. The reported data from this table were obtained by at random measurements along the sinusoids. "n.d." = no data available. Data are expressed as mean ± S.D. In case of baboon, human and rainbow trout the data correspond with the minimum and maximum diameter or number of fenestrae measured.

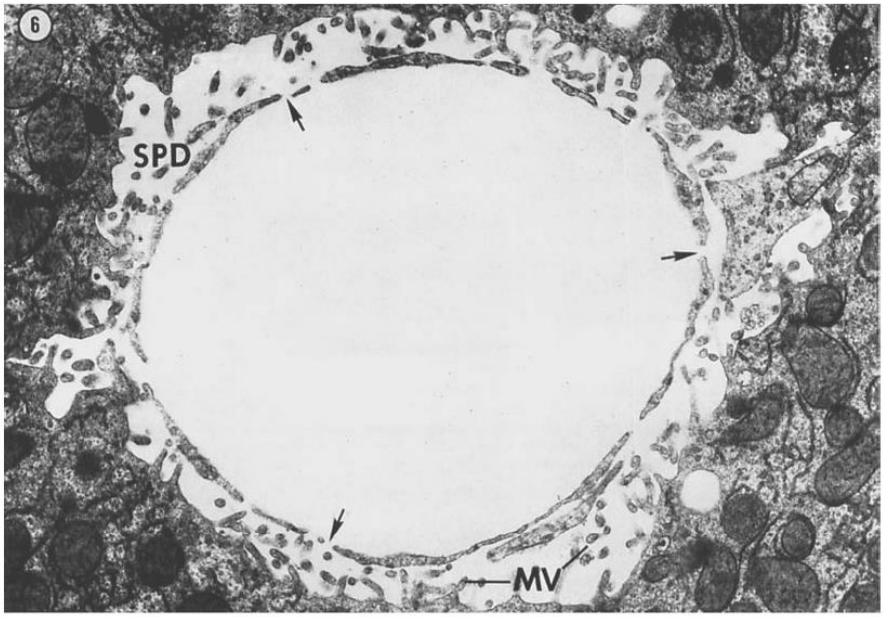
Species (ref.)	Diameter (nm)	Number of fenestrae / μm^2
Rat [25]	98.0 ± 13.0	20.0 ± 6.3
Mouse [26]	99.0 ± 18.0	14.0 ± 5.0
Rabbit [27]	59.4 ± 4.8	17.3 ± 3.8
Chicken [23]	89.6 ± 17.8	2.9 ± 0.3
Baboon [20]	92 – 116	1.4 – 1.9
Human [28]	50 – 300	15 – 25

SPACE OF DISSÉ



Pseudo-colored Scanning EM of an hepatic plate, accentuating the **fenstrated sinuoidal endothelium (blue)**, **hepatocyte (brown)** and a **collagen fibril (lavender)**.

Hepatocytes surface enlarged via **microvilli**.



6 Intermediate sinusoid. The lining cells possess fenestrations (arrows) and there is no basement membrane. The space of Dissé (*SPD*) is voluminous. The surface of the parenchymal cells is characterized by numerous microvillae (*MV*). The blood plasma has free access to the liver cells. Glutaraldehyde; OsO_4; Epon; 13,500 ×.

The fine structure of rat liver sinusoids, space of Dissé and associated tissue space.
Burkel, W. E. & Low, F. N.; *Am J Anat,* **1966***, 118*, 769-783
http://www.easloffice.eu/jhep/contest/website/see_photos.html

SINUSOID ULTRASTRUCTURE

- fenestraetion & absence of basement membranes provide direct access of blood plasma to hepatocytes

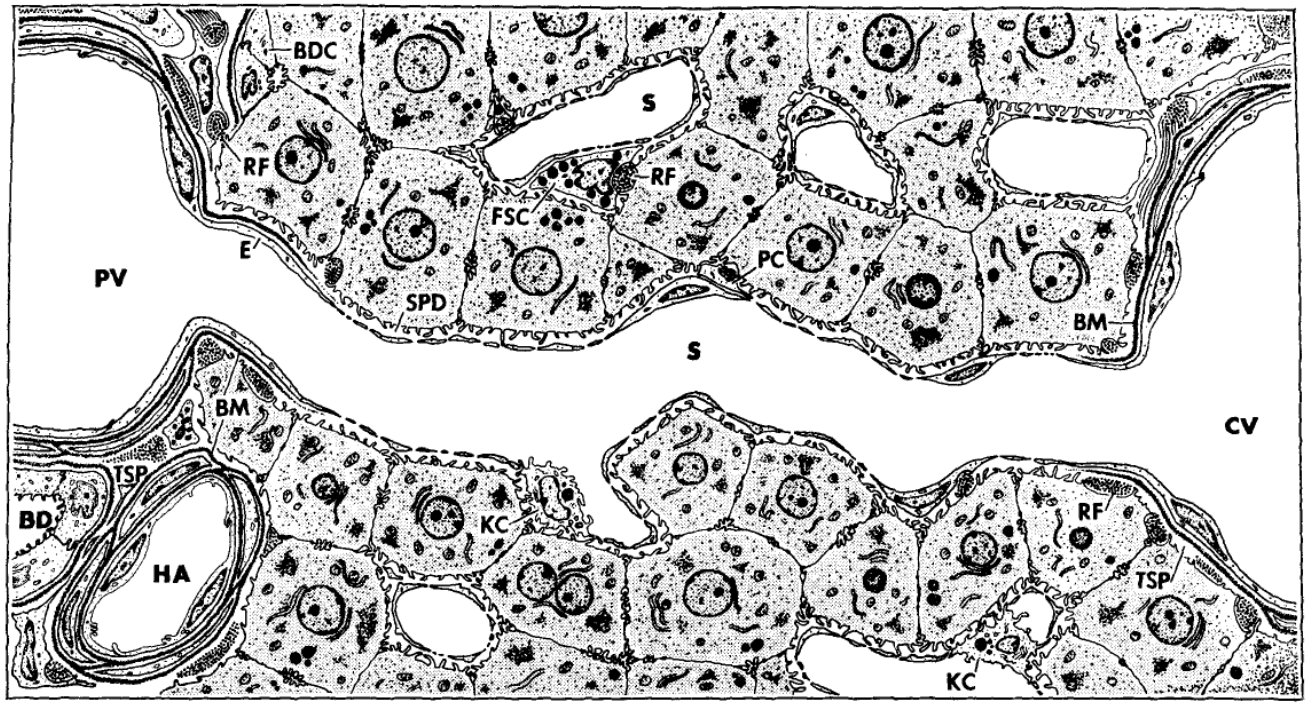
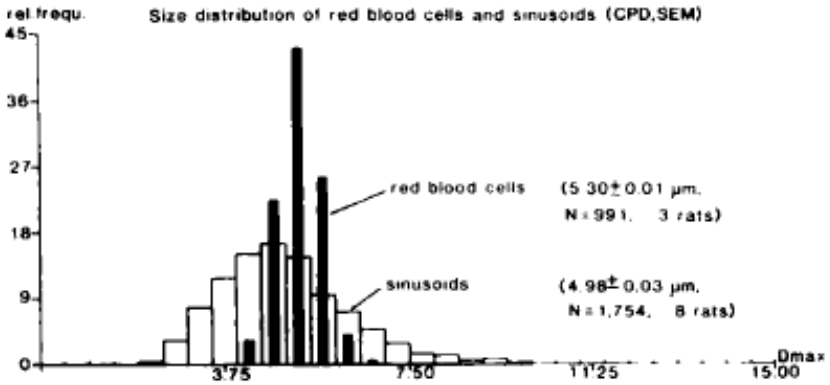


Fig. 1 *Fine structure in the rat liver lobule.* The periphery of the lobule is at the left where branches of portal vein (*PV*) hepatic artery (*HA*) and bile duct (*BD*) lie in the tissue space (*TSP*). Above, bile duct cells (*BDC*) abut on liver cells. The sinusoid (*S*) connects the portal vein with the central vein (*CV*). In the peripheral portion of the sinusoid both the endothelium (*E*) and its basement (boundary) membrane (*BM*) are continuous with those of the portal vein. In the intermediate portion the lining is fenestrated and there is no basement membrane. Centrally the cellular lining is continuous with the endothelium of the central vein and a basement membrane is present. Reticular fibers (*RF*) are found in the tissue space and in the space of Dissé (*SPD*) which surrounds the sinusoids. In places the sinusoids are lined by the cells of von Kupffer (*KC*). Perisinusoidal cells (*PC*) and fat storage cells (*FSC*) are in the space of Dissé. See text for interpretation.

The fine structure of rat liver sinusoids, space of Dissé and associated tissue space.
Burkel, W. E. & Low, F. N; *Am J Anat,* **1966***, 118*, 769-783

TIGHT SINUSOIDS



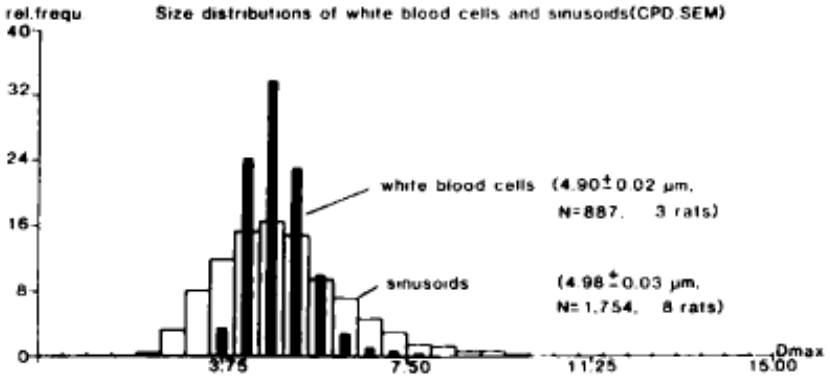


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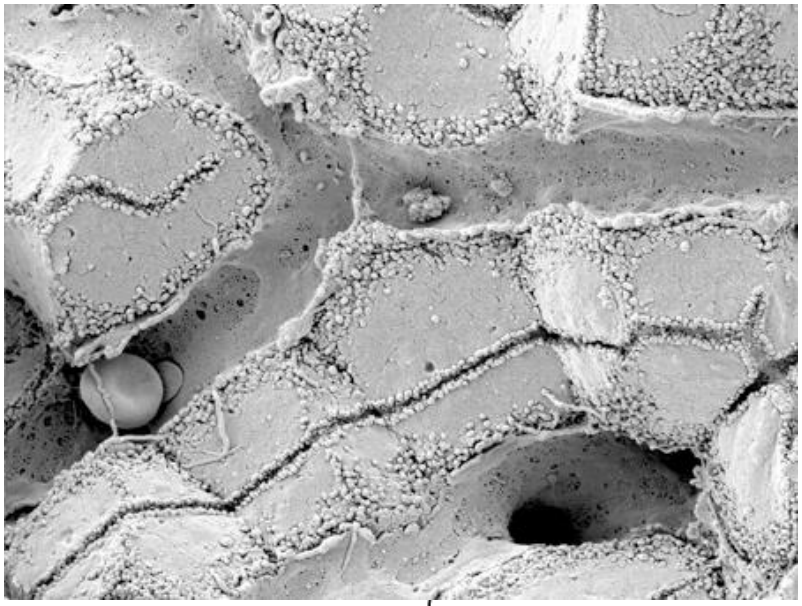
FIG. 9. Size distribution of sinusoids and red and white blood cells, extending the data of Table 1. From these two graphs, one might conclude that starting at 3.75 µm blood cells are larger than a certain percentage of sinusoids. At about the size of 7 µm, white blood cells are bigger than most sinusoids, and these cells will progressively plug sinusoids in the range of approximately 4 µm (in SEM preparations).



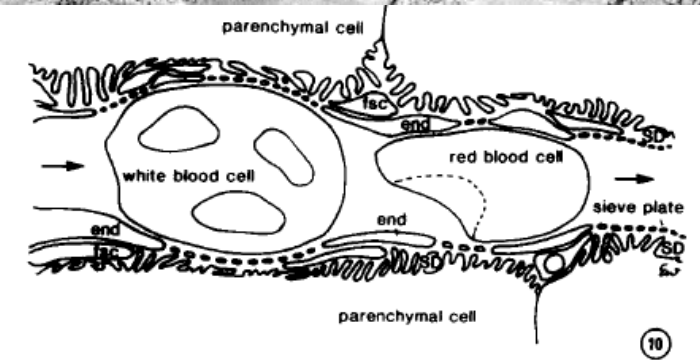


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FIG. 10. shows the interaction of blood cells and the fenestrated sinusoidal wall. As observed in the *in vivo* microscope, red blood cells pass by in a single row and show typical deformation morphology. Their observed flexibility is enormous, and in slow streaming sinusoids one observes that red blood cells adapt their diameter constantly to the diameter of the sinusoid by expanding and narrowing. This implies that uptake and exchange is taking place from volumes of plasma in between the red blood cells. White blood cells are much more rigid than red blood cells and are thought to compress the space of Disse, thereby performing endothelial massage (Fig. 12).

The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Wisse, E. et al; *Hepatology,* **1985***, 5*, 683-692

BLOOD FLOW

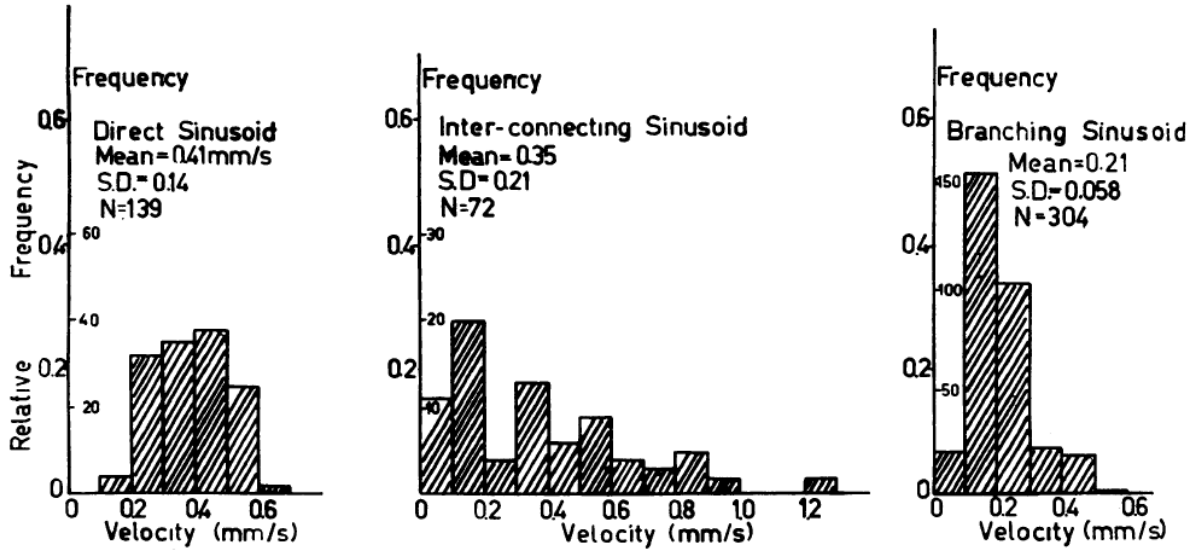

FIG. 1. Frequency distribution of the velocity of the erythrocytes in the direct sinusoids, the branching sinusoids and the interconnecting sinusoids.

The terminal hepatic microcirculation in the rat.
Koo, A.; Liang, I. Y. & Cheng, K. K.; *Q J Exp Physiol Cogn Med Sci,* **1975**, *60*, 261-266
Intermittence of blood flow in liver sinusoids, studied by high-resolution in vivo microscopy. MacPhee, P. J.; Schmidt, E. E. & Groom, A. C.; **1995**, *269*, G692-G698

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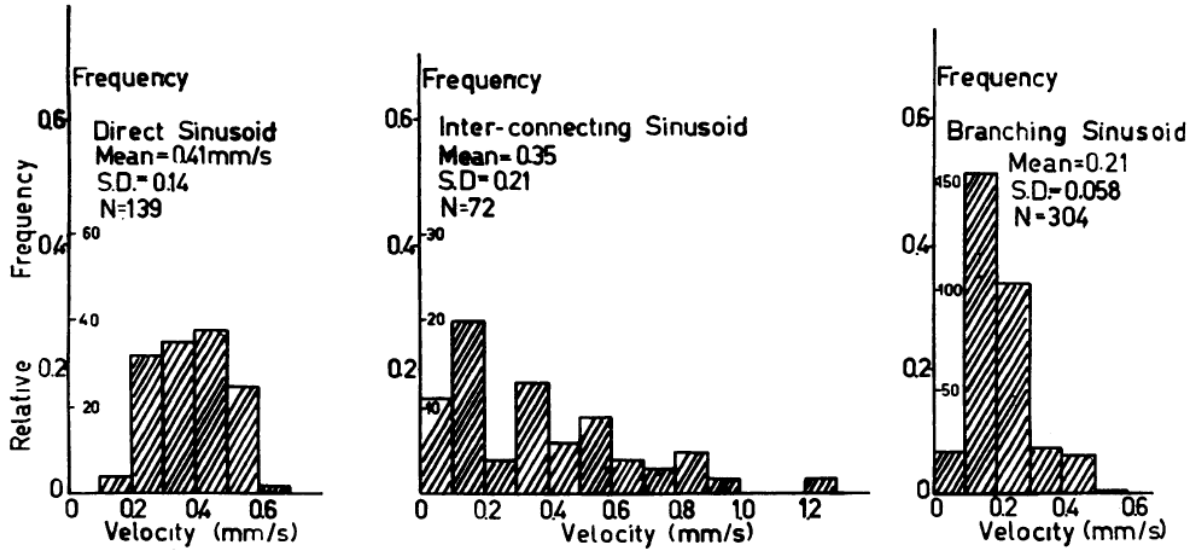

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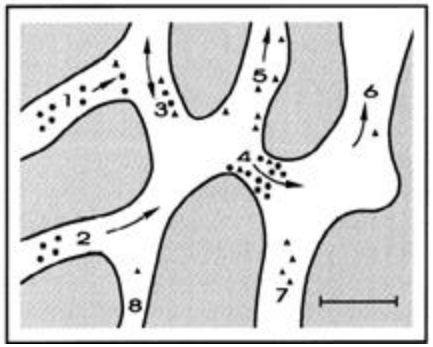


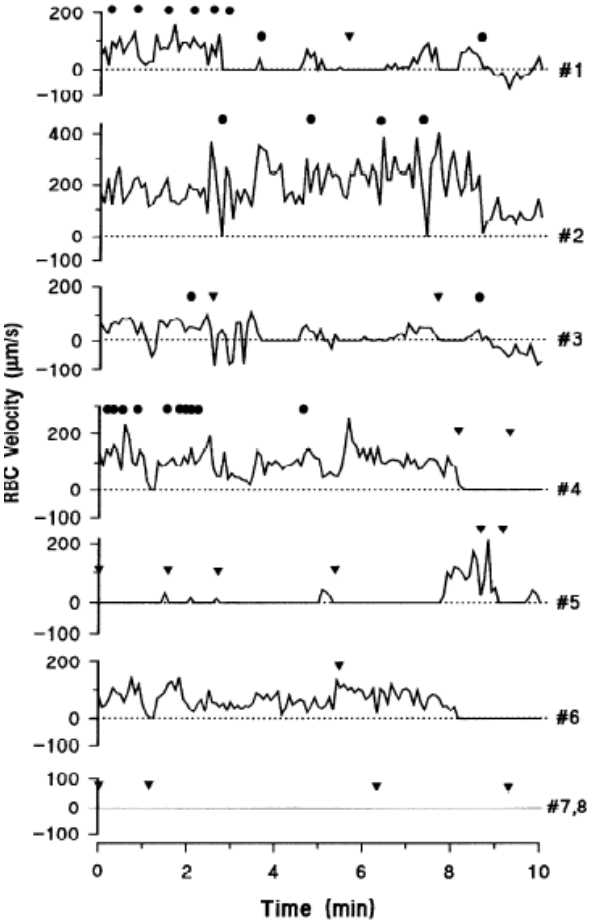


Fig. 3. Temporal overview of RBC velocity fluctuations over a 10-min period, in sinusoidal network from zone 1 of mouse liver (shown in Fig. 2). Measurements in each sinusoid were made every 5 s. Changes of flow in one sinusoid often produced changes in others. Instants are indicated at which a migrating Kupffer cell obstructed flow (▼) or a circulating leukocyte slowed or stopped temporarily (●). *Sinusoids 7* and *8* had no flow throughout the 10-min period. Kupffer cells are seen in *sinusoid 7*, and in *sinusoid 8* one Kupffer cell blocked flow throughout.

The terminal hepatic microcirculation in the rat.
Koo, A.; Liang, I. Y. & Cheng, K. K.; *Q J Exp Physiol Cogn Med Sci*, **1975**, *60*, 261-266
Intermittence of blood flow in liver sinusoids, studied by high-resolution in vivo microscopy. MacPhee, P. J.; Schmidt, E. E. & Groom, A. C.; **1995**, *269*, G692-G698

LYMPH

- substantial amount of hepatic lymph is generated in space of Dissé
  - space of Dissé is continuos with the tissue space at both ends of the sinusoid !
  - high protein content of hepatic lymph
- **lymphatic vessels** originate as blind-ending capillaries in the connective tissue spaces (portal tracts) associated with the portal veins and hepatic arteries
  - fluid contained in these lymphatics flows toward the **hepatic hilus** and eventually into the **cisternae chyli**

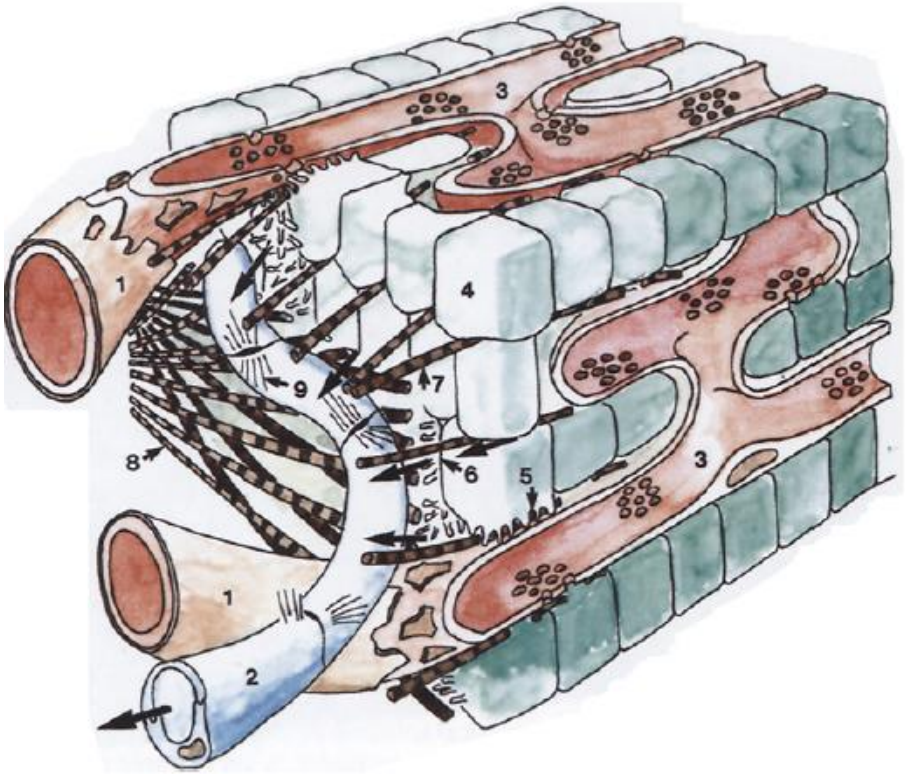
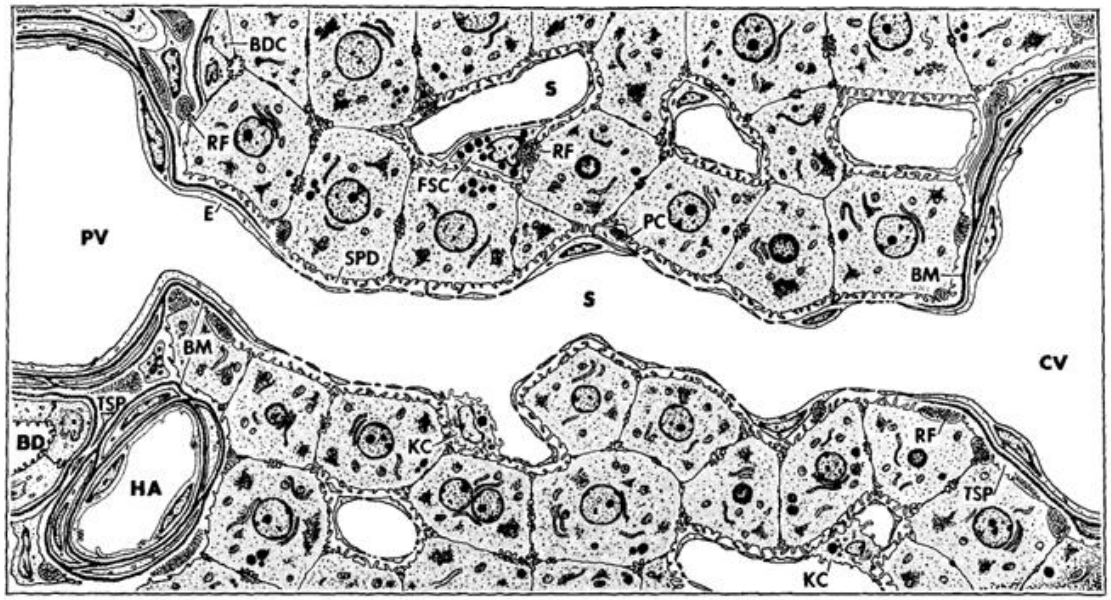


Fig. 5. Terminal lymphatics of the periportal area. The *thick arrows* indicate the possible lymph flow, coming from the space of Disse and entering a terminal lymphatic. The continuity between the space of Disse and the periportal area is represented by collagen fibers. *1*, blood capillary entering the liver parenchyma; *2*, terminal lymph vessel; *3*, sinusoid; *4*, periportal hepatocyte; *5*, space of Disse; *6*, space of Mall; *7*, collagen fibers entering the limiting plate; *8*, network of periportal collagen fibers; *9*, anchoring filaments

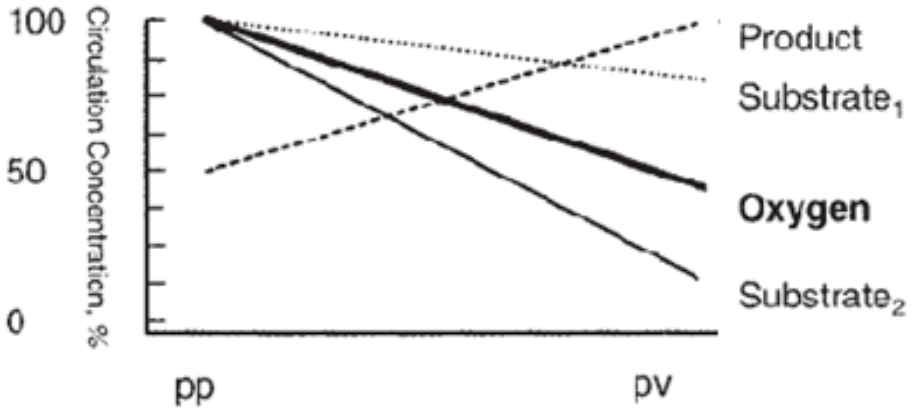
Lymph circulation in the liver.
Ohtani, O. & Ohtani, Y.; *Anat Rec;* **2008***, 291*, 643-652
The lymphatics of the liver.
Trutmann, M. & Sasse, D. ; *Anat Embryol (Berl),* **1994***, 190*, 201-209



METABOLIC ZONATION OF GLUCOSE METABOLISM

ZONATED METABOLITES

- production/utilization by hepatocytes along sinusoid
  - depending on hepatic metabolism & exchange
  - glucose & lactate between 10-50% increase/decrease
  - O2 ~50% decline with strong longterm & shortterm effects of O2 on glucose metabolism



Role of oxygen in the zonation of carbohydrate metabolism and gene expression in liver. Jungermann, K. & Kietzmann, T. *Kidney Int,* **1997***, 51*, 402-412

Long-term effects of physiological oxygen concentrations on glycolysis and gluconeogenesis in hepatocyte cultures. Wölfle, D. & Jungermann, K.; *Eur J Biochem,* **1985***, 151*, 299-303

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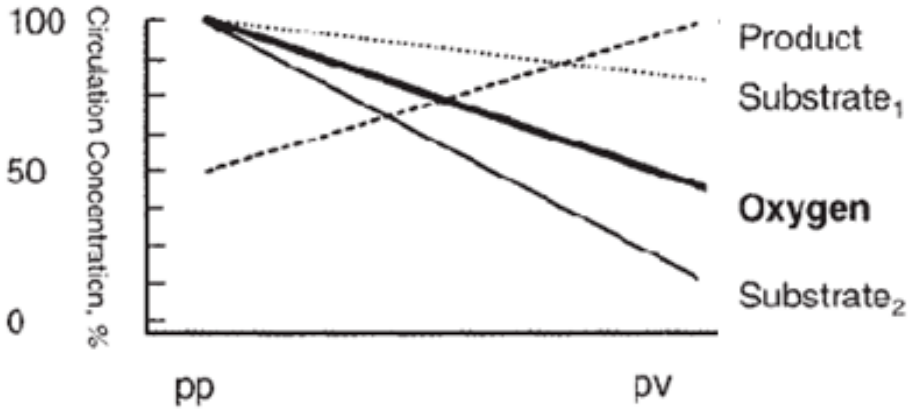

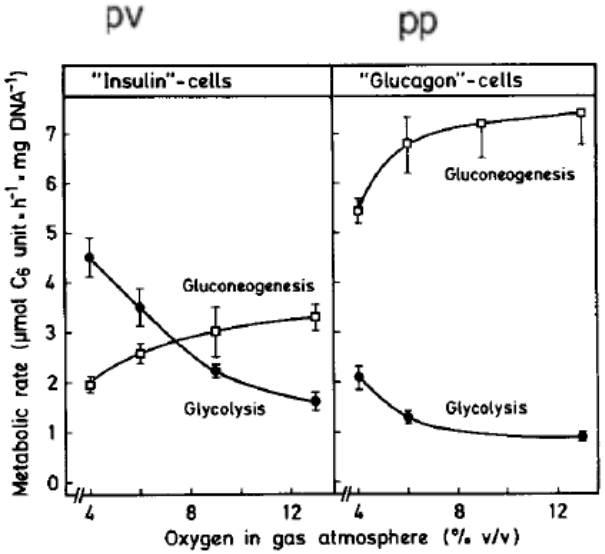

Fig. 1. *Overall long-term effects of different oxygen tensions on metabolic rates in hepatocytes.* Cells were cultured for 46 h with insulin or glucagon as the major hormone (10 nM) and dexamethasone (10 nM) under the indicated oxygen atmospheres with gentle shaking (20 rev./min). Metabolic rates were measured for 2 h after the last medium change with 5 mM glucose and 2 mM lactate under the same indicated oxygen concentrations. Either glucose or lactate were ^{14}C-labeled. Values are means ± SEM of eight cultures from at least three different cell preparations

Role of oxygen in the zonation of carbohydrate metabolism and gene expression in liver. Jungermann, K. & Kietzmann, T. *Kidney Int,* **1997***, 51*, 402-412

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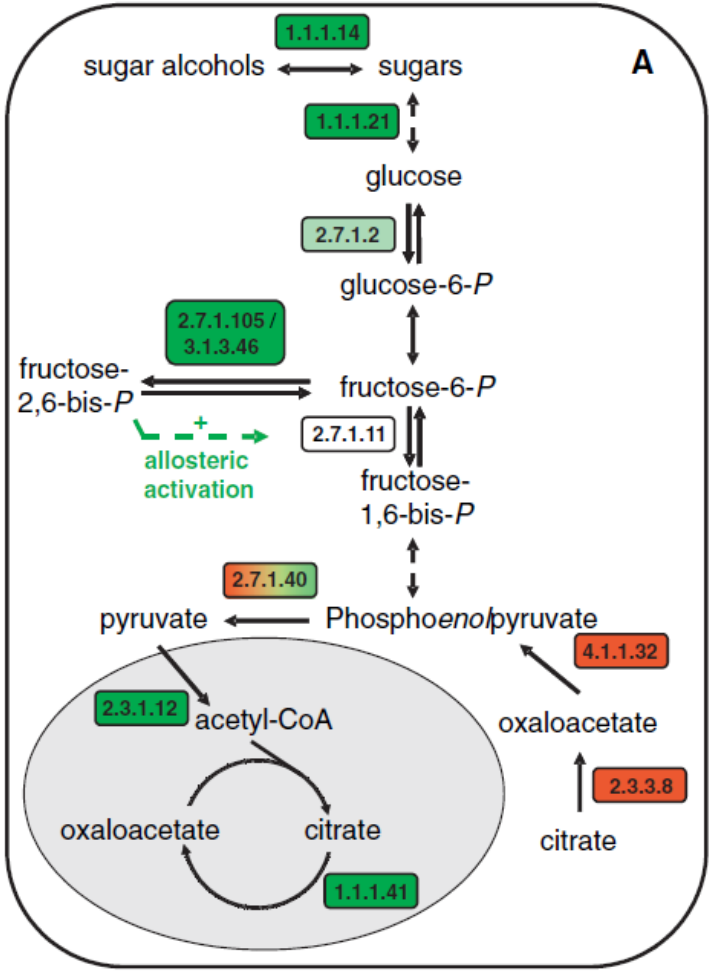
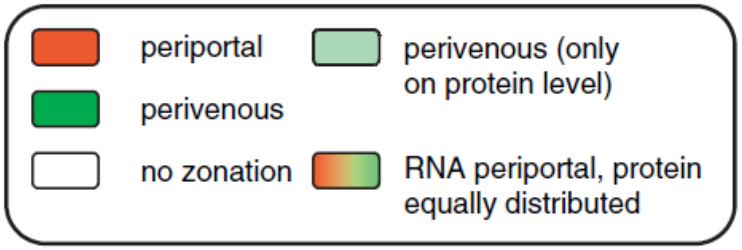
ZONATED GENE EXPRESSION

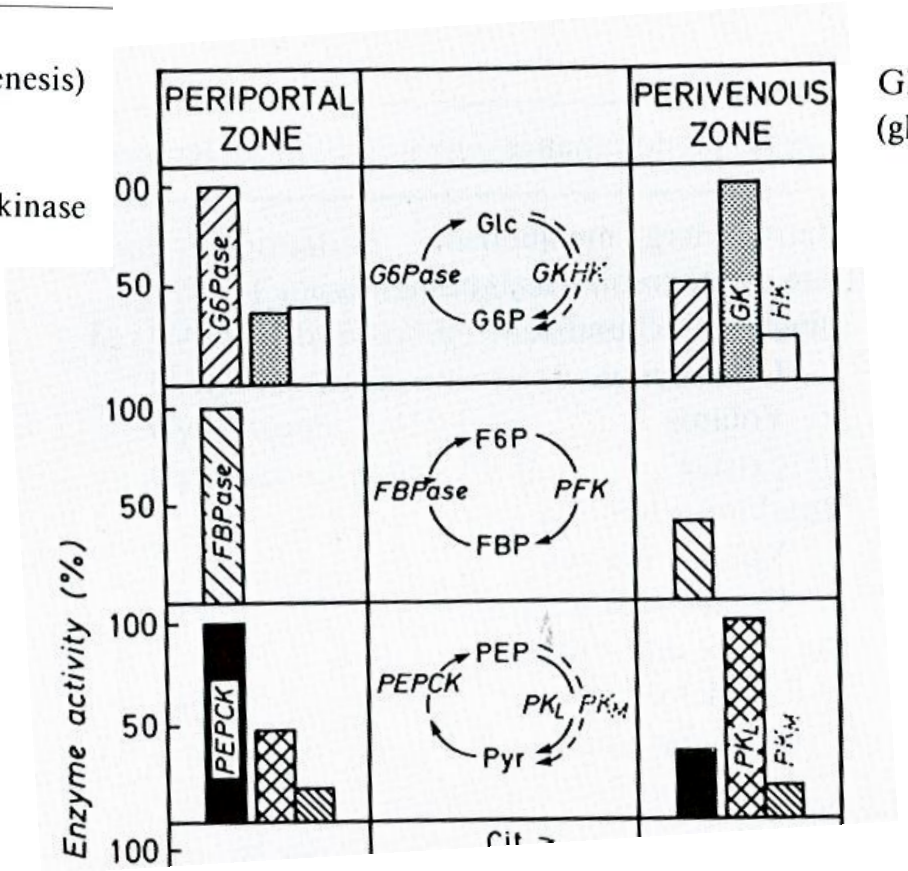




Differential gene expression in periportal and perivenous mouse hepatocytes. Braeuning, A. et al.; , **2006**, *273*, 5051-5061

ZONATED PROTEIN LEVELS

Glucose output
(glycogen degradation, gluconeogenesis)
- Glucose-6-phosphatase
- Fructose-1,6-bisphosphatase
- Phosphoenolpyruvate carboxykinase
- Lactate dehydrogenase[1]

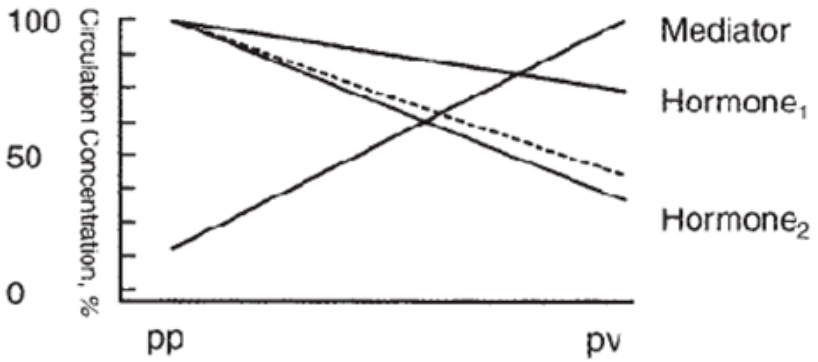


Glucose uptake
(glycogen synthesis, glycolysis)
- Glucokinase
- Pyruvate kinase L

Functional heterogeneity of periportal and perivenous hepatocytes.
Jungermann, K.; *Enzyme,* **1986**, *35*, 161-180

ZONATED SIGNALLING

- Hormonal gradients
  - liver main role in clearance of hormones
    - insulin (85-50% fasting, postprandial)
    - glucagon (~50%) & epinephrine
  - but also producer of hormones
    - large adenosine gradient
  - changing ratio (glucagon+epinephrine)/insulin



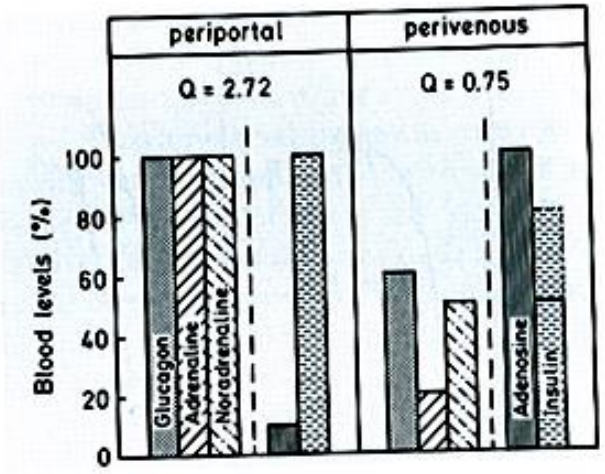


Fig. 3. Zonal heterogeneity of signals. The higher hormone concentrations either in the portal vein or in the hepatic vein were set equal to 100%. Values are from fed rats (at the shift from the absorptive to the postabsorptive phase), only with insulin the value of eating animals (absorptive phase) is indicated in addition by the dotted line. Since glucagon and the catecholamines can be regarded as antagonists of insulin and adenosine, a normalized ratio Q was defined: Q = (glucagon + adrenaline + noradrenaline)/(insulin + adenosine).

Hepatocyte heterogeneity in the metabolism of carbohydrates.
Jungermann, K. & Thurman, R. G.; *Enzyme,* **1992***, 46*, 33-58

INDUCTION OF PP/PV VIA INSULIN & GLUCAGON

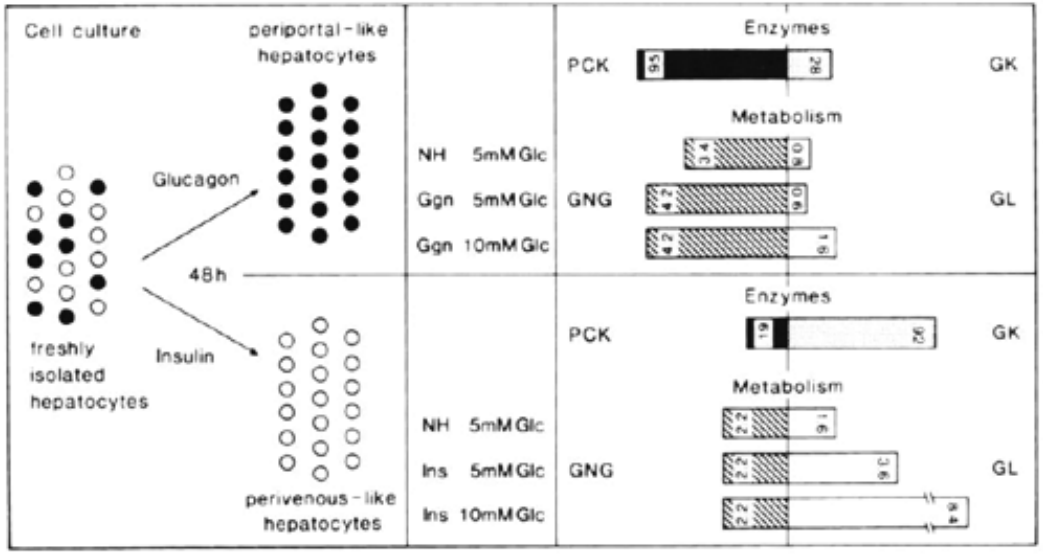
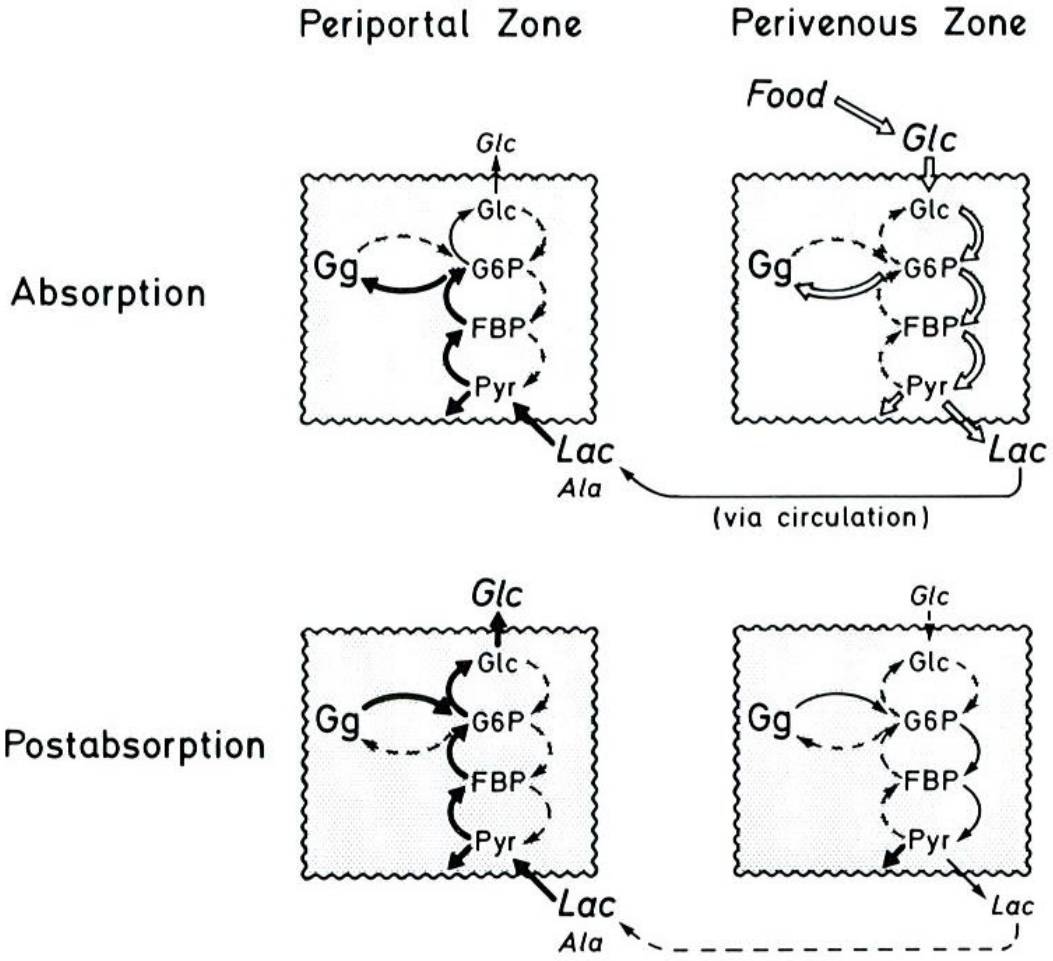


Fig. 5. Enzyme contents and metabolic rates in cultured rat hepatocytes resembling periportal and perivenous cells. The heterogeneous population of freshly isolated hepatocytes were cultured for 48 h with either high glucagon (10 nmol/l) levels to obtain periportal-like cells or with high insulin (10 nmol/l) levels to obtain perivenous-like cells. After a medium change, enzyme activities (µmol × min^{-1} × mg DNA^{-1}) and metabolic rates (µmol C_6 unit × h^{-1} × mg DNA^{-1}) were determined under substrate conditions mimicking the postabsorptive (5 mmol/l glucose, 2 mmol/l lactate) and absorptive (10 mmol/l glucose, 2 mmol/l lactate) conditions with hormone levels varying from 1 pmol/l to 100 nmol/l. Glucagon was half-maximally effective at 0.3 nmol/l and insulin at 1 nmol/l. The metabolic rates are shown for conditions that could best be regarded as an extrapolation to the in vivo situation (see text). PCK = Phosphoenolpyruvate carboxykinase; GK = Glucokinase; GNG = gluconeogenesis; GL = glycolysis; NH = no hormones; Ggn = glucagon; Ins = insulin; Glc = glucose [for details see reference 44].

Hepatocyte heterogeneity in the metabolism of carbohydrates.
Jungermann, K. & Thurman, R. G.; *Enzyme,* **1992***, 46*, 33-58

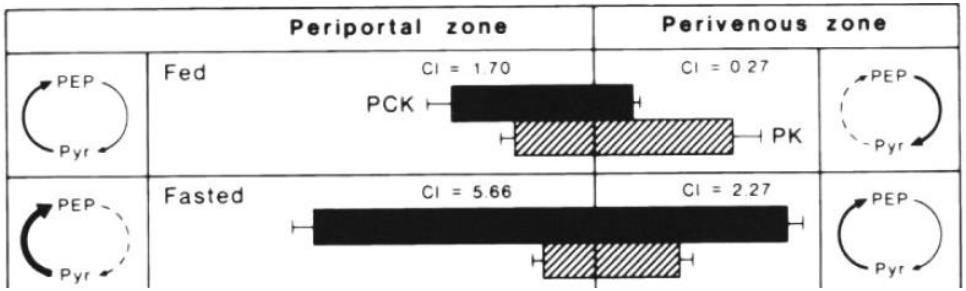
ZONATED GLUCOSE METABOLISM



Functional heterogeneity of periportal and perivenous hepatocytes.
Jungermann, K.; *Enzyme,* **1986***, 35*, 161-180

DYNAMIC ZONATION

- Adaptations of zonation depending on physiological state
  - fed -> fast transition associated with decreasing insulin & increasing glucagon
- hormone/oxygen dependent expression
  - adapting enzyme activities via hormone gradients on longer time scale (**expresson signal integrator** over the last hours)
  - in addition to fast regulation via interconvertible enzymes



Hepatocyte heterogeneity in the metabolism of carbohydrates.
Jungermann, K. & Thurman, R. G.; *Enzyme,* **1992***, 46*, 33-58