Quarterly Journal of Experimental Physiology (1975) **60**, 261–266

THE TERMINAL HEPATIC MICROCIRCULATION IN THE RAT.

By ANTHONY KOO, ISABELLA Y. S. LIANG and K. K. CHENG. From the Department of Physiology, Faculty of Medicine, Hong Kong University, 5 Sassoon Road, Hong Kong.

(*Received for publication 4th February* 1975)

The hepatic microcirculation was observed microscopically in the transilluminated liver of the rat. The portal and hepatic venous microvessels were classified into four orders according to their branching hierarchy, and the hepatic sinusoids into branching, direct and interconnecting types according to their topographic arrangements. The diameters of the various orders of microvessels and types of sinusoids were measured by serial photomicrography, and the velocity of the erythrocytes in these various microvessels and sinusoids by the dual-slit photometric technique. The microvascular volume flows were calculated from these data. In both portal and hepatic venous systems, the erythrocyte velocity and the volume flow significantly decreased in successive orders of the microvessels in apparent relation to the cross-sectional areas. The diameters of the three types of sinusoids did not significantly differ, but the velocity of the erythrocytes in the direct sinusoid was significantly faster than that in the branching sinusoid while that in the inter-connecting sinusoid fluctuated widely.

Although the terminal ramification of the hepatic microvasculature was studied many years ago by Mall [1906], it was Elias [1949] who first classified the various types of terminal portal aborization, but he did not extend the study to the entire hepatic microvasculature. Also, a systematic quantitative study of the microvascular flow in the intact liver has not been done. The present paper reports studies on the topographic classification and distribution of the hepatic microvessels and sinusoids *in vivo*, and on the quantitative measurements of their diameters and the velocity of the erythrocytes in these vessels.

METHODS

Fifty male Wistar rats of about 175 g body-weight were anaesthetized with sodium pentobarbital (40 mg/kg ip). The animal breathed spontaneously through a tracheotomy tube and the body temperature was maintained by a heating bed. As described by Cheng, Ho and Ma [1973], the anterior margin of the liver was transilluminated for direct microscopic observation through a cover-glass, using an Olympus Vanox microscope with an Oel+W 22×/0·65 (Leitz) or an FL 40×/0·75 (Olympus) objective, and the centre-line velocity of the erythrocytes in a hepatic microvessel or sinusoid projected on a screen was measured by a two-slit photometric method [Wayland and Johnson, 1967] with processing of the photometric signals by an on-line cross-correlation computer technique [Intaglietta, Tompkins and Richardson, 1970; Ma, Koo, Kwan and Cheng, 1974]. The diameter of the hepatic microvessels and sinusoids was measured with a caliper from enlarged photomicrographs taken serially with a camera fitted on top of the microscope and connected to an automatic exposuremeter system (Model PM–10–A, Olympus), using Kodak Plus-X film (ASA–125) and a Kodak Wratten green filter (No. 58). The microvascular volume flow (Q) in the hepatic microvessel was calculated from Wayland's [1973] equation: $Q = 1/2\,(V_{CL}/0{\cdot}8)A$, where V_{CL} is the apparent centre-line velocity of the erythrocytes and A the cross-sectional area of the microvessels calculated from its measured

diameter. The erythrocyte velocity and the microvascular diameter were recorded in the same preparation but not simultaneously.

The systemic arterial and the portal venous pressures were recorded from a common carotid artery and a branch of the superior mesenteric vein, through Statham P23A and P23B pressure transducers, respectively. The arterial blood pH and $PaCO_2$ were determined by the micro-Astrup apparatus (Radiometer, Copenhagen), and the venous haematocrit by the capillary tube micro-method. These five parameters did not differ significantly before and after all the experiments (all $P > 0{\cdot}5$).

Results

Plate 1 shows the general topographic distribution of the portal venous and hepatic venous systems observed microscopically *in vivo* in the transilluminated anterior liver edge. It is to be noted that central venules occur on the external surface but portal venules are usually situated below the surface making their quantitative measurements difficult. In the present study, the hepatic microvessels are classified into four orders according to their branching hierarchy and relative dimensions [Wiedeman, 1963, 1968; Duling, 1972]. Thus, in the portal venous system, a large branch of the portal vein is considered as a first order vessel, and subsequent generations of venous branchings from this vessel are assigned the ordinal numbering of orders 2, 3 and 4. The hepatic venous system is also similarly classified into four orders. Plate 2 depicts their classified topographic distributions.

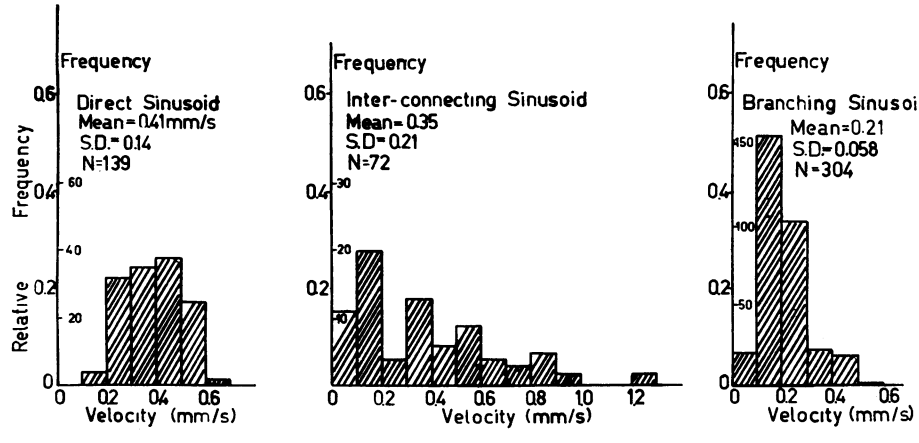


FIG. 1. Frequency distribution of the velocity of the erythrocytes in the direct sinusoids, the branching sinusoids and the interconnecting sinusoids.

Table I summarizes the microvascular diameter, the velocity of the erythrocytes, and the calculated mean volume flow as related to the different orders of the portal and hepatic venous microvessels. The velocity of the erythrocytes and the microvascular diameter between the corresponding orders of the portal venous and the hepatic venous systems did not differ significantly, but the two parameters decrease significantly ($P < 0{\cdot}001$) between the successive ascending orders of microvessels in the same system. The calculated volume flow also falls

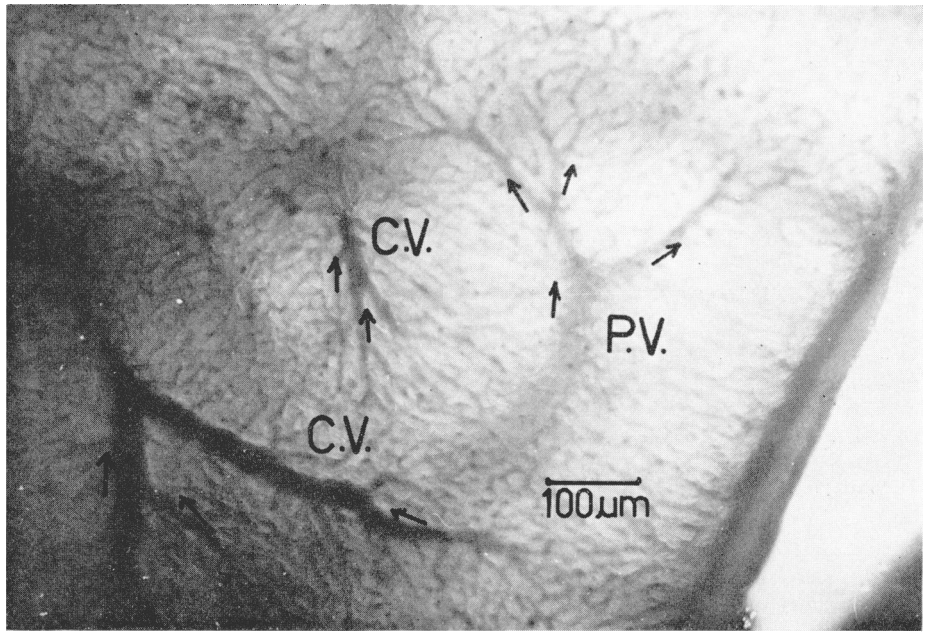


PLATE 1. Photomicrograph showing the topographic distribution of the hepatic microcirculation. (P.V. and C.V. denote the portal and the hepatic venous system respectively, with arrows pointing the direction of blood flow.)

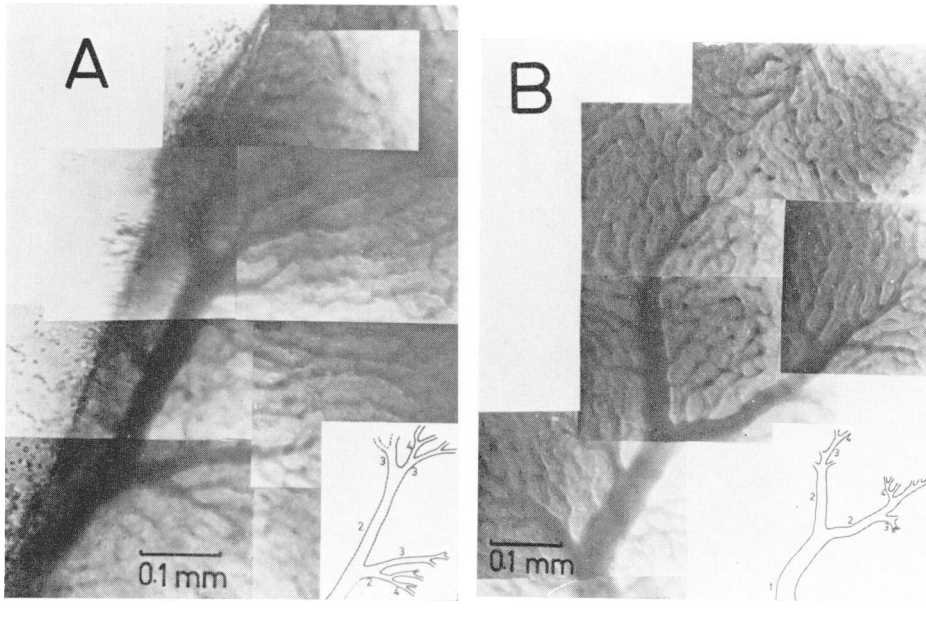
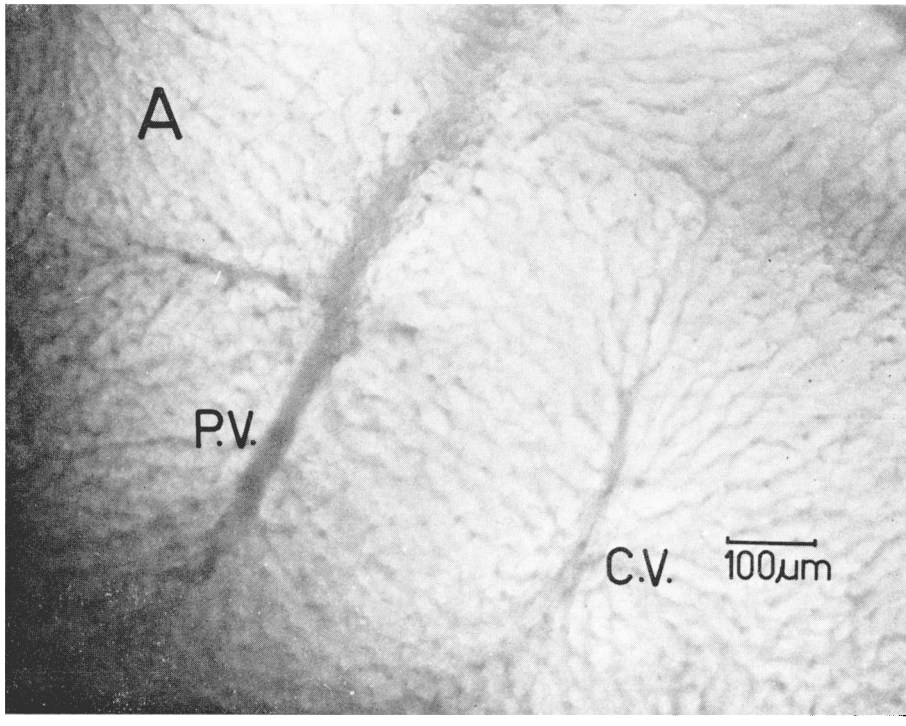


PLATE 2. Photomicrographs and line drawings showing the classification of the portal (A) and the hepatic (B) venous microvessels into orders according to their branching hierarchy and relative dimensions.

[*To face page* 262



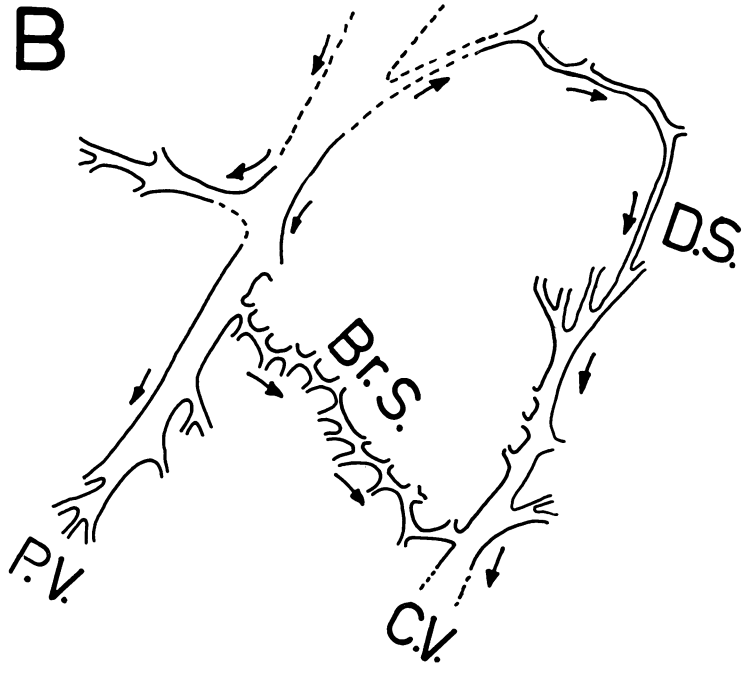


PLATE 3 [*Caption opposite*]

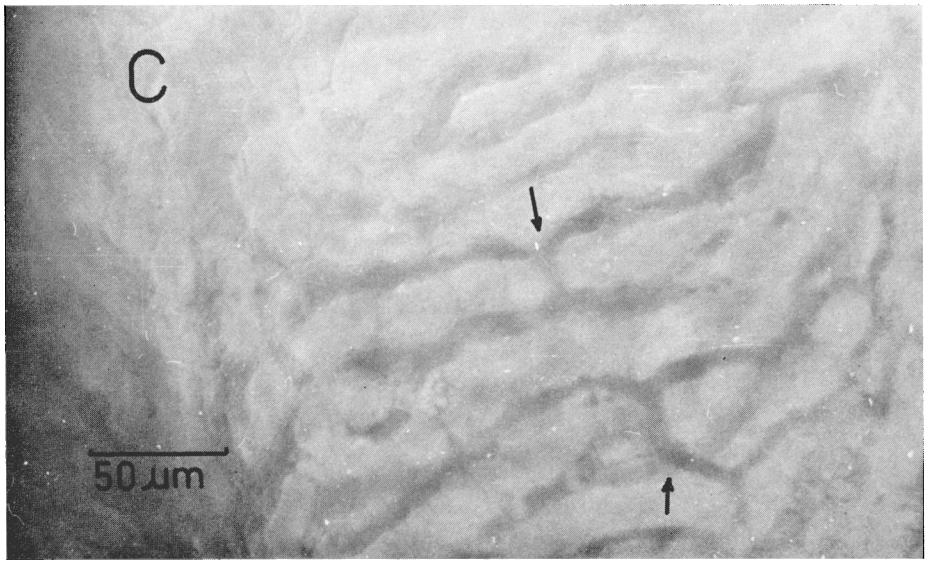


PLATE 3. Photomicrograph (A) and line drawing (B) showing the direct sinusoid (D.S.) and the branching sinusoids (Br.S.) (P.V. and C.V. denote the portal and hepatic veins respectively, with arrow pointing the direction of blood flow), and photomicrograph (C) showing the interconnecting sinusoids (at arrows).

between the successive ascending orders of the same system, that is, there is a longitudinal decreasing gradient of volume flow as the microvessel branches into tributaries in the portal and the hepatic venous systems.

TABLE I. *Classification of the portal and the hepatic venous microvessels into four orders with their respective microvascular diameter, velocity of the erythrocytes and calculated microvascular volume flow in fifty rats, body wt.* $172 \pm 2{\cdot}5$ *g, mean arterial pressure* $115{\cdot}5 \pm 3{\cdot}40$ *mm Hg, mean portal venous pressure* $9{\cdot}8 \pm 0{\cdot}34$ *mm Hg, arterial pH* $7{\cdot}4 \pm 0{\cdot}001$, $PaCO_2$ $33{\cdot}8 \pm 0{\cdot}20$ *mm Hg and venous haematocrit* $44 \pm 0{\cdot}2\%$ (*mean* $\pm$*S.E.M.*) (**the value for the velocity of the erythrocytes of order* 1 *portal venule, because the vessels are situated mostly under the hepatic surface, is obtained from an extrapolation of the correlation curve shown in Text fig.* 2)

Order of vessels	Microvascular diameter (μm)	Erythrocyte velocity (mm/s)	Volume flow (mm³/s)	Number of vessels
Portal venous system				
1	$45{\cdot}6 \pm 1{\cdot}06$	*2·66	$2{\cdot}7 \times 10^{-3}$	(5)
2	$29{\cdot}2 \pm 0{\cdot}88$	$1{\cdot}29 \pm 0{\cdot}022$	$0{\cdot}54 \times 10^{-3}$	(9)
3	$18{\cdot}8 \pm 0{\cdot}74$	$0{\cdot}62 \pm 0{\cdot}005$	$0{\cdot}11 \times 10^{-3}$	(27)
4	$9{\cdot}7 \pm 0{\cdot}18$	$0{\cdot}39 \pm 0{\cdot}004$	$0{\cdot}018 \times 10^{-3}$	(29)
Hepatic venous system				
1	$42{\cdot}0 \pm 0{\cdot}79$	$2{\cdot}03 \pm 0{\cdot}077$	$1{\cdot}76 \times 10^{-3}$	(19)
2	$29{\cdot}6 \pm 0{\cdot}53$	$1{\cdot}10 \pm 0{\cdot}030$	$0{\cdot}47 \times 10^{-3}$	(47)
3	$18{\cdot}8 \pm 0{\cdot}40$	$0{\cdot}61 \pm 0{\cdot}058$	$0{\cdot}11 \times 10^{-3}$	(49)
4	$9{\cdot}2 \pm 0{\cdot}26$	$0{\cdot}41 \pm 0{\cdot}049$	$0{\cdot}017 \times 10^{-3}$	(21)

In the present study, the observed topographic arrangement of the hepatic sinusoids shows that hepatic sinusoids could be classified into three types, namely, the branching sinusoid, the direct sinusoid and the interconnecting sinusoid. Thus, the direct sinusoid apparently forms a direct short transit channel from a terminal order 4 portal venule to a terminal order 4 hepatic

TABLE II. *Classification of the hepatic sinusoids with their respective diameter and the velocity of the erythrocytes*

Type	Microvascular diameter (μm)	Erythrocyte velocity (mm/s)	Number of vessels
Direct sinusoid	$6{\cdot}6 \pm 0{\cdot}09$	$0{\cdot}41 \pm 0{\cdot}039$	(139)
Branching sinusoid	$6{\cdot}3 \pm 0{\cdot}07$	$0{\cdot}27 \pm 0{\cdot}058$	(304)
Interconnecting sinusoid	$6{\cdot}3 \pm 0{\cdot}12$	$0{\cdot}37 \pm 0{\cdot}025$	(72)

venule, while a branching sinusoid arising from an order 4 portal venule divides into branches along a long tortuous course before the terminal branches join several adjacent order 4 hepatic venules. In contrast, interconnecting sinusoids link two parallel adjacent branching sinusoids (Plate 3) like the rungs of a ladder. Figure 1 shows the frequency distribution of the three types of sinusoids. Table II shows that the diameter of the three types of sinusoids did not differ significantly ($P > 0{\cdot}1$), but the velocity of the erythrocytes is significantly greater in the direct sinusoid than in the branching sinusoid ($P < 0{\cdot}001$),

whereas that in the interconnecting sinusoid fluctuates widely from 0·05 to 1·25 mm/s but with a mean velocity intermediate between those in the other two types of sinusoids.

Discussion

In the present study, the portal venous and the hepatic venous systems are considered as the inflow and the outflow vessels for the hepatic microvasculature, excluding the hepatic arterial system which could not be defined, though sometimes the recorded sinusoidal flow velocity is so rapid as to suggest its probable arterial origin. The importance of classifying the microvessels into different orders is that it is meaningless to define a particular vessel on the basis of its diameter alone, without determining its position and function in the microvascular system [Wiedeman, 1963], because vasomotion can easily invalidate the diameter measurements [Wiederhielm and Weston, 1973]. The order 1, 2, 3 and 4 portal microvessels in the present classification correspond to the large branch of a portal vein, the conducting vein, the axial or marginal vein and the terminal or inlet venule respectively in Elias' [1949] classification based on a histological injection study. However, the present *in vivo* study did not detect two types of portal aborization of the sinusoids reported by Elias [1949]. The terminal ramification of the hepatic venous system is apparently little studied [but see Rappaport, 1973]. The present study shows that it can be classified into four orders in the same pattern as in the portal venous system.

The present quantitative result supports the histological [Maegraith, 1958] and direct microscopic [Ho and Ma, 1972] findings of the presence of direct hepatic sinusoids which run almost as short thoroughfares from the portal venules to the hepatic venules, in contrast to the long tortuous branching sinusoid arising from a portal venule to drain into several central venules.

Both the diameter and the erythrocyte flow velocity significantly decrease (both $P < 0{\cdot}001$) in the ascending orders of the portal and the hepatic venous microvessels, hence the smaller the microvessel the slower is its erythrocyte velocity. The velocity of the erythrocytes in a particular order of the portal or the hepatic venous system, though not linearly related to the microvascular diameter, is related significantly to the square of the microvascular diameter, that is, to the cross-sectional area of the microvessel (Fig. 2, portal venous system: $P < 0{\cdot}01$, $r = 0{\cdot}996$; hepatic venous system: $P < 0{\cdot}001$, $r = 0{\cdot}999$). A similar longitudinal decreasing gradient of erythrocyte flow velocity as the microvessels become smaller has been observed in the mesentery [Gaehtgens, Meiselman and Wayland, 1970] and in the brain [Ma, Koo, Kwan and Cheng, 1974].

The different velocities of the erythrocytes in the different types of hepatic sinusoids are unrelated to the diameters which are about the same (Table II). Therefore, the significant slowing of the velocity of the erythrocytes in the branching sinusoid compared to that in the direct sinusoid ($P < 0{\cdot}001$) is apparently related to the difference in their total cross-sectional areas because there are many more branching sinusoids than direct sinusoids between the

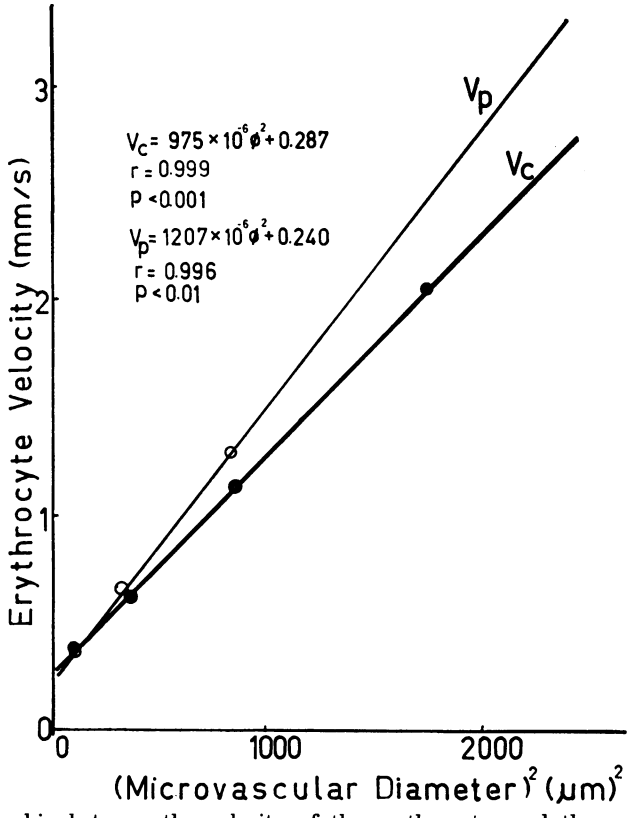


FIG. 2. Relationship between the velocity of the erythrocytes and the cross-sectional area (diameter^2) in the portal and the hepatic venous systems. V_p and V_c refer to the velocity in the portal and the hepatic venous systems. (ϕ = diameter).

order 4 portal and hepatic venules. Since their diameters did not differ significantly, the volume flows in the two sinusoids should be related to their erythrocyte flow velocities. The velocity of the erythrocytes in the direct sinusoids did not differ significantly from that in the order 4 portal or hepatic venules ($P > 0{\cdot}8$), although the diameters of the two order 4 venules are significantly greater (both $P < 0{\cdot}001$), showing that there is a rapid blood flow in the direct sinusoids for the direct transit of blood between the portal and hepatic venules. The observation supports the contention of Rappaport, Knoblauch, Black and Ohira [1970] that direct sinusoids represent an intrahepatic anastomosing channel between the portal and the hepatic venous systems. The branching sinusoids, which have a significantly slower erythrocyte flow velocity compared to the direct sinusoids ($P < 0{\cdot}001$) and the order 4 portal and hepatic venules (both $P < 0{\cdot}01$), are obviously the true venous exchange capillaries of the liver. The interconnecting sinusoids run a short course, and their conspicuous fluctuating flow rate apparently depends on the haemodynamic state of the sinusoidal channels at its two ends. A more meaningful haemodynamic analysis of the hepatic microcirculation requires the simultaneous micro-pressure data which are at present unavailable.

Acknowledgments

The authors wish to thank Mr W. S. Chan for his collaboration in this study, and Messrs C. Y. Choi, K. K. Lam and C. Y. Wat for technical assistance. The investigation was supported by grants from the Hong Kong University Research Grants Committee (Code nos. 158/233 and 167/175).

REFERENCES

Cheng, K. K., Ho, M. S. L. and Ma, Y. P. (1973). The velocity of erythrocytes in a hepatic sinusoid, and the effect of intraportal injection of adrenaline. *Quarterly Journal of Experimental Physiology*, **58**, 1–6.

Duling, B. R. (1972). Microvascular response to alteration in oxygen concentration. *Circulation Research*, **31**, 481–489.

Elias, H. (1949). A re-examination of the structure of the mammalian liver. II. The hepatic lobule and its relation to the vascular and biliary system. *American Journal of Anatomy*, **85**, 379–456.

Gaehtgens, P., Meiselman, H. J. and Wayland, H. (1970). Erythrocyte flow velocities in mesenteric microvessels of the cat. *Microvascular Research*, **2**, 151–162.

Ho, M. S. L. and Ma, R. Y. P. (1972). A study of the hepatic microcirculation. *Quarterly Journal of Experimental Physiology*, **57**, 233–236.

Intaglietta, M., Tompkins, W. R. and Richardson, D. R. (1970). Velocity measurements in the microvasculature of the cat omentum by on-line method. *Microvascular Research*, **2**, 462–473.

Ma, Y. P., Koo, A., Kwan, H. C. and Cheng, K. K. (1974). On-line measurement of the dynamic velocity of erythrocytes in the cerebral microvessels in the rat. *Microvascular Research*, **8**, 1–13.

Maegraith, B. G. (1958). Sinusoids and sinusoidal flow. In *Liver Function* (ed. R. W. Brauer), pp. 135–139. Washington, D.C.: American Institute of Biological Sciences.

Mall, F. P. (1906). A study of the structural unit of the liver. *American Journal of Anatomy*, **5**, 227–308.

Rappaport, A. M. (1973). The microcirculatory hepatic unit. *Microvascular Research*, **6**, 212–228.

Rappaport, A. M., Knoblauch, M., Black, R. C. and Ohira, S. (1970). Hepatic microcirculatory changes leading to portal hypertension. *Annals of New York Academy of Science*, **170**, 48–64.

Wayland, H. (1973). Photosensor methods of flow measurement in the microcirculation. *Microvascular Research*, **5**, 336–350.

Wayland, H. and Johnson, P. C. (1967). Erythrocyte velocity measurement in microvessels by a two-slit photometric method. *Journal of Applied Physiology*, **22**, 333–337.

Wiedeman, M. P. (1963). Dimensions of blood vessels from distributing artery to collecting venules. *Circulation Research*, **12**, 375–378.

Wiedeman, M. P. (1968). Blood flow through terminal arterial vessels after denervation of the bat wing. *Circulation Research*, **22**, 83–89.

Wiederhielm, C. A. and Weston, B. V. (1973). Microvascular, lymphatic, and tissue pressures in the unanesthetized mammals. *American Journal of Physiology*, **225**, 992–996.