Regional blood flow measurements with 15 μm and 50 μm microspheres in pregnant guinea-pigs

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Summary. Two sizes of microsphere were used to determine regional blood flow in pregnant guinea-pigs. Tissue perfusion measured with 50 μm microspheres was significantly greater than that measured with 15 μm microspheres in the small intestine, uterus, vagina, and placenta. A significantly larger proportion of the smaller microspheres passed through the systemic vasculature and could be detected in the lungs.

Introduction

The tracer microsphere technique for the determination of regional blood flow (Heymann, Payne, Hoffmann & Rudolph, 1977) requires that microspheres injected into the left ventricle will be distributed evenly in the arterial blood. It is assumed that their partition between the various regions of the systemic circulation will then be identical to the partition of the cardiac output. Blood flows are measured by calculating the number of microspheres extracted by an organ. The values obtained are thus dependent upon the relation of microsphere size to the diameter of the organ's blood vessels. To evaluate the influence of this relationship upon the measurement of regional blood flow, we have compared the values obtained with microspheres of different diameter in pregnant guinea-pigs. It was of particular interest to establish whether the trophoblastic channels of the haemochorial guinea-pig placenta are able to trap small microspheres, or whether it is advisable to use larger microspheres for the determination of maternal placental blood flow.

Materials and Methods

The animals were of mixed breed and were anaesthetized with sodium pentobarbitone (30–40 $\text{mg} \cdot \text{kg}^{-1}$ i.p.) and ventilated through a tracheal cannula. Details of the experimental technique are presented elsewhere (Sjöquist, Bjellin & Carter, 1977a, b). Microspheres labelled with ^{141}Ce (3M Co., St. Paul, Minnesota, U.S.A.) and suspended in 10% dextran were injected through a catheter in the left ventricle. Two sizes of microsphere were used to measure regional blood flow: 4×10^5 – 1×10^6 with a diameter of $15 \pm 5 \mu\text{m}$ in 9 guinea-pigs (Group 1), and 8×10^4 – 2×10^5 with a diameter of $50 \pm 10 \mu\text{m}$ in 10 animals (Group 2). A reference sample of about 1 ml femoral arterial blood was withdrawn at a constant rate ($0.84 \text{ ml} \cdot \text{min}^{-1}$) from just before the microsphere injection until shortly after its completion. The animal was then killed, and the organs listed in Table 2 were dissected free and weighed. The radioactivity in the organs and in the reference blood sample was measured in a well scintillation counter and used to calculate regional blood flow as previously described (Bjellin, Sjöquist & Carter, 1975). The statistical significance of differences between the tissue perfusions measured with the microspheres

of different size was assessed by Student's *t* test for independent observations. Within each group, the significance of the correlation between the perfusion rates of the left and right kidneys, and of the left and right halves of the brain, was determined as recommended by Colquhoun (1971, p. 276).

Results and Discussion

The basic data pertaining to the animals of the two groups are given in Table 1 and the blood flow values obtained are shown in Table 2. In most of the organs the tissue perfusion measured with the 50 μm microspheres was greater than that measured with the smaller microspheres. The greatest discrepancies occurred in the gut, uterus, vagina and placenta.

Table 1. Data relating to the two groups of guinea-pigs

	Group 1	Group 2
Microsphere size (μm)	15 $\pm$ 5	50 $\pm$ 10
No. of animals	9	10
Gestational age (days)	49–62	46–64
Mean arterial blood pressure (kPa)	6.6 $\pm$ 0.6	6.7 $\pm$ 0.5
Heart rate (Hz)	3.7 $\pm$ 0.2	3.4 $\pm$ 0.1
Correlation between right and left renal perfusion	$\left\{ \begin{array}{l} r = 0.99 \\ P < 0.001 \end{array} \right.$	$\left\{ \begin{array}{l} r = 0.92 \\ P < 0.001 \end{array} \right.$
Correlation between right and left cerebral perfusion	$\left\{ \begin{array}{l} r = 0.97 \\ P < 0.001 \end{array} \right.$	$\left\{ \begin{array}{l} r = 0.91 \\ P < 0.001 \end{array} \right.$

Table 2. Regional blood flows (mean $\pm$ s.e.m.) in pregnant guinea-pigs measured with 15 μm microspheres (Group 1, N = 9) and 50 μm microspheres (Group 2, N = 10)

Organ	Blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)		
	Group 1	Group 2	% difference [†]
Brain	0.48 $\pm$ 0.05	0.54 $\pm$ 0.08	–11
Myocardium	1.62 $\pm$ 0.46	1.28 $\pm$ 0.32	+27
Kidneys	2.61 $\pm$ 0.26	2.13 $\pm$ 0.23	+23
Adrenal glands	1.00 $\pm$ 0.11	1.00 $\pm$ 0.15	$\pm$ 0
Stomach	0.84 $\pm$ 0.09	1.30 $\pm$ 0.21	–35
Duodenum	2.40 $\pm$ 0.28	3.10 $\pm$ 0.39	–23*
Ileum	1.19 $\pm$ 0.27	2.49 $\pm$ 0.43	–52*
Ascending colon	0.80 $\pm$ 0.09	0.63 $\pm$ 0.09	+27
Descending colon	0.60 $\pm$ 0.06	0.82 $\pm$ 0.14	–27
Pancreas	0.76 $\pm$ 0.12	0.93 $\pm$ 0.17	–18
Spleen	1.61 $\pm$ 0.27	2.09 $\pm$ 0.23	–23
Urinary bladder	0.23 $\pm$ 0.04	0.31 $\pm$ 0.05	–26
Vagina	0.30 $\pm$ 0.05	0.64 $\pm$ 0.13	–53*
Uterus	0.14 $\pm$ 0.02	0.24 $\pm$ 0.04	–42**
Placenta [‡]	0.80 $\pm$ 0.08	1.10 $\pm$ 0.11	–27*
Lungs [‡]	1.37 $\pm$ 0.44	0.36 $\pm$ 0.08	+281*

[†] Difference between mean blood flows expressed as a percentage of blood flow in Group 2: * $P < 0.05$, ** $P < 0.01$.

[‡] Represents blood flow through systemic channels with a diameter exceeding that of the microspheres plus the bronchial arterial supply.

Microspheres that fail to remain in the precapillary vessels of the systemic circulation will be trapped during their subsequent passage through the lungs. Rudolph & Heymann (1967) ascertained that >99.5% of 50 μm microspheres injected in the inferior vena cava of the guinea-pig lodged in the pulmonary vascular bed. A similar result has been obtained in analogous experiments with 15 μm microspheres (L. Bjellin, unpublished data). The values for pulmonary perfusion presented in Table 2 therefore reflect the extent to which microspheres are short-circuited in the systemic circulation, although the lungs do receive a small direct contribution from the bronchial artery. The 'pulmonary perfusion' measured with 50 μm microspheres was significantly smaller than that measured with the 15 μm microspheres, which indicates that larger microspheres are extracted with greater efficiency by the systemic vasculature.

A distinctive feature of the mesenteric circulation is the presence of arterio-venous bridges or thoroughfare channels (Wiedeman, 1963). The partition of blood flow between these channels and the capillaries is thought to vary, thereby facilitating a great expansion in the number of capillaries with an active circulation (Chambers & Zweifach, 1947). It has been shown, using glass microspheres, that one-sixth to one-fourth of the blood flow to the canine ileum by-passes the capillaries through vessels with a diameter greater than 12 μm (Grim & Lindseth, 1958). These vessels are presumably thoroughfare channels, as true arterio-venous anastomoses are of little importance in the mesenteric circulation of the dog (Delaney, 1969). Direct arterio-venous channels have been described in the mesentery of the guinea-pig (Lee & Lee, 1947), and the difference between ileal and duodenal blood flows determined with 15 μm and 50 μm microspheres may represent blood flow through similar channels in the gut wall.

The uterine circulation does not appear to have been examined for arterio-venous bridges (Wiedeman, 1963). Nevertheless, our results do indicate that there are channels in the uterine wall, with a diameter of 15–50 μm , whose blood supply is almost as great as that of channels with a diameter of 15 μm or less. These wider channels are probably arterio-venous bridges but it is difficult to assign them a physiological function. They may facilitate rapid variations in capillary blood flow, for example, during the phases of myometrial contraction and relaxation.

The greater value for maternal placental perfusion with the larger microspheres (Table 2) probably reflects blood flow through labyrinthine channels with a diameter of 15–50 μm . Larger channels can be disregarded because in the perfused guinea-pig placenta 50 μm microspheres injected into a maternal artery do not appear in the venous effluent (Schröder & Leichtweiss, 1977).

Although 50 μm microspheres were efficiently extracted by most organs, including the placenta, they do not necessarily yield the most reliable estimates of regional blood flow. This size of microsphere was less evenly distributed, as demonstrated by the relatively poor correlation between perfusion of the right and left kidneys and of the right and left halves of the brain (Table 1). The less even distribution of large microspheres has been attributed to their greater tendency to axial migration within the blood stream (Phibbs & Dong, 1970) and is a source of error in the measurement of regional blood flow within the myocardium (Domenech *et al.*, 1969) and brain (Marcus, Heistad, Erhardt & Arboud, 1976). When the distribution of microspheres is uneven, the theoretical assumptions of the tracer microsphere technique become invalid. It is therefore preferable to use small microspheres and to define the population of blood vessels in which blood flow can be measured.

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