

Measurement of Organ Blood Flow using Tritiated Water. II.* Uterine Blood Flow in Conscious Pregnant Ewes

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Abstract

Total uterine blood flow was measured with a tritiated water (TOH) diffusion method and with radioactive microspheres in six, conscious, pregnant ewes.

With continuous infusion of TOH, equilibrium between the TOH concentration in utero-ovarian venous blood and arterial blood was attained within 50 min of the start of the infusion. The concentration of TOH in uterine and foetal tissue and in foetal blood water was the same as that in uterine venous water by 40 min; at this time, the concentration of TOH in the water of amniotic and allantoic fluids was 96% of that in uterine venous blood water.

Estimates of total uterine blood flow obtained using TOH were highly correlated with those obtained with microspheres ($r = 0.889$, $P < 0.001$, $n = 8$) and the corresponding mean (\pm s.e.m.) flow values obtained with the two techniques (1030.5 ± 110.6 and 1006.2 ± 100.0 ml min⁻¹, respectively) did not significantly differ. The percentage of the total uterine blood flow passing through arteriovenous anastomoses ranged from 1.4 to 3.3%.

Introduction

Methods which allow sequential measurements of uterine blood flow are required for the study of uterine nutrient metabolism. The necessity for slaughter limits the usefulness of the microsphere and other indicator fractionation techniques where it is essential to determine the concentration of the tracer in the tissue.

For the measurement of uterine blood flow in the pregnant uterus, nitrous oxide has many disadvantages. Equilibrium between blood and tissue concentration of the gas does not occur within a workable interval of time (Huckabee *et al.* 1961), analysis of the blood samples is tedious and there are difficulties in the administration of the gas to conscious animals (Setchell and Linzell 1974). 4-Amino-antipyrine and antipyrine have been used to measure uterine blood flow by the Fick technique (Huckabee and Barron 1961; Huckabee *et al.* 1961, 1972; Meschia *et al.* 1967) but neither of these substances fully equilibrates with the amniotic and allantoic fluids of the gravid uterus during the period in which blood flow is measured. Consequently, the total uterine mass to which the blood flow is related is not known precisely. Further, 4-amino-antipyrine interferes with the enzymatic estimation of glucose (Setchell and Waites 1964).

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During studies on metabolism in the gravid ovine uterus, Setchell *et al.* (1972) obtained measures of uterine blood flow using tritiated water (TOH) as a tracer. However, neither the tissues into which the TOH diffused nor the time at which the concentration of TOH in arterial and uterine venous blood were in equilibrium was reported. Additionally, it is not known how the estimates of blood flow obtained with the TOH method in the gravid uterus relate to these obtained by methods that do not rely upon diffusion of a tracer.

Accordingly, in the present study on conscious pregnant ewes, estimates of uterine blood flow obtained with the TOH method and those obtained with microspheres in the same animal were compared.

Materials and Methods

Animals and Surgical Preparation

The 12 mature, Merino ewes used in this study were 112–114 days pregnant and their live weights ranged from 40 to 50 kg. Each ewe was fed 800 g of chaffed lucerne hay per day, had free access to water and was accustomed to the experimental environment for 2–4 weeks before the experiment. In six ewes, the concentration of TOH in uterine and foetal tissue, maternal venous and foetal blood, as well as in allantoic and amniotic fluid at various times during infusions of TOH was determined (expt. I). In the remaining six animals (expt. II), uterine flow was measured using both the microsphere and TOH methods.

With the ewes under halothane anaesthesia, polyethylene catheters (1.0 mm i.d.; 1.5 mm o.d., Dural Plastics and Engineering, Dural, N.S.W.) filled with heparin saline were inserted into (a) the left ventricle via the left carotid artery, (b) the abdominal aorta via the right saphenous artery, (c) the right jugular vein and (d) one utero-ovarian vein. The latter catheter was inserted into a branch of a uterine vein and advanced so that the tip lay approximately 5 cm within the utero-ovarian vein. Only one utero-ovarian vein was cannulated for in an earlier study in which TOH was infused into conscious, pregnant ewes, the estimates of uterine blood flow based on samples taken from the two veins were 17.2 ± 0.4 (s.e.m.) and 17.6 ± 0.5 ml $100 \text{ g}^{-1} \text{ min}^{-1}$, $n = 11$ (V. H. Oddy, unpublished data).

On recovery from anaesthesia the ewes were kept in metabolism cages and the catheters were flushed daily with heparin saline until used about 7 days later. The ambient temperature in the room was maintained between 18 and 25°C.

Experiment I

To determine the concentration of TOH in the water of maternal arterial blood, uterine tissue, foetal blood and tissue or allantoic and amniotic fluids relative to that in uterine venous blood, TOH was infused into the jugular vein for 60 min at an exponentially decreasing rate (Oddy *et al.* 1981) while blood was sampled continuously at a rate of 1.02 ml min^{-1} from a utero-ovarian vein and the left ventricle. The blood was heparinized and dispensed at 3-min intervals into separate tubes on a refrigerated fraction collector, described by Oddy *et al.* (1981). The ewes were killed with an overdose of Pentobarbitone Sodium at either 20 ($n = 3$), 30 ($n = 2$), or 40 ($n = 1$) min after the start of the infusion and triplicate subsamples of each tissue and fluid were taken within 5 min of death. The water content in these samples was vacuum-distilled (Oddy *et al.* 1981) and counted together with the blood samples in a liquid scintillation counter (Model PW 4510, Philips, Holland) to determine the level of radioactivity. The concentration of TOH in whole blood was then measured using the method of Pappenheimer and Setchell (1972). Dry matter content of these samples was determined as described by Oddy *et al.* (1981).

Experiment II

Uterine capillary blood flow was determined using the microsphere method described by Hales (1974). A known dose of 15 ± 3.0 (mean \pm s.d.) μm diameter microspheres (approximately 15

million and labelled with either ^{113}Sn , ^{46}Sc or ^{141}Ce ; New England Nuclear Co., Boston, Massachusetts) was delivered via the indwelling catheter into the left ventricle while blood was being withdrawn from the abdominal aorta at approximately 25 ml min^{-1} ('reference organ' sample). Microspheres of this size provide measures of capillary blood flow in tissues, as the diameter of capillaries rarely exceeds $8 \mu\text{m}$ and that for patent anastomoses falls between 25 and $150 \mu\text{m}$ (Hales 1974). Simultaneously, a blood sample was withdrawn from a utero-ovarian vein at approximately 15 ml min^{-1} to enable estimates of total uterine blood flow and the anastomosal component of that flow to be made (Archie *et al.* 1973). Ten minutes later, TOH was infused into the jugular vein while blood was withdrawn from a utero-ovarian vein and the left ventricle as described in experiment I. In two ewes, a second set of blood flow measurements were obtained with the two methods; the first and second dose of microspheres being distinguished by different nuclides.

On completion of the final blood flow measurement, the animal was killed and the uterus was removed within 2 min and weighed. The foetus, uterine fluids and placenta were separated and weighed and the uterus, including the maternal cotyledons, was divided into left and right uterine horns and uterine body. These portions of the uterus were weighed, separately minced and quadruplicate subsamples of each were counted together with the arterial and venous blood samples in an autogamma spectrometer (Model 5320, Packard Instruments, La Grange, Illinois, U.S.A.) for their radioactive content. Blood sampled during the TOH infusion was processed as described in experiment I. Three of the ewes were killed 3 weeks after the experiment. For these, an assessment of the total uterine weight at the time of the experiment was made using the uterine growth curves for Merino ewes reported by Cloete (1939).

Analysis of the Data

Microsphere method

Uterine capillary blood flow \dot{Q}_c (ml min^{-1}) and total uterine blood flow \dot{Q}_T (ml min^{-1}) were calculated using the following equations [(1), see Hales (1974); (2), see Archie *et al.* (1973)]:

$$\dot{Q}_c = \dot{Q}_{ar}(I_t/I_{ar}) \quad (1)$$

and

$$\dot{Q}_T = I_t/[(I_{ar}/\dot{Q}_{ar}) - (I_{vr}/\dot{Q}_{vr})] \quad (2)$$

where \dot{Q}_{ar} represents the rate of withdrawal (ml min^{-1}) of the arterial reference sample; \dot{Q}_{vr} is the rate of withdrawal (ml min^{-1}) of the venous reference sample; I_t represents the counts per minute of radioactivity in the tissue; I_{ar} represents the counts per minute of radioactivity in the arterial reference sample; and I_{vr} is the counts per minute of radioactivity in the venous reference sample.

The percentage of the total flow that passed through arteriovenous anastomoses (AVAs) was calculated from the expression $[(\dot{Q}_T - \dot{Q}_c)/\dot{Q}_T] \times 100$.

TOH method

Total uterine blood flow F ($\text{ml g}^{-1} \text{ min}^{-1}$) was calculated using the Fick equation (Kety and Schmidt 1945) as described in detail by Oddy *et al.* (1981).

$$F = \left\{ [C_v(\text{eq.}) \cdot S] / \left[\int_0^{t(\text{eq.})} (C_a - C_v) dt \right] \right\} \times 100,$$

where C_a and C_v are the concentrations of TOH in arterial and venous whole blood during the approach to equilibrium; $C_v(\text{eq.})$ is the concentration of TOH per millilitre of venous blood at equilibrium; S (partition coefficient) is the ratio of the TOH concentration per gram of tissue to venous concentration per gram at equilibrium.

From preliminary experiments (V. H. Oddy, unpublished observations), the mean (\pm s.e.m.) values for the fraction of water in the pregnant uterus and in arterial blood was calculated to be 0.869 ± 0.003 ($n = 6$) and 0.834 ± 0.002 ($n = 15$), respectively. Using these values and the correction factor 1.04 to account for the dilution of blood with heparin, the corrected partition coefficient S required in the calculation of blood flow was 1.0. The weight (or derived weight) of the uterus and conceptus was then used to calculate flow in absolute terms (ml min^{-1}).

Estimates of total blood flow obtained with the two methods were compared using paired *t*-test and regression analysis.

Results

Experiment I

The relative concentrations of TOH, expressed as $100 \times (\text{TOH per millilitre of water in blood, tissue or uterine fluid} / \text{TOH per millilitre of water in uterine vein blood})$, at 20, 30 and 40 min after the start of the infusion for the maternal arterial

Table 1. TOH concentration ratio in uterine and foetal tissue, foetal blood and allantoic and amniotic fluid at 20, 30 and 40 min after the start of a TOH infusion

Values represent means (range) for three (20 min), two (30 min) and one observation at 40 min. Values for percentage water content of components represent means of six observations

Time after start of infusion (min)	Maternal arterial blood	Uterus	TOH concentration ratio in:			
			Foetal tissue	Foetal blood	Allantoic fluid	Amniotic fluid
20	105 (101-117)	80.9 (78-84.8)	77.7 (73.8-81.4)	89.8 (88.2-90.8)	57.2 (52-67.3)	32.4 (25-38.9)
30	101 (100-102)	81.8 (73.5-100)	85.7 (71.4-100)	95.2 (90.3-100)	73.4 (49.8-97)	71.2 (45.4-97)
40	100	100	100	100	96	96
Water content (%) \pm s.e.m.		84.9 0.17	83.4 0.26		96.4 0.22	97.6 0.49

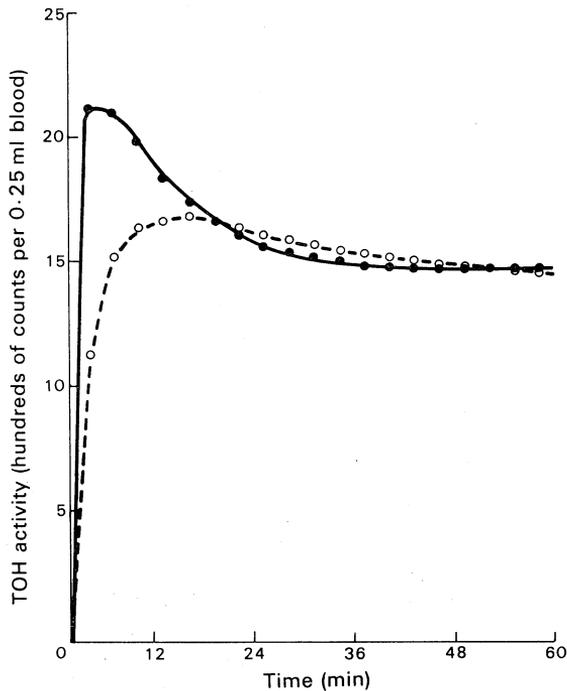


Fig. 1. Time course of TOH activity in ventricular arterial (●) and uterine venous blood (○) in one ewe. Infusion of TOH continued for the whole of the blood sampling period (60 min).

blood, uterine and foetal tissues, foetal blood, allantoic and amniotic fluids are shown in Table 1. Concentration of TOH in uterine and foetal tissue and maternal arterial, uterine venous and foetal blood were in equilibrium by 40 min, while the TOH concentration in the water in the uterine fluids was 96% of that in uterine venous blood water. Consequently, TOH was infused for 60 min into each ewe in experiment II to ensure that equilibrium conditions were achieved.

Table 2. Estimates of total uterine blood flow measured by the TOH and microsphere techniques in six conscious, pregnant ewes

Sheep No.	Days after mating	Total uterine weight including fluids (g)	Microspheres			TOH technique	
			Capillary flow (ml min ⁻¹)	AVA flow (%)	Total flow (ml min ⁻¹)	Total flow (ml 100 g ⁻¹ min ⁻¹)	Total flow (ml min ⁻¹)
264	113	3130	704.4	2.4	721.4	27.2	851.0
	114	3130	805.6	2.2	824.2	32.8	1077.6
7497	113	3998	1125.3	2.2	1150.0	28.1	1121.8
	114	3998	1024.8	2.3	1049.1	21.4	856.4
7422	114	3400	856.4	2.4	877.1	23.0	782.7
44	113	3500 ^A	954.3	3.3	986.9	26.9	941.5
28	112	6500 ^{A, B}	1593.6	1.4	1616.7	25.9	1748.5
74	112	3500 ^A	806.4	2.2	824.3	24.7	864.5
Mean			983.9	2.3	1006.2	26.3	1030.5
			± s.e.m.				
			± 99.3	± 0.2	± 100.0	± 1.23	± 110.6

^A Calculated weight (see Materials and Methods).

^B Twins.

Experiment II

The concentration of TOH in maternal arterial (ventricular) and uterine venous blood plotted as a function of time, is shown for one ewe in Fig. 1. The time taken for the TOH concentration in the arterial and uterine venous blood to reach equilibrium depended upon the rate of blood flow and ranged from 18 to 50 min. In some experiments, the TOH concentration in venous blood exceeded that in arterial blood within the first 20 min and remained at a slightly higher level until equilibrium conditions were reached at 40–50 min after the start of the infusion.

The total uterine blood flow values (Table 2) obtained with the TOH method were well correlated with those obtained with microspheres ($r = 0.889$, $P < 0.001$) and the mean values (\pm s.e.m.) did not differ significantly (1030.5 ± 110.6 ml min⁻¹ and 1006.2 ± 100.0 ml min⁻¹, respectively). The proportion of the total uterine blood flow that passed through AVAs was particularly small and ranged from 1.4 to 3.3% of the mean flow (Table 2).

Discussion

The present study shows that TOH not only diffuses into the tissues which are perfused with maternal blood but also into the conceptus including the amniotic and allantoic fluids. Diffusion of the TOH into these fluid compartments does not diminish the usefulness of TOH as a tracer for measuring blood flow in the pregnant uterus for, with time, the concentration of TOH in these fluids equilibrates with that in arterial and venous blood water.

Although blood flow in the gravid uterus can undergo periodic fluctuations (Assali and Morris 1964) and the microsphere and TOH methods provide measures of flow over different durations of time, there was good agreement between assessments of total uterine blood flow made with the two techniques in the same animal. As the amount of the total uterine blood flow passing through AVAs in the gravid uterus around 112–114 days is extremely small (1.4–3.3%), it may not always be necessary to make an assessment of this component. On the other hand, AVA flow may be quite an important component of total uterine blood flow at other stages of pregnancy or in the non-gravid uterus. While catheterization of the utero-ovarian veins induced abnormally high flow through the ovaries (Mattner *et al.* 1976), it is clear that cannulation of this vein in the present study did not result in the occurrence of abnormal levels of flow through the uterus. The values for total uterine blood flow ($\text{ml } 100 \text{ g}^{-1} \text{ min}^{-1}$) obtained with the TOH method in this study (mean, 26.3) and in that of Setchell *et al.* (1972) (mean, 27.0) were of the same order as those measured with 25- μm microspheres in sheep at a similar stage of pregnancy (Rosenfeld *et al.* 1974; Rosenfeld 1977). Since the microsphere method is based upon simple mechanical principles, it is generally considered to provide accurate measures of blood flow. In view of the present findings, it appears, therefore, that the TOH method also provides satisfactory estimates of total flow in the gravid uterus in conscious sheep.

The TOH method suffers from the disadvantage that surgery is required for cannulation of the uterine blood vessels. However, the method has the advantage of low cost and because TOH equilibrates with all water of the pregnant uterus, there is no need to kill the animal to determine the accumulation of the marker in this organ. Accordingly, the method can be used in chronic preparations to provide sequential measurements of both blood flow and metabolic uptake in the pregnant uterus.

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