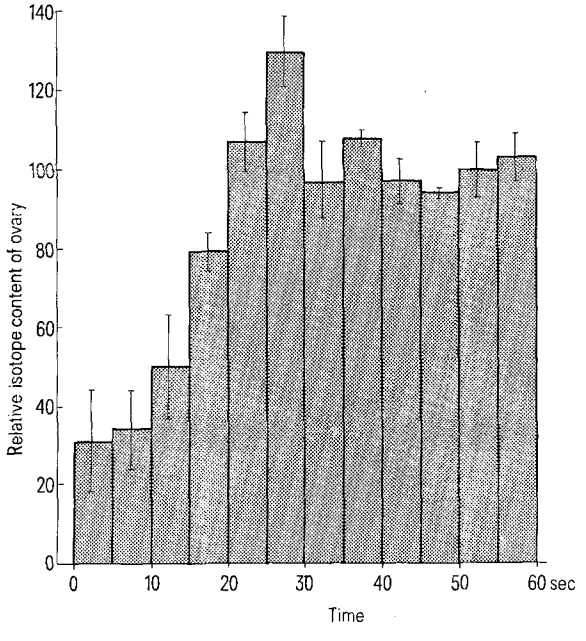
Capillary Blood Flow in Sheep Ovaries, Measured by Iodoantipyrine and Microsphere Techniques

Ovarian venous outflow has been measured at different stages of the oestrous cycle in sheep[1]. However, there are numerous large arteriovenous anastomoses (AVA's) in the interstitial tissue of ovine ovaries and these may shunt relatively large amounts of blood directly to the venous drainage[2]. Thus the ovarian venous outflow is not necessarily a measure of the capillary or nutrient blood flow.

Estimates of blood flow obtained with microspheres of small diameter or with soluble indicators such as [^{86}Rb]-rubidium chloride or [^{125}I]-iodoantipyrine[3] should represent capillary blood flow. However, in preliminary observations in sheep[4], microspheres of 15 μm diameter provided much higher estimates of blood flow through corpora lutea than did soluble indicators. Furthermore, rubidium chloride[5] and microspheres[6] yielded markedly dissimilar estimates of blood flow in ovaries of pregnant rabbits. The results obtained in the present study support the previous finding in sheep and offer an explanation for the differing assessments provided by the two types of indicator.

Materials and methods. a) *Changes in [4-^{125}I]-iodoantipyrine content of the ovary with time.* In each of 4, mature, Merino ewes anaesthetized with sodium pentobarbitone, a catheter (1.40 mm I.D., 1.90 mm O.D.) was inserted into the posterior vena cava via a recurrent tarsal vein.

An ovary was exteriorized through a small mid-ventral abdominal incision and shielded from body radiation by heavy-gauge lead sheeting. A dose of [4-^{125}I]-iodoantipyrine (60 μCi) (Radiochemical Centre, Amersham) contained in 4 ml of 0.9% saline was injected through the catheter and the radioactivity levels of the ovary were continuously monitored using a sodium iodide collimator connected to a pulse height analyser (Nuclear Chicago Des Plains, Illinois, U.S.A.) and a digital recorder. The collimator was positioned close to the ovary, parallel with the ventral surface of the abdominal wall. The level of background radiation was obtained prior to delivery of the test material.



Amount of radioactivity (± S.E. of the mean) in ovaries from 4 ewes at various times after i.v. injection of [^{125}I]-iodoantipyrine. The counts registered during each 5 sec interval are expressed as a percentage of the mean counts per 5 sec interval registered during plateau conditions (between 30 and 60 sec).

b) *Estimation of capillary blood flow by fractional distribution of [4-^{125}I]-iodoantipyrine and [^{141}Ce]-cerium labelled microspheres.* 8 conscious, mature Merino ewes, 4 at oestrus and 4 at day 9 of the oestrous cycle (oestrus = day 0), were used. Doses of microspheres (15 ± 5 μm diameter) labelled with ^{141}cerium (3 *M* Company, St. Paul, Minnesota) were prepared as previously described[7]. Each dose contained approximately 15×10^6 microspheres. The doses of iodoantipyrine (60 μCi) were each prepared in 4 ml of 0.9% saline in a syringe.

Several hours prior to the experiment, 3 catheters – 1 in the left cardiac ventricle, 1 in a femoral artery and 1 in a recurrent tarsal vein – were implanted in each ewe. A stainless steel 'T' piece, filled with heparinized 0.9% saline was connected to the ventricular catheter. The microsphere dose was injected through one arm of the 'T' piece and washed into the ventricular catheter with 0.2 ml saline which was insufficient to wash any of the test dose into the circulation. The iodoantipyrine dose was then injected into the ventricular catheter through the second arm and followed immediately by 3 ml of 0.9% saline to introduce both indicators to the circulatory system simultaneously. 27 sec later, 40 ml of saturated KCl was delivered through the recurrent tarsal catheter to stop the heart within 35 to 40 sec after delivery of the indicators (while the iodoantipyrine content of the ovaries was constant; see Figure). The ovaries were removed during the following minute. When an ovary contained a corpus luteum (CL), the luteal and non-luteal (interstitial tissue) portions were separated and weighed before the counts of radioactivity attributable to each indicator was determined in an autogamma spectrometer (Packard Instruments; ^{125}I, 6–78 kev; ^{141}Ce, 110–187 kev). The radioactivity remaining in the ventricular catheter, the 'T' piece and syringes was also counted so that the effective dose could be determined.

For the determination of cardiac output according to the method described by HALES[7], blood was withdrawn from the femoral artery catheter. Capillary blood flow in the ovarian tissues was calculated by multiplying the amount of radioactivity in the tissues (expressed as a fraction of the dose) by the cardiac output.

Results. a) Changes in the [4-^{125}I]-iodoantipyrine content of the ovary with time. The level of radioactivity in the ovary increased gradually until 25–30 sec after the initial injection and then remained relatively constant over the next 30 sec (Figure); this pattern was identical whether or not the ovary contained a CL. In the subsequent experiments, therefore, the ewes were killed 35–40 sec after delivery of the indicator.

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Capillary blood flow (ml/min) in the ovaries of ewes at oestrus (day 0) and at day 9 of the oestrous cycle, as determined by the use of [^{141}Ce-] microspheres and [^{125}I]-iodoantipyrine

Tissue	Day 0		Day 9	
	[^{141}Ce]	[^{125}I]	[^{141}Ce]	[^{125}I]
Ovary containing either a large follicle (day 0) or a CL (day 9)				
1 Total ovary	0.27 ± 0.15	0.12 ± 0.06	4.02 ± 0.65 [a]	0.75 ± 0.08
2 Interstitial tissue	—	—	0.14 ± 0.02	0.19 ± 0.04
3 CL	—	—	3.88 ± 0.63 [a]	0.56 ± 0.10
Contralateral Ovary	0.13 ± 0.08	0.07 ± 0.03	0.01 ± 0.00	0.02 ± 0.01

Means ($\pm$ SEM) based on 4 ewes. [a] $P < 0.02$.

b) Estimation of capillary blood flow using [4-^{125}I]-iodoantipyrine and microspheres. In each of the oestrous ewes, there was only 1 Graafian follicle larger than 6 mm in the ovaries while each of the ewes at day 9 of the oestrous cycle had only 1 large CL. The capillary blood flow values for whole ovaries and for luteal and non luteal (interstitial) portions of ovaries are shown in the Table. The estimates of blood flow obtained with the 2 indicators in either whole ovaries at oestrus or in non-luteal tissue at day 9 were not significantly different ($P > 0.25$ by paired t-test). However, the estimates of blood flow in CL obtained with the microspheres were significantly greater (7-fold on average) than those obtained with iodoantipyrine ($P < 0.02$ by paired t-test).

Discussion. In ovaries containing CL, the estimates of capillary blood flow that were obtained using 15 µm microspheres were markedly greater than those obtained using iodoantipyrine as the indicator. Partitioning of the flow in these ovaries showed that this was due to the 2 indicators providing different assessments of blood flow in the luteal tissue only.

While there are no obvious reasons to suggest that the microsphere method gave falsely high estimates of capillary blood flow in the CL, there are several instances when the use of soluble indicators has underestimated capillary blood flow in certain tissues[8–10]. The microsphere method relies simply upon mechanical trapping of particles within the capillary bed of the tissues, while estimates of capillary blood flow obtained with soluble indicators could be influenced by factors that affect the permeability of the tissue to the indicator. As the venous outflow from ovine ovaries with active CL is about 8 ml/min[1], a capillary blood flow value of 4 ml/min in such ovaries does not appear to be unduly high. Nor is this flow, which is equal to 7.6 ml/min/g of tissue excessively high when compared with capillary blood flow in the thyroid and kidney (4.6 and 5.5 ml/min/g respectively)[7] or in peri-renal brown adipose tissue of lambs when it is being actively metabolized (11.3 ml/min/g)[11].

With soluble indicators, reliable estimates of capillary blood flow are obtained only if the organ in question and the body (as a whole) have the same extraction ratio for the indicator[3]. This does not apply with all indicators and tissues, for rubidium chloride is almost entirely rejected by the brain as a whole[8] and by some compartments of both the pineal body[9] and the testis[10]. In these organs, the radioactivity rises to a peak level within a few seconds after isotope delivery and then falls rapidly to plateau levels[9]. However, as the curve for iodoantipyrine content of ovaries with CL rose continuously until plateau levels were reached (as in the Figure), it is clear that the CL did extract iodoantipyrine from the blood and furthermore, acted as a one compartment tissue in respect to the uptake of this indicator. Thus if the use of iodoantipyrine does provide an unreasonably low estimate of luteal capillary blood flow, then the CL must have a lower permeability to iodoantipyrine than the tissues of the body as a whole.

It appears that there may also be some limitation to uptake of rubidium chloride in the CL as the pattern of change in the amount of radioactivity in ovine ovaries following i.v. injection of this indicator closely paralleled that observed after the administration of iodoantipyrine. In addition, the estimates of capillary blood flow obtained for ovine ovarian tissue during within-animal comparisons of these 2 indicators were almost identical[2]. Thus rubidium chloride, like iodoantipyrine, is probably not suitable for determining capillary blood flow through CL of sheep.

Résumé. La circulation capillaire du sang dans les ovaires des moutons a été mesurée en se servant des techniques d'iodoantipyrine radioactive et de microsphère (15 $\pm$ 5 µm diamètre). Pour les tissus non-lutéine, les estimations de circulation capillaire du sang obtenues par les deux méthodes furent similaires; pour les corpora lutea, les estimations obtenues avec les microsphères étaient, en moyenne, 7 fois plus grande que celles obtenues avec l'iodoantipyrine comme indicateur.

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