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PERMEABILITY OF THE SHEEP PLACENTA TO UNMETABOLIZED POLAR NON-ELECTROLYTES

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SUMMARY

1. The rate of appearance in uterine venous blood of radioactively labelled, polar, non-electrolytes has been measured following their injection into the foetal circulation in the chronically catheterized sheep near term. Uterine blood flow was measured by an antipyrine technique.

2. Estimates of a placental permeability constant not corrected for area were $61{\cdot}2 \pm 5{\cdot}5$ ml. min^{-1} (mean ± S.E. of mean) for [^{14}C] urea, $1{\cdot}85 \pm 0{\cdot}16$ for [^{14}C]erythritol and $0{\cdot}21 \pm 0{\cdot}03$ for [^{3}H]mannitol. Results are also presented for [^{14}C]ethylene glycol, [^{14}C]L-glucose, [^{14}C]mannitol, [^{51}Cr] EDTA and [^{3}H] and [^{14}C]sucrose.

3. In four sheep, permeability measurements for several solutes were made and the results were analysed in terms of restricted diffusion via cylindrical, water-filled pores. Calculation of pore radius was made by a minimum variance method and values ranging from 0·43 to 0·45 nm were obtained.

4. Stability and absence of protein binding of probe molecules was investigated by gel permeation on Sephadex columns.

INTRODUCTION

A knowledge of the permeability of the placenta to substances thought to cross it solely by diffusion through the aqueous phase is a useful basis from which to study more complicated forms of transfer. Furthermore, the finding that diffusion of polar substances across the rabbit placenta shows progressive restriction with increasing molecular size (Stulc, Friedrich & Jiricka, 1969; Faber, Green & Lang, 1971) suggests the possible existence of transplacental water-filled channels, or pores, of molecular dimensions similar to those proposed for capillary walls by Pappenheimer (1953).

This paper reports measurements of the permeability of the sheep placenta to such substances following their injection into the foetus, and

the measurement of their rate of transfer into the mother by application of Fick's principle to the uterine circulation (Meschia, Battaglia & Bruns, 1967). Studies were made on conscious sheep up to 6 days after the implantation of sampling catheters in the vascular system on both the maternal and foetal sides of the placenta (Meschia, Makowski & Battaglia, 1969–70).

METHODS

Surgical procedure

Experiments were made on pregnant ewes of Dorset, Clun or mixed breeds, near term (range 128–139 days gestational age). Surgical techniques were based on those of Meschia *et al.* (1969–70). Ewes were starved for 48 hr pre-operatively. After sedation with approximately 500 mg pentobarbitone I.V., maternal spinal anaesthesia was achieved by the injection of 0·4 ml. 0·5% cinchocaine hydrochloride B.P. in 6% glucose. Further pentobarbitone was given I.V. during surgery as necessary, to a total dose of 1–2 g. Catheters, 1·5 mm o.d. (maternal) and 0·95 mm o.d. (foetal), of vinyl (Vygon) or of polyethylene (Portex), were siliconized, implanted under sterile conditions and brought out through the mother's flank for subsequent sampling. They were kept filled with heparinized saline (1000 u. $ml.^{-1}$) and flushed through daily. Ampicillin (500 mg) was added to the amniotic fluid at the time of surgery, and benzylpenicillin (300 mg) and streptomycin (500 mg) were injected I.M. into the mother for 4 days after surgery.

In most preparations five catheters were placed. A catheter was first inserted into a maternal femoral artery by a direct approach just below the inguinal ligament and passed 15 cm up the vessel. A catheter was then placed in a uterine vein, via a needle venepuncture near the ovary, so that its free end was about 7 cm inferolateral to the ovary, lying in the large vein which runs towards the pelvic wall in the broad ligament. The puncture wound was closed by a purse string suture. In two animals a second catheter was placed in another uterine vein to check the importance of exact catheter position. If twins were present the foetus lying on the side of the catheterized uterine vein was selected. The uterus was then opened and two venous catheters passed 10–25 cm up the pedal veins of one or both hind limbs of the foetus. A foetal arterial catheter was similarly passed up a hind limb pedal artery. All catheters were tied in position and secured with Eastman tissue glue. In those animals whose autopsies were performed before delivery of the lamb, catheter position and vessel patency was confirmed. During study periods samples were collected aseptically.

Experimental protocol

The injection of an isotope into the foetus was followed by measurement of the permeability of the placenta to it over a period of from 30 to 400 min, depending on the isotope. This was done on one or several occasions in an individual sheep, each injection and subsequent measurement being designated as a study period. In a single study period the uterine blood flow, the uterine veno-arterial concentration difference and the foeto-maternal concentration difference were measured on a number of occasions. From these data a permeability constant (K) of the placenta to the individual isotope was calculated.

Injection of isotope. 40–1000 μc of isotope was injected in different study periods into a foetal vein. Isotopes mixed with cold carrier were dissolved in up to 5 ml. 0·9% NaCl (10 ml. for [^{51}Cr]EDTA) and injected as a bolus over about 1 min,

followed by 2 ml. 0·9 NaCl. Times of injection and sampling were recorded as the mid-time of injection and withdrawal. All injections were sterilized by filtration through 0·22 μm disposable bacterial filters (millipore, Millex).

Two extra studies were made following the injection of [^{14}C]urea together with 10 g unlabelled urea. In these studies, chemical estimation of urea was made, by a minor modification of the method of Searcy, Foreman, Ketz & Reardon (1967), in addition to radioactive counting. A number of studies were also made of urea permeability, and one of ethylene glycol permeability, following constant infusion of ^{14}C isotopes of these substances into a foetal vein.

In some animals, permeability to [^{14}C]mannitol, [^{14}C]erythritol and [^{14}C]urea was measured in turn on the same day. To do this, the background foetal count rate for each substance was calculated by extrapolation of the foetal plasma disappearance curve of the previously injected isotope or isotopes. By injecting the isotopes in order of increasing permeability, the background maternal uterine veno-arterial differences of radioactivity resulting from the previous injections were small in relation to the rise in difference, following injection.

Measurement of radioactivity. Blood samples were collected after first flushing the catheter with at least 1 ml. (foetal) or 2 ml. (maternal) of blood, which was subsequently returned. Plasma from foetal samples was separated by centrifugation and deproteinized by adding to 0·4 ml. plasma, 1·6 ml. water, 1·0 ml. 0·16 M barium hydroxide and 2·0 ml. 0·16 M zinc sulphate. In the case of [^{14}C] urea, [^{14}C]erythritol and [^{14}C]mannitol, it was shown that centrifugation immediately or after a lapse of 1 hr made no significant difference to the plasma count rate. Maternal samples were prepared for counting and antipyrine estimation by direct deproteinization of whole blood. To each 2·0 ml. blood were added 2·0 ml. water, 2·0 ml. 0·16 M barium hydroxide and 4·0 ml. 0·16 M zinc sulphate. Recovery for all isotopes used was not significantly different from 100%. ^{14}C and ^{3}H radioactivity was counted on a Packard Tricarb scintillation counter, after addition of 2·0 ml. water and 10 ml. of the scintillant P.C.S. (Amersham Searle) to 1·0 ml. deproteinized supernant in the case of feotal samples, and of 10·0 ml. P.C.S. to 3·0 ml. supernant in the case of maternal samples. Counting efficiencies on a given occasion were shown to be the same for both foetal and maternal samples, and were approximately 73% for ^{14}C and 14% for tritium. [^{51}Cr]EDTA was counted in a Packard autogamma counter, using foetal plasma and maternal whole blood. Foetal arterial pH was determined using a Radiometer micro-electrode and was above 7·25 during all studies reported.

Blood flow measurement. Uterine blood flow was estimated by the steady-state diffusion method of Meschia, Cotter, Makowski & Barron (1967). A steady infusion of antipyrine into a foetal vein was started at time zero. After allowing approximately one hour for equilibration, the uterine blood flow, Q_m (ml. min^{-1}), was calculated from eq. (1)

$$Q_m = \frac{R_i - R_m - R_1}{[V] - [A]}. \quad (1)$$

R_i is the rate of infusion of antipyrine into the foetus, R_m is the rate of metabolism, and R_1 is the rate of accumulation of antipyrine by the foetus, estimated as described by Meschia Cotter *et al.* (1967). For this purpose, foetal weight was calculated from the weight of the lamb at autopsy or delivery, using the equation of growth against gestational age given by Gresham, Rankin, Makowski, Meschia & Battaglia (1972). [V] and [A] are the maternal uterine venous and femoral arterial whole blood concentrations, in this case of antipyrine. R_m and R_1 were usually less than 10% of R_i. The rate of accumulation of antipyrine by the placenta was ignored.

Antipyrine was measured on deproteinized whole blood by a modification of the

automated nitrite method of Meschia (1964). The dialysis step was omitted and, as the deproteinant zinc sulphate was noted to increase the method's sensitivity, standards and wash solutions were made up in zinc sulphate (10 g/l).

Properties of isotopes

^{14}C-labelled urea, erythritol, mannitol, ethylene glycol, L-glucose and sucrose, tritiated sucrose and [^{51}Cr]EDTA were supplied by Radiochemical Centre, Amersham; [^{3}H]mannitol was obtained from New England Nuclear Co.

Gel filtration of radioactive samples up to 0·3 ml. in volume was performed by using Sephadex G15 columns approximately 30 cm × 1 cm. These were eluted with Sorensen's phosphate buffer (pH 7·4), at flow rates approximately 30 ml. hr^{-1}, into fractions of 0·6 ml.

The elution volume, V_e, was estimated by eye from a plot of radioactivity against fraction number. K_{av} was calculated from eqn. (2).

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}. \quad (2)$$

The void volume, V_o was estimated with Blue Dextran, and the total liquid volume, V_t was estimated with radioactive iodide. The results of several estimations of K_{av} for each isotope are summarized in Table 1, together with published values for molecular radius (a_s) of urea, erythritol, mannitol and sucrose.

TABLE 1. Some properties of radioactive test molecules

(1) Molecule	(2) Mol. wt.	(3) a_s (nm)	(4) K_{av} Mean ± S.E. of mean (n)	(5) $\alpha \times 10^5$ Mean ± S.E. of mean (n)
[^{51}Cr]EDTA	387*	—	0·175 ± 0·009 (4)	2·89 ± 1·16 (3)
Sucrose	342	0·51	0·212 ± 0·01 (5)	1·55 — (2)
Mannitol	182	0·42	0·246 ± 0·006 (11)	3·05 ± 0·12 (5)
Glucose	180	—	0·230 ± — (2)	2·22 — (2)
Erythritol	122	0·35	0·277 ± 0·008 (4)	2·64 ± 0·17 (7)
Ethylene glycol	62	—	0·303 ± 0·009 (3)	—
Urea	60	0·22	0·444 ± 0·007 (5)	13·5 ± 1·08 (4)

* As disodium ethylene diamine tetraacetate complexed 1:1 with chromium.

(3) Molecular radius (a_s) from sources quoted in Normand, Olver, Reynolds & Strang (1971).

(4) K_{av} on G15 Sephadex gel.

(5) Olive oil:water partition coefficient (α) measured using ^{14}C isotopes apart from EDTA.

Gel filtration was carried out on foetal plasma samples collected at the end of a study period with each isotope, except sucrose, L-glucose and ethylene glycol, in order to exclude degradation of the radio-labelled test substance, and to look for evidence of protein binding. As the bulk of the radioactivity of foetal plasma samples in each case eluted as a sharp peak in the expected position, their K_{av} values have been used together with those of standard samples in the constitution of Table 1.

After injection into the foetus of [^{14}C]urea and [^{14}C]erythritol, gel filtration was performed on the supernatant from deproteinized maternal blood samples, after concentration by freeze drying using a Chemlab 5B4 drier. Maternal counts after foetal

injections of radio-labelled mannitol, sucrose and EDTA were found to be too low for adequate concentration to be practicable.

Olive oil : water partition coefficients. These were measured by the method implied in Collander (1949). A known volume x (ml.) of olive oil (Fison's), previously equilibrated with an aqueous solution of isotope of subsequently measured radioactivity C_1 (counts min^{-1} $ml.^{-1}$), was thoroughly shaken with a known volume of distilled water, y (ml.), at 18° C. The phases were separated by centrifugation, and radioactivity was measured in the aqueous phase (C_2 counts $min.^{-1}$ $ml.^{-1}$). The partition coefficient (α) was obtained by an application of the law of conservation of matter:

$$\alpha = \frac{y}{x}\frac{C_2}{C_1+C_2}. \tag{3}$$

Derivation of placental permeability constant (K)

The isotope concentration of a substance (s) in foetal arterial plasma water, $[a]_{ps}$, is assumed to be equal to that found throughout the foetal placental capillaries. The same assumption about maternal arterial plasma water concentration, $[A]_{ps}$, is made for the maternal side. Furthermore, the placenta is assumed to comprise a single quantitavely important barrier separating two well-mixed foetal and maternal compartments. Concentration in the foetal compartment at time t is $[a]_{pst}$, and in the maternal $[A]_{pst}$.

A permeability constant, K_s (ml. min^{-1}) for an unmetabolized substance (s) and for a given placenta is defined as

$$K_s = \frac{J_t}{[a]_{pst}-[A]_{pst}}, \tag{4}$$

where J_t is the net flux from foetus to mother at time t.

J_t is estimated by Fick's principle:

$$J_t = Q_{mt}([V]_{bst}-[A]_{bst}), \tag{5}$$

where Q_{mt} (ml. min^{-1}) is the maternal uterine blood flow at time t, and $[V]_{bst}$ and $[A]_{bst}$ are respectively the uterine venous and femoral arterial whole blood concentrations of s at time t, in counts/min $ml.^{-1}$ or mg $ml.^{-1}$

Only in the case of urea and ethylene glycol did maternal radioactivity exceed 5 % of the foetal levels. For these substances maternal whole blood and maternal plasma counts were very similar, and substitution of $[A]_{bst}$ for $[A]_{pst}$ produces a trivial error. In all other cases $[A]_{bst}$ and hence $[A]_{pst}$, was negligible in relation to $[a]_{pst}$.

The permeability constant of a placenta defined in this way is identical with the Diffusion Clearance of Meschia, Battaglia *et al.* (1967).

Calculation of pore radius from K by minimum variance method

If it is supposed that lipid insoluble substances cross the membrane solely by passive diffusion through uniform cylindrical water filled pores in the absence of solvent flow, K may be described in terms of the total area of pores, A, their length, Δx, the free diffusion coefficient of the substance at body temperature, D_s, and the Faxen Ferry term which describes the restriction to diffusion of a substance s of molecular radius a_s by a pore of radius r (Solomon, 1968).

$$K_s = \frac{A}{\Delta x} D_s F\left[\frac{a_s}{r}\right], \tag{6}$$

where

$$F\left[\frac{a_s}{r}\right] = \left[1-\frac{a_s}{r}\right]^2\left[1-2{\cdot}104\left(\frac{a_s}{r}\right)+2{\cdot}09\left(\frac{a_s}{r}\right)^3-0{\cdot}95\left(\frac{a_s}{r}\right)^5\right]. \tag{7}$$

For a given placenta at a given time $A/\Delta x$ should be constant. Its value was computed as follows: for a given permeant s, a_s was taken from Table 1 and D_s from Normand, Olver, Reynolds & Strang (1971). K_s was experimentally measured and $A/\Delta x$ values were estimated for a series of values of r. In each case the value of $A/\Delta x$ has a variance reflecting the variance of the experimental measurement of K_s and the appropriateness of the r value. $A/\Delta x$ estimations were performed for the permeants mannitol, erythritol and urea. If eqn (6) is appropriate, r should be the same for all permeants and its best value was thus taken to be that generating the smallest deviation in $A/\Delta x$ between different permeants, each $A/\Delta x$ being weighted. (Snedecor, 1956) according to the variance of the experimental mean value used in its calculation.

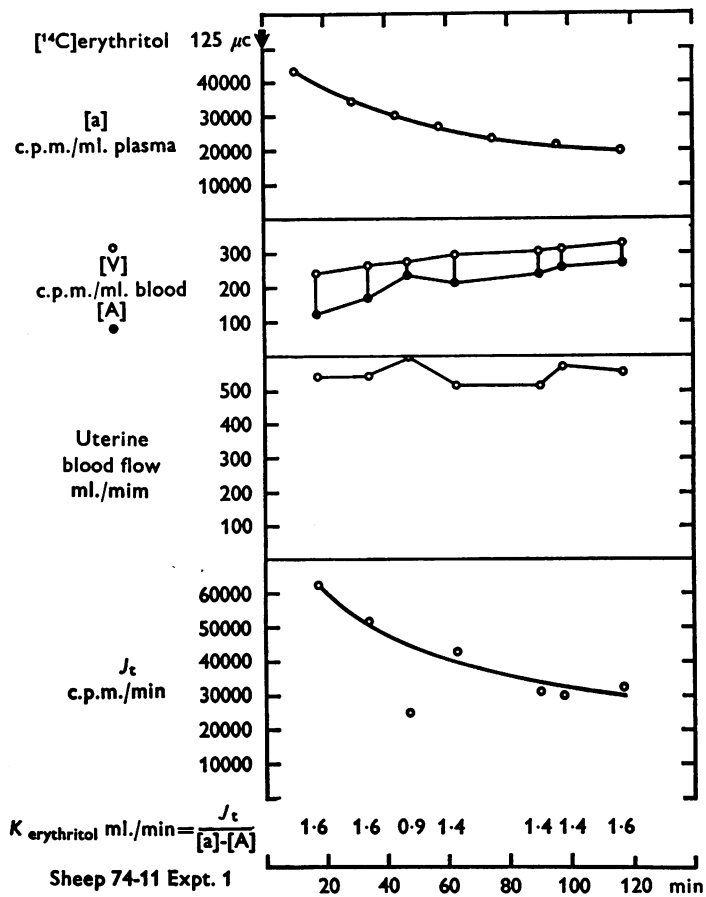

Fig. 1. Single study period following I.V. injection into foetus 74-11 of 125μc [^{14}C]erythritol at time zero on the day after surgery. [a] is foetal arterial plasma radioactivity. [V] and [A] are maternal whole blood radioactivity in uterine venous and femoral arterial blood respectively. Uterine blood flow is estimated from eqn. (1) and J_t is calculated from equation (5). Individual measurements of permeability constant, (K) calculated by means of eqn. (4), are shown. The mean value for this study period (presented in Table 2) is $K = 1{\cdot}44 \pm 0{\cdot}10$ ml. min^{-1} ($n = 7$).

RESULTS

Permeability measurements

[^{14}C]*erythritol.* Erythritol permeability was measured during twelve study periods in ten animals, a typical experiment being shown in Fig. 1. The mean value of the permeability constant for erythritol ($K_{\text{erythritol}}$) for this and for each of the other eleven erythritol studies is given, together with results for other isotopes, in Table 2.

In six study periods one or two measurements of $K_{\text{erythritol}}$ were made in the first 10 min after injection of isotope. This is the period during which foetal plasma concentration of isotope is falling most rapidly, and during which measured $K_{\text{erythritol}}$ will be too low if an intermediate compartment between foetus and mother exists. In fact values measured in the first 10 min after injection are not significantly different from the mean for the whole study period (paired t test, $P > 0{\cdot}5$, $n = 6$). Conversely, values measured a long time after injection of [^{14}C]erythritol would be most liable to error if the isotope was substantially metabolized by the lamb. In fact, $K_{\text{erythritol}}$ values measured more than 60 min after isotope injection had a mean value 87 % of earlier values, which was not a significant change (paired t test $P < 0{\cdot}2$; $n = 12$). During two study periods $K_{\text{erythritol}}$ values were measured using uterine venous blood collected from catheters placed in two different sites in the uterine venous drainage on the same side as the foetus. For one the mean values for $K_{\text{erythritol}}$ of simultaneously collected samples were 2·32 and 2·31 ml. min^{-1} ($n = 3$) for the other, 1·54 and 1·42 ml. min^{-1} ($n = 5$). Neither difference is significant.

$K_{\text{erythritol}}$ measured in four study periods immediately after surgery with the ewe semiconscious, was not significantly different from that obtained from eight measurements made in animals up to 5 days postoperatively ($P < 0{\cdot}5$). On the other hand, $K_{\text{erythritol}}$ was significantly higher ($P < 0{\cdot}025$) on day 2 than day 1, in one of two sheep studied twice on different days. The other did not alter significantly. Regression of $K_{\text{erythritol}}$ against gestational age over the small range studied (128–139 days) was insignificantly different from zero.

Non-radioactive erythritol in variable amounts between 50 and 250 mg was injected as cold carrier with the isotope. There was no significant regression of mean $K_{\text{erythritol}}$ on amount of carrier injected.

Gel filtration of a foetal plasma sample collected 45 min after injection of [^{14}C]erythritol to foetus 74–13 demonstrated 93 % recovery of applied counts in a single symmetrical peak with a K_{av} compatible with erythritol. There was no evidence of binding to other plasma constituents. Chromatography of the low counts in uterine venous blood was also performed after concentration by freeze drying of the supernatant following

TABLE 2. Mean placental permeability constant ($K \pm$ S.E. of mean) following each bolus injection of isotope into foetus. n = number of observations per bolus. G.A. = gestational age at operation. d = days after operation (which counts as day 1). K values not corrected for foetal weight. Units ml. min^{-1}. * = includes data from two venous catheters. † = infusion study. ‡ = excluded from mean values, see Results.

Sheep t = twin s = single	G.A. (wt. kg)	(d)	[^{14}C]urea $K \pm$ S.E. of mean (n)	(d)	[^{14}C]ethylene glycol $K \pm$ S.E. of mean (n)	(d)	[^{14}C]erythritol $K \pm$ S.E. of mean (n)	(d)	[^{14}C]L-glucose $K \pm$ S.E. of mean (n)	(d)	[^{3}H]mannitol $K \pm$ S.E. of mean (n)	(d)	[^{14}C]mannitol $K \pm$ S.E. of mean (n)	(d)	[^{51}Cr]EDTA $K \pm$ S.E. of mean (n)	(d)
72-2	131		—		—		2·12 ± 0·07		—		—		—		—	
(s)	(4·0)	(2)					(7)	(1)								
73-7	129		—		—		1·99 ± 0·12		—		—		—		—	
(t)	(4·0)	(5)					(11)	(1)								
73-8	135		—		—		1·99* ± 0·26		—		—		—		—	
(t)	(2·7)	(4)					(10)	(1)								
			—		—		2·88 ± 0·24		—		—		—		—	
							(9)	(2)								
73-12	127		—		—		1·37* ± 0·15		—		—		—		—	
(t)	(2·5)	(4)	—		—		(14)	(2)								
73-14	131		—		—		—		0·32 ± 0·07							
(s)	(5·3)	(4)							(10)	(1)						
74-2	138		—		—		2·52 ± 0·14		—		—		—		—	
(s)	(5·2)	(5)					(9)	(2)								
74-4	129		91·8 ± 2·9		—		—		0·52 – 0·1		—		—		—	
(t)	(3·2)	(3)	(6)	(2)	—		—		(10)	(2)	—		—		—	
74-5	127		—		—		—		—		0·293 – 0·076		—		—	
(s)	(3·0)	(2)									(10)	(1)				

TABLE 2 (*continued*)

74-6	128		54·6 ± 7·6	—	2·16 ± 0·30	—	0·188 ± 0·086	—	0·0059 ± 0·006
(s)	(3·7)	(8)	(6) (1)	—	(7) (2)	—	(9) (1)	—	(7) (2)
			77·9 ± 6·6	—	—	—	—	—	—
			(6) (6)						
74-9	137		57·1 ± 1·9	—	1·88 ± 0·14	—	0·172 ± 0·141	—	—
(t)	(3·8)	(6)	(7) (2)	—	(8) (2)	—	(10) (1)	—	—
74-11	135		78·1 ± 5·6	—	1·44 ± 0·10	—	0·196 ± 0·034	—	—
(t)	(3·7)	(8)	(7) (2)	—	(7) (2)	—	(7) (2)	—	—
			80·1 ± 5·1	—	1·59 ± 0·21	—	—		0·0086 ± 0·012
			(7) (5)	—	(7) (5)	—	—	—	(5) (5)
74-13	131		60·1 ± 1·1	—	1·28 ± 0·12	—	1·28‡ ± 0·22	—	—
(t)	(3·7)	(4)	(7) (2)	—	(6) (2)	—	(7) (2)	—	—
			41·0 ± 1·4	—	—	—	—	—	—
			(7) (3)						
74-14	126		—	—	—	—	0·87‡ ± 0·24	0·14 ± 0·028	—
(t)	(3·4)	(—)	—	—	—	—	(10) (3)	(9) (5)	—
74-16	132		36·9 ± 4·7	—	1·03 ± 0·07	—	—	0·024 ± 0·008	—
(t)	(3·2)	(10)	(7) (3)	—	(7) (3)			(7) (3)	
74-19	132		51·5 ± 3·6	—	—	—	—	—	—
(s)			(8) (3)						
74-22	127		43·9 ± 5·2	24·7† ± 1·4	—	—	—	—	—
(s)	(3·1)	(8)	(7) (2)	(8) (3)	—	—	—	—	—
Over-all Mean ± S.E. of mean (No. of studies)			61·2 ± 5·5 (11)	24·7 (1)	1·85 ± 0·16 (12)	0·42 (2)	0·21 ± 0·03 (4)	0·084 (2)	0·007 (2)

deproteinization. 99 % of the counts frozen were recovered within a small peak of appropriate K_{av}.

[^{14}C]urea. The results of measurement of K_{urea} following eleven bolus injections of [^{14}C]urea to eight foetuses are given in Table 2. The K_{urea} was measured in each study on six or more samples collected between 2 and 40 min following isotope injection. Four of these studies were followed by further measurements of K_{urea} during constant infusion of [^{14}C]urea into

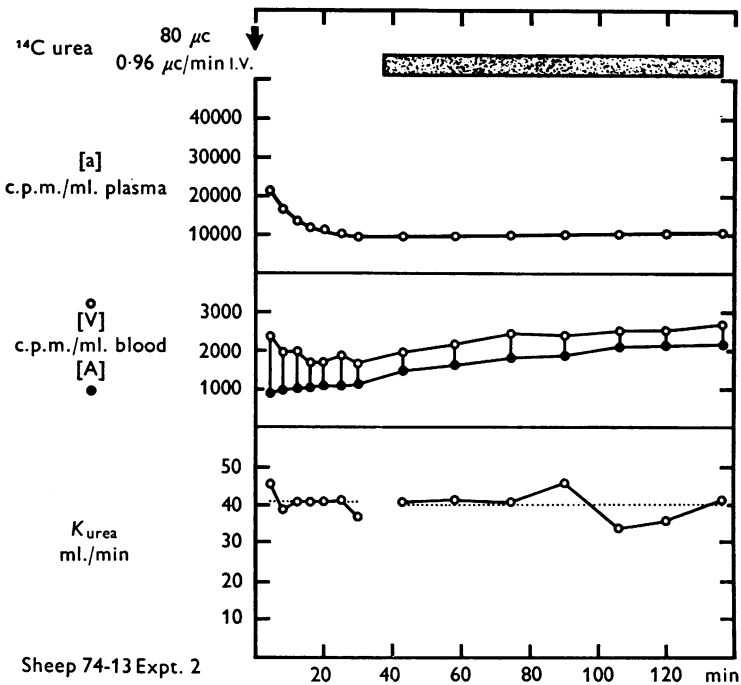

Fig. 2. Study period after I.V. injection of a bolus of 80 μc [^{14}C]urea to foetus 74-13 at time zero followed by constant infusion of [^{14}C]urea at approximately 0·96 μc min^{-1} from 37 to 135 min after the bolus. [a], [V] and [A] as for Fig. 1, values of K_{urea} estimated from eqn. (4) are plotted. Mean value of $K \pm$ S.E. of mean following the bolus was 41·0 ± 1·4 ml. min^{-1} and during the infusion 40·0 ± 1·5 ml. min^{-1}.

the foetus for a period of up to 2 hr, as shown in Fig. 2. In each case the K_{urea} measured during constant infusion (mean of at least 6 measurements) was within 10 % of that measured following bolus injection and in no case was the infusion result significantly different from the bolus result. There was little delay between foetal injection of [^{14}C]urea and its appearance in uterine venous blood. Thus in Fig. 2 the first estimate of K_{urea} 4 min after foetal injection is just as high as subsequent values. In ten studies where

measurements of K_{urea} were made between 2 and 5 min after injection the mean K_{urea} value for these early measurements was $122 \pm 9{\cdot}3$ % of the overall K_{urea} value (significance of difference $P < 0{\cdot}1$).

Of the three animals studied a second time, the K_{urea} was significantly higher in one, significantly lower in one and the same in a third. The overall mean K_{urea} from measurements performed on day 3 or later was 16·9 % lower at $65{\cdot}6 \pm 3{\cdot}6$ ml. mm^{-1} than the mean for earlier measurements: $68{\cdot}1 \pm 3{\cdot}1$ ml. min^{-1} ($t = 2{\cdot}4$, $P < 0{\cdot}025$, $n = 68$).

Sephadex filtration of foetal arterial and maternal arterial and venous samples from two animals produced single peaks with the K_{av} appropriate for urea. In another animal, however, gel filtration studies demonstrated the gradual appearance of a second higher molecular weight [^{14}C] peak, during the 30 min after bolus injection. This second peak had a K_{av} of 0·16 on G15 sephadex gel. It was visible as a trace contaminant (0·2 %) in the material injected into the foetus. It accounted for 2 % of foetal counts by 5 min and 12 % by 29 min after bolus injection. Maternal blood samples collected at approximately the same times after injection and fractionated after freeze drying also showed a subsidiary peak of radioactivity in the same position.

In two animals, not shown in Table 2, simultaneous measurements were made of K_{urea} by chemical analysis of urea and by radioactive counting following the injection of radioactive and unlabelled urea together.

The results were $27{\cdot}5 \pm 6{\cdot}2$ ml. $min^{-1} \pm$ S.E. of mean for unlabelled urea and $27{\cdot}8 \pm 6{\cdot}4$ for [^{14}C]urea in one animal. In the other they were respectively $86{\cdot}6 \pm 2{\cdot}6$ and $91{\cdot}3 \pm 2{\cdot}5$. In each study $n = 5$; in neither was the pair of results significantly different from each other. Despite the raising of the foetal arterial plasma urea to 51 and 41 mM respectively, followed by a rapid decline, there was no inverse dependence of K_{urea} on foetal urea concentration in these two experiments.

[^{3}H]- *and* [^{14}C]*mannitol.* Mannitol permeability studies were performed in the same way as for erythritol except that samples were collected for up to 400 min after bolus injection. Mean results for each of six ^{3}H and two ^{14}C, study periods are in Table 2. Unlike K_{urea} and $K_{erythritol}$, $K_{[^3H]mannitol}$ values declined with time after injection of bolus. Thus the mean of $K_{[^3H]mannitol}$ measurements made more than 150 min after injection was 53 % of the mean for the first 150 min (paired t test $t = 2{\cdot}66$, $P < 0{\cdot}05$, $n = 6$).

When the relatively high $K_{mannitol}$ for animal 74–13 became apparent, a sample of the isotope administered to this animal was fractionated by gel filtration and found to have a second subsidiary peak, corresponding to a smaller molecular size. This apparent contamination or degradation of some samples of [^{3}H]mannitol was confirmed in animal 74–14 where both

[^{14}C]- and [^{3}H]mannitol permeability studies were performed. On gel filtration, both the injected isotope solution and the foetal plasma (Fig. 3) showed a subsidiary peak of ^{3}H counts, in contrast to a discrete symmetrical peak of ^{14}C counts. In this animal, the permeability coefficient, K, for [^{3}H]mannitol was six times that for [^{14}C]mannitol. Samples from the other studies with [^{3}H]mannitol were not analysed by gel filtration, and there is therefore no direct evidence for such contamination in these cases. However, the declining K values with time, not seen in [^{14}C]mannitol studies, which in any case gave lower mean values for K_{mannitol}, suggests

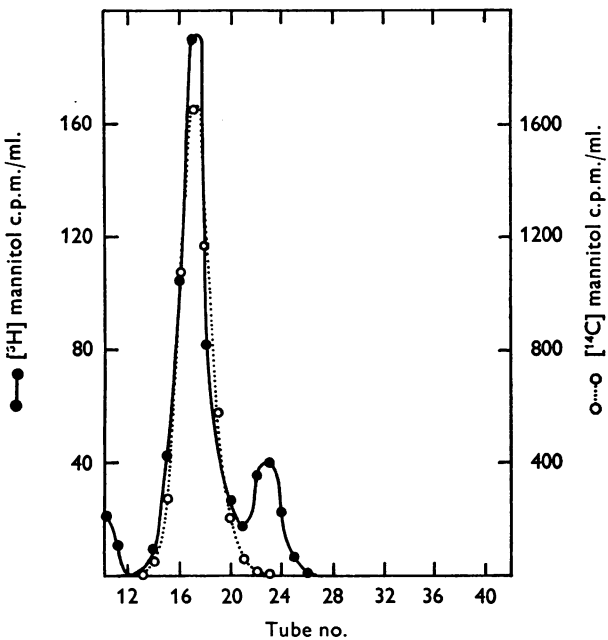

Fig. 3. Elution of radioactivity from Sephadex G15 gel column after application of 150 μl. plasma collected from foetus 74–14, 134 min after injection into it of [^{14}C]mannitol and 2 days after injection of [^{3}H]mannitol. The [^{14}C]mannitol is eluted as a symmetrical peak but there are ^{3}H counts present in a subsidiary peak of smaller molecular size.

that some degree of contamination was indeed present. All [^{3}H] K_{mannitol} values should therefore be considered as upper limits to the true value for K_{mannitol}. The two studies 74-13 and 74-14 in which gel filtration demonstrated unequivocal degradation were excluded from further consideration. Gel filtration of a foetal plasma sample from 74-6 gave a single discrete [^{3}H] peak with 85 % recovery of applied counts.

[^{51}Cr]EDTA. As shown in Table 2, $K_{[^{51}\text{Cr}]\text{EDTA}}$ was insignificantly

different from zero in two study periods. Samples were collected from 5 until 160 min after bolus injection and there was no significant change in K over this period. Gel filtration of foetal plasma samples collected 19 min after injection gave a single symmetrical peak with 95 % recovery.

Other substances. Two measurements of the K values for ^{14}C-labelled L-glucose are reported in Table 2. In each case there was a significant rise in K over 2 hr after isotope injection. A bolus injection of [^{14}C]ethylene glycol was associated with sudden death of the foetus but a measurement of permeability to it during constant infusion is also given in the Table. In an earlier series of animals, four measurements of K for [^{14}C]sucrose were made, the over-all mean value being $0{\cdot}19 \pm 0{\cdot}05$ ml. min^{-1}. A single measurement of [^{3}H]sucrose permeability showed a significantly higher mean value of $1{\cdot}59 \pm 0{\cdot}4$ ml. min^{-1}.

TABLE 3. Value of pore radius (r) which, when substituted in eqn. (6), together with K values for, in turn, erythritol, mannitol and urea generates the smallest mean weighted variance in $A/\Delta x$ for individual animals

Sheep no.	Gestation (days)	Weight at autopsy (kg)	Pore radius (nm)	$A/\Delta x$ (km)
74-6	128	3·7	0·45	526
74-9	138	3·8	0·43	717
74-11	136	3·7	0·44	492
74-16	134	3·2	0·45	217

Estimation of placental pore radius

Four animals had satisfactory studies of placental permeability to mannitol, erythritol and urea made within 24 hr of each other. The calculated placental pore radius for each of these animals is given in Table 3 together with minimum variance values of $A/\Delta x$. In Fig. 4, the line calculated from eqn. (6) using the value of r which generates the minimum variance in $A/\Delta x$ is drawn on a graph of the experimental values of K for animal 74-6 plotted against molecular radius of the test molecules urea, erythritol and mannitol.

In animal 74-22, K values are available for ethylene glycol and urea only. Taking the molecular radius of ethylene glycol as being 0·02 nm more than urea (a difference given by Goldstein & Solomon (1960) whose absolute value for the radius of urea is slightly smaller than the one we have selected), the calculated pore radius for this placenta is between 3·0 and 5·2 nm. This range was derived by using the measured values of $K \pm 1$ S.E. of mean for urea and ethylene glycol in the calculation.

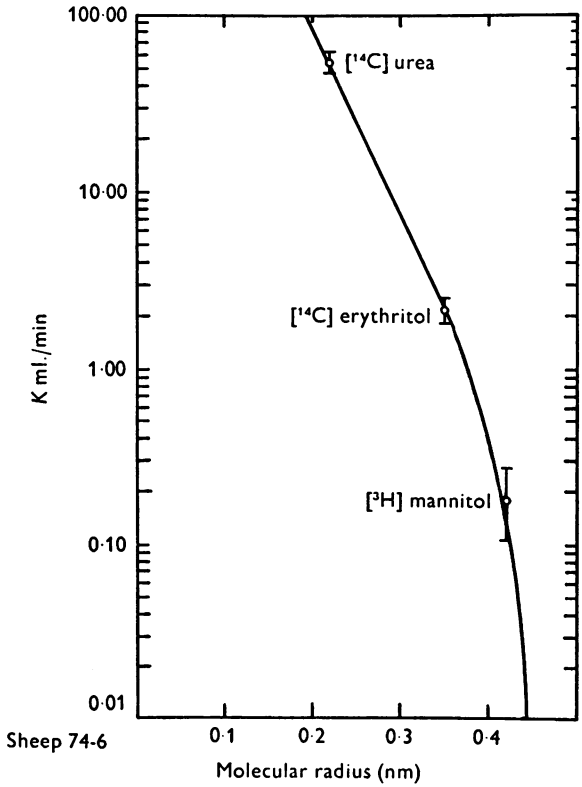

Fig. 4. Animal 74–6. Permeability constant plotted on a log. scale against molecular radius. The line is the solution to eqn. (6) when pore radius is 0·45 nm and $A/\Delta x$ is 526 km. The data points ± S.E. of mean are experimental values from Table 2.

DISCUSSION

Purity and stability of permeants

In any permeability study with isotopes, critical attention must be paid to whether they are indeed free in solution and to whether they are pure and undegraded during study. Gel filtration was performed to investigate these points. There was no evidence for the molecules whose isotopes were studied in detail (Urea, Erythritol, Mannitol, Cr EDTA) being bound to plasma protein, at any rate under conditions of gel filtration. Erythritol is present in low concentration in the blood of foetal lambs (Britton, 1967). Despite this, degradation was excluded for [^{14}C]erythritol by measurements of both foetal and maternal samples, although in the latter case total counts were low. Some degradation of [^{3}H]mannitol was demonstrated in the foetal circulation and was probably associated with labelling

of a much more permeant contaminant, since the permeability constant estimated with [^{3}H]mannitol was higher than that found using [^{14}C]mannitol. Even this latter value must be considered to be an upper limit for K_{mannitol} as there were too few counts in the maternal circulation to fractionate and if these few counts were in the form of a more permeant contaminant of mannitol we would not know. K for [^{51}Cr]EDTA is similarly an upper limit measurement but was in any case insignificantly different from zero. It is in the case of [^{14}C]urea that the largest area of doubt about isotope stability exists, for in one sheep there was gel filtration evidence of significant ^{14}C counts in a higher molecular weight substance within 10 min of injection. This requires further study, but the restriction of radioactivity to the urea peak on filtration of samples from two other animals, and the similar values found for K_{urea} by radioactive and by chemical measurement in each of another two animals, leads us to feel this is not a serious source of error.

Results for sucrose, L-glucose and ethylene glycol must be considered provisional as their isotopes were not analysed by gel filtration.

Estimation of K_s using eqns. (4) *and* (5). Equation (4) is a statement of Fick's law of diffusion. Its direct application to transplacental flux assumes the correctness of a two compartment model and the absence of a carrier system across the intervening barrier. The former assumption appears to be broadly true for the range of permeants studied, in that (with the exception of [^{3}H]mannitol) K estimated early after bolus injection when foetal plasma levels are rapidly falling, is close to that measured later on when plasma levels are falling slowly, or in the case of [^{14}C]urea, held constant by infusion. This finding is incompatible with any intermediate compartment of quantitative importance in the transfer of substances with this order of permeability constant. The latter assumption is justified by analogy with other epithelia and is supported, in the case of erythritol and urea, by the absence of any inverse dependence of K on the amount of unlabelled carrier injected.

In the measurement of J_t it is necessary to study a sample of mixed uterine venous blood and this is critically dependent on catheter placement. In a given case only a proportion of the placenta may be being studied. In addition venous samples will contain shunted blood and $[V]_{bst} - [A]_{bst}$ will underestimate the true placental arteriovenous difference. Conversely, while the antipyrine steady-state diffusion technique has been validated for umbilical blood flow measurement by comparison with electromagnetic flow-meters (Rudolph & Heymann, 1967), its use to estimate Q_{mt} is less secure and in any case the flow calculated will be overestimated if venous samples include blood shunted past the placenta. Fortunately the over-estimate, due to shunting, in Q_{mt} should exactly

balance the underestimate in $[V]_{bst}$. As a further check, when K was recalculated assuming a standard flow for all studies in a given animal, the ratio of K for different permeants and thus the derived pore radius are trivially changed.

The materno-foetal concentration difference ($[a]_{pst} - [A]_{pst}$) will be in error to the extent that plasma water content is different in mother and foetus; this is ignored. In addition, it is assumed that the permeants studied are not flow limited to an important extent. By this is meant that $[a]_{pst}$ is a good estimate of a uniform concentration of s on the foetal side of the barrier at time t and similarly for $[A]_{pst}$ on the maternal side, or, in other words, that there is not an arterio-venous capillary difference of importance in relation to the over-all foeto-maternal difference. This assumption is least likely to be true for the substance with the highest K value, urea. K_{urea} was therefore corrected according to the model of Meschia *et al.* (1967) for partial flow limitation. The increase in value of K_{urea} found was small in relation to the other possible errors and flow limitation for urea and other permeants has been ignored.

Appropriateness of pore model. It is apparent from Table 2 that there is a range of over 1000-fold between the permeability constants for [^{14}C]mannitol and [^{14}C]urea in animal 74-16. There is, indeed, a 300 times difference between the over-all mean constants for [^{3}H] mannitol (which may be an over-estimate) and for [^{14}C]urea. This wide difference is the central finding of the study. It requires interpretation and could not be explained by even quite substantial experimental errors. The wide differences found between $K_{mannitol}$ and K_{urea} compares with a threefold difference in their molecular weights, a twofold difference in free diffusion coefficient and estimated molecular radius, and a fourfold difference in olive oil:water partition coefficient.

We cannot exclude a complicated system of carrier mediated transfer to explain our results, but at the present state of knowledge the empirical equations of pore theory appear to provide a reasonable model. The narrow scatter for pore radius in Table 3 also strengthens the case for the application of this model. The pore radius may also be used as a means of quantifying the permeability relationships of a given placenta, immune from the criticism that between animal differences are being ignored.

It may reasonably be asked how well the values of K presented of other permeants fit with the proposed pore radius of approximately 0·44 nm. The single measurement on ethylene glycol fits well. The molecular radius of [^{51}Cr]EDTA is unknown but K_{av} and molecular weight measurements suggest it will be larger than sucrose, which has a radius of 0·51 nm (Table 1). The K value for [^{51}Cr]EDTA, insignificantly different from zero, is thus appropriate. A zero value would also be expected but is

not found for $K_{sucrose}$. The sucrose results, however, are likely to be overestimates as sucrose easily undergoes some decomposition and the difference between ^{14}C and ^{3}H results support this possibility. The L-glucose results are also rather higher than would be expected but their increase in value with time after injection again implies a possible degradation.

The estimates of pore radius, here reported, are comparable in size to some estimates for rat intestinal cells (Lindemann & Solomon, 1962), for toad epithelial cells (Whittembury, 1962), or indeed for the red blood cell (Solomon, 1968). They are much smaller than those proposed for capillary walls, e.g. Normand *et al.* (1971). There have been few studies of this sort on the placenta. Stulc *et al.* (1969) were unable to fit permeability data for the perfused rabbit placenta to an isoporous model, while Faber *et al.* (1971), studying the same species, estimated a pore radius of approximately 20 nm, forty times larger than our data suggest. It is not yet clear whether the large difference apparent between sheep and rabbit in these results merely reflects diversity of experimental approach, or is a genuine indication of differences between species.

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