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Received Nov. 25, 1968. P.S.E.B.M., 1969, Vol. 131.

Lack of Active Transfer of ^{14}C Tetraethylammonium and Para-Aminohippuric Acid by the Term Sheep Placenta* (33802)

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The placenta is generally considered to behave as a lipoid membrane analogous to the blood–brain barrier in being poorly permeable to ionized substances (1). However, placental transfer is not completely passive, since active transport has been demonstrated for a number of materials, *l*-amino acids being one example (2). The possibility that the placenta may actively remove substances from the fetal circulation has seldom been studied. Of substances known to be actively transported by such organs as the liver and kidney, only BSP has been administered to the fetus (3). It was demonstrated that BSP was not actively transported from the fetal compartment. The present study was designed to determine whether the term sheep placenta actively removes a typical organic acid or base from the fetal compartment.

Methods. Mixed breed pregnant ewes, weighing 48–68 kg, 128–140 days from dated breeding (normal duration of pregnancy is approximately 150 days), were fasted for 36 hr, and anesthetized with phencyclidine HCl (Sernylan), 1 mg/kg, intramuscularly and chloralose, 50 mg/kg intravenously. Intermittent supplemental chloralose was administered as needed. Respiration was maintained by a Bird respirator (Mark 8). Surgical preparation included laparotomy and hysterotomy with catherization of maternal (femoral) artery (MA), uterine vein (UV), fetal (cotyledonary) artery (FA), and umbilical (cotyledonary) vein (FV). Additional maternal veins and a fetal femoral artery and vein were cannulated for measurement of pressures and infusion of drugs. A total of 12 experiments were performed, comprising 4 equal groups. Para-aminohippuric acid (PAH) was administered via the maternal circulation in one group and by the fetal circulation in another group. The remaining two groups received ^{14}C tetraethylammonium bromide (^{14}C TEA) sp act 2.47 mCi/mmole, (New England Nuclear Corp.). The labeled compound was administered via the maternal circulation in one group and the fetal circulation in the other. In each experiment, infusion into the fetus of antipyrine (A) at a constant rate was employed to measure effective maternal and fetal placental flows, according to the method developed by Meschia (4) and validated by Rudolph (5). Transplacental gradients of A were also utilized as indices of the passive transfer characteristics of the placenta, providing a basis for evaluation of possible active transfer of PAH or ^{14}C TEA.

The experimental protocols were as follows: A was infused via a fetal vein at 2 $\times$ maintenance rate for 5 min, and at maintenance rate (see section "Calculations" below) for the remainder of the experiment. Twenty min was allowed to elapse for equilibration of A concentrations. The PAH or ^{14}C TEA was then administered as a single bolus injection to either mother or fetus. A maintenance

* Supported by NIH Grant GM 14270.

infusion was administered only after administration of PAH to the mother, since neither the plasma half-life ($t½$) of TEA in the mother nor $t½$ of either drug in the fetus was known in advance. The dosages used were: maternal: PAH, 25 mg/kg + 0.5 mg/kg/min; fetal: PAH, 25 mg/kg (body wt assumed 4 kg); maternal ^{14}C TEA, 30 μCi; fetal: ^{14}C TEA, 10 μCi. One mg of carrier TEA chloride was added per 10 μCi of ^{14}C TEA, with one exception.[1] After a 20-min initial equilibration period, timed serial specimens were drawn from MA, UV, FA and FV over an additional 40-min period. One hr after administration of PAH or ^{14}C TEA, antagonists of the active transport of acids and bases were administered as follows: (a) following PAH to the mother, 25 mg/kg of probenecid was administered intravenously to the mother accompanied by discontinuation of maintenance PAH infusion; (b) following fetal PAH administration, 25 mg/kg (assumed fetal body wt, 4 kg) of probenecid was administered to fetus; (c) following both maternal and fetal administration of ^{14}C TEA, 2 mg/kg of cyanine 863 was administered intravenously to the fetus (assumed fetal body wt 4 kg). The latter compound was available in limited amounts obviating maternal administration of the accepted blocking dose (2 mg/kg) (6). For the succeeding hour serial blood specimens from the 4 vessels supplying the placenta were drawn to assess possible effects of probenecid and cyanine 863.

Calculations. The calculation of effective maternal and fetal placental flow utilized the Fick principle which depends on knowledge of the rate of transfer of A between the fetal and maternal compartments. The latter rate is considered to be equal to the rate of the fetal infusion when a constant FA_A is attained. In our experiments we employed A infusion rates of 22.8 or 45.6 mg/min. These rates were greater than those used by others, 11–15 mg/min (5). Our intention was to increase FA_A and thereby permit chemical analysis on smaller volumes of plasma samples. However, the rate of A infusion exceeded the maternal capacity for disposition and as a consequence accumulation occurred in both maternal and fetal circulations. We observed that the rate of increase of the log of A concentration was constant and equal in all 4 vessels over the period of study. Linear regressions of the logarithms of A concentrations vs time were calculated by least squares. The mean slopes, b, indicating change of $\log_{10}$ of A concentration $\times$ min $^{-1}$ (SE) were; MA, 0.0028 (0.0004); UV, 0.0028 (0.0004); FA, 0.0030 (0.0007); UV, 0.0023 (0.0004); A paired t test indicated that the last two slopes were not significantly different ($p > 0.30$). All analyses presented in subsequent tables were performed on data representing calculated drug concentrations at the end of the sampling period, 60 min following administration of PAH or ^{14}C TEA. We approximated the rate of fetal retention of A at that time by the following formula:

$$0.80 \text{ ml/g}^2 \times (\text{fetal wt.} + \text{placental wt.}^{3}) \text{ g} \times \text{A concentration mg/ml} \times [(\text{antilog } b) - 1] \text{ min}^{-1} = \text{mg min}^{-1}.$$

We found that the average fetal retention rate of A at the time of analysis was 0.24 $\pm$ 0.07 (mean $\pm$ SE) of the infusion rate. Fetal to maternal transfer rate was considered equal to infusion rate minus fetal retention rate. Effective maternal and fetal placental flows were calculated from the equation:

$$\text{Transfer rate}_A = \text{Flow}_{\text{fetal}} (FV_A - FA_A) = \text{Flow}_{\text{maternal}} (UV_A - MA_A).$$

To compare the fetal to maternal placental transfer of PAH and TEA to that of A, we employed the transfer constant used by Pappenheimer (9) in his analysis of transfer (of substance X) from CSF to blood; *i.e.* K_X (ml $\times$ min $^{-1}$) = transfer rate per unit concentration difference. The same constant has been employed by Meschia *et al* (7) in analysis of transplacental transfer of urea. The concentrations in maternal and fetal blood, respectively, were defined as the means of the respective blood concentrations

[1] In one experiment 10 μCi were injected into the fetus without carrier; the results were not different from those in which carrier was used.

[2] Based on fetal composition studies (7).

[3] Estimated at 8% fetal weight (8).

entering and leaving the placenta (7, 9).

We related the K_X (where X = PAH or TEA) to K_A by the ratio:

$$\frac{K_X}{K_A} = \frac{\text{Flow}_{\text{maternal}} \times (\text{UV}_X - \text{MA}_X) / \text{Fetal to maternal concentration gradient}_X}{\text{Flow}_{\text{maternal}} \times (\text{UV}_A - \text{MA}_A) / \text{Fetal to maternal concentration gradient}_A}.$$

The ratio, K_X/K_A is independent of flow, since the latter cancels in the above equation. In order to compare the maternal to fetal transfer of TEA with the fetal to maternal transfer of A, the maternal A concentration difference equivalent to that resulting from an equal amount of A transferred from fetus to mother was calculated from the equation:

$$\text{Flow}_{\text{fetal}} \times (\text{FV}_A - \text{FA}_A) = \text{Flow}_{\text{maternal}} \times (\text{UV}_A - \text{MA}_A).$$

Analytical methods. Concentration of A was determined by the method of Brodie (10), PAH by an AutoAnalyzer modification of the Bratton–Marshall (11) reaction. ^{14}C TEA was measured by a Beckman LS 250 liquid scintillation spectrometer. Samples of plasma (0.5 ml) were added to 12 ml of a solution prepared as follows: to 1 liter of toluene (Mallinckrodt, A. R.) were added 7 g of PPO, 0.36 g of POPOP and 200 ml of Beckman BBS - 3. Counting of low activity specimens was performed for at least 100 min. The presence of A and cyanine 863 had no influence on counting efficiency. The results were expressed in counts per minute above background (30 cpm) corrected for quenching of appropriately diluted stock ^{14}C TEA solution by 0.5 ml of plasma. Past work by other investigators has suggested that TEA is not metabolized (12).

Since our experimental observations frequently concerned concentrations of PAH and ^{14}C TEA which were at or near blank levels, it was important that we express the latter explicity. Maternal and fetal blanks did not differ significantly and were pooled. Since there were 4 vessel blanks per experiment and 6 experiments each for PAH and ^{14}C TEA, a total of 24 blank values were available for each compound. The mean ± SD blank for PAH was 0.02 ± 0.02 mg/100 ml. The mean blank above background (± SD) for ^{14}C TEA, calculated by a standard method (13) was 3.27 ± 0.02 cpm.

Results. The average fetal weight was 4.9 kg. The mean ± SE uterine blood flow was 914 ± 201 ml/min, and the mean ± SE umbilical blood flow as 500 ± 104 ml/min. The umbilical blood flows were somewhat below the published normal value (4, 5, 14).

Administration of PAH or ^{14}C TEA via maternal circulation. During the last 40 min of the first hour after administration of PAH, MA_{PAH} was constant, the average value being 10.02 mg/100 ml. Only minimal FA_{PAH} was observed, the average concentration at 1 hr being 0.02 mg/100 ml, identical with the one standard deviation of the PAH blank. This agrees with the finding that there was no increase in PAH concentration of fetal blood traversing the placenta, the average concentration difference ($\text{FV}_{\text{PAH}} - \text{FA}_{\text{PAH}}$) being −0.01 mg/100 ml.

In the case of ^{14}C TEA, the material was administered to the ewes as a single bolus. Subsequently, a first order disappearance from plasma was observed, the average $t\frac{1}{2}$ being 187 min, and the apparent volume of distribution 0.057 total maternal body weight. At 1 hr the average concentrations of ^{14}C TEA were; MA, 1551 cpm and FA, 16.1 cpm. The average concentration difference ($\text{FV}_{\text{TEA}} - \text{FA}_{\text{TEA}}$) was 10.4 cpm. The latter 2 findings were consistent with a slow but measurable transplacental passage of ^{14}C TEA from mother to fetus. After correction for the ratio of fetal to maternal placental flow rates, we found the average ratio K_{TEA}/K_A to be 5.2×10^{-3}, indicating low transplacental passage of ^{14}C TEA relative to A.

Administration of PAH and ^{14}C TEA into the fetal circulation. Following single bolus injections to the fetus, the average $t\frac{1}{2}$ of PAH and ^{14}C TEA were 94.1 and 70.0 min, respectively. The average volumes of distribution as fractions of body weight were 0.476 and 0.069, respectively.

Table I indicates that following administration to the fetus, there was a slight tendency for PAH to appear in maternal blood and a small positive concentration gradient

TABLE I. Concentration of PAH in Maternal Plasma 1 hr after Fetal Administration (av of 3 expts.).[a]

	Maternal artery (MA)			Uterine vein (UV)			
	Blank	1 hr	Net Δ	Blank	1 hr	Net Δ	ΔUV—ΔMA
PAH (mg/100 ml)	0.02	0.05	0.03	0.02	0.06	0.04	0.01

[a] PAH blank for entire series, mean ± SD: 0.019 ± 0.020 mg/100 ml.

TABLE II. Comparison of Fetal Transfer Constants (K) of PAH and Antipyrine (av of 3 expts.).[a]

	Δ Conc (UV—MA)	Fetal to maternal conc gradient	$K = (UV—MA)/conc$ gradient	
PAH (mg/100 ml)	0.01	4.73	2.1×10^{-3}	(a)
A (mg/100 ml)	7.2	8.6	0.84	(b)

[a] Ratio: K_{PAH}/K_A (a/b) $= 2.5 \times 10^{-3}$.

($UV_{PAH} - MA_{PAH}$). Table II shows that the transfer constant for PAH from fetus to mother was quite small relative to that of A.

Analogous data for fetal to maternal transfer of TEA are presented in Table III. A small increase in MA_{TEA} concentration above control and a slightly positive ($UV_{TEA} - MA_{TEA}$) were observed. In Table IV the average transfer constant of TEA is shown to be small compared to that of A.

Effect of probenecid and cyanine 863. During the 60-min period following the administration of the inhibitors of active transport, neither had an observable effect on placental transfer of PAH or ^{14}C TEA, respectively, as evaluated by accumulation in maternal or fetal arterial plasma or by transplacental transfer constants.

Discussion. Our results indicate that the sheep placenta impedes the transfer of PAH and TEA between mother and fetus in either direction. The data concerning maternal to fetal transfer are consistent with observations relating to other compounds which are highly ionized at body pH, and support the analogy often made between the placental and blood–brain barriers (1). However, the demonstration (9) that an active transport system exists for removal of acids from cerebrospinal fluid (CSF) to blood and the subsequent proof of a similar active transport system for bases (15) raised the possibility that the placenta might also possess an active mechanism for extrusion of acids and bases from the fetal compartment. Our results offer strong evidence against such a possibility. Pappenheimer (9) in studies of the transfer of Diodrast and phenolsulfonphthalein from CSF to blood, compared the clearance rates of the actively transported compounds to those of creatinine, a passively transferred compound. Our comparison of the transfer constants of PAH and TEA to those of A was intended to serve a similar purpose. Meschia (4) has found that A transfer by the placenta is equal in rate to that of ^3H_2O. The very small placental clearance constants of fetally administered PAH and TEA relative to those

TABLE III. Concentration of ^{14}C TEA in Maternal Plasma 1 hr after Fetal Administration (av of 3 expts.).[a]

	Maternal artery (MA)			Uterine vein (UV)			
	Blank	1 hr	Net Δ	Blank	1 hr	Net Δ	ΔUV—ΔMA
^{14}C TEA (cpm)	3.2	4.2	1.0	3.3	4.9	1.6	0.6

[a] Blank for entire series, mean ± SD: 3.27 ± 0.20 cpm.

TABLE IV. Comparison of Fetal Transfer Constants (K) of ^{14}C TEA and Antipyrine (av of 3 expts.).[a]

	Δ Conc (UV—MA)	Fetal to maternal conc gradient	$K = (UV—MA)/conc$ gradient	
^{14}C TEA (cpm)	0.6	2884.0	2.1×10^{-4}	(a)
A (mg/100 ml)	3.6	6.5	0.55	(b)

[a] Ratio: $K_{^{14}C\ TEA}/K_A$ (a/b) $= 3.8 \times 10^{-4}$.

of A are clear evidence against an active mechanism for their placental removal from fetal plasma.

The possibility could be raised that active transport from fetal to maternal blood might be present but obscured by dilution of transferred material due to the high blood flow rates on both sides of the placenta. Quantitative calculations taking into consideration placental weight and the transfer maximum/g of other transporting tissues (9) makes this possibility very remote. In addition, in the case of CSF to blood transfer, the appearance of transported material in jugular venous blood following administration via the CSF was observed. Considering the relative masses of the placenta and choroid plexus, one would expect a similar observation if an active component were present in transplacental transfer. Therefore, it would appear that the necessary exchanges of naturally occurring acids and bases can occur with very small extractions from fetal blood in a single transplacental passage, perhaps due to the relatively great fraction of fetal cardiac output that normally traverses the placenta, and its exposure to roughly twice as great a maternal blood flow.

PAH and TEA are representative, respectively, of broad groups of organic acids and bases which are actively transported by the kidney, biliary system, choroid plexus, and ciliary body. The function of any of these transport systems may be affected by inhibitors or compounds competing for transport, with resultant changes in concentration of drugs and other biologically important substances in appropriate fluids or tissues. For example, the administration of probenecid has been shown to elevate CSF concentration of the serotonin metabolite, 5-HIAA (16). However, on the basis of our data, one would not assume that inhibitors or substances competing for active transport of either acids or bases would directly affect the transfer of drugs, natural products, or their metabolites between the maternal and fetal compartments.

Summary. The placental transfer of PAH and ^{14}C TEA by term sheep placenta was evaluated by administration of each compound via the maternal and fetal circulations. A, which is transferred passively by the placenta, was used as a reference compound. We found that the placental transfer rates of PAH and TEA were very low bidirectionally. This observation is interpreted as virtually excluding an active transport mechanism for transfer of organic acids and bases from the fetal compartment to the maternal circulation. Data obtained following probenecid and cyanine 863 administration were consistent with this conclusion.

We wish to acknowledge the able technical assistance of Mrs. J. Steinhorst. We thank Dr. J. Baer of Merck, Sharp and Dohme for the probenecid, and Dr. Leslie Brooker, Eastman Kodak, for the cyanine 863.

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Received Nov. 1, 1968. P.S.E.B.M., 1969, Vol. 131.

Renal Excretion of Phosphate, Calcium and Sodium During and After a Prolonged Thyrocalcitonin Infusion in Man (33803)

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Various experiments suggested that thyrocalcitonin acts on the kidney, and that its action is therefore not limited to bone. However, results are different according to the various species studied. In the rat, the renal excretion of phosphate increases (1, 3–5) or remains unchanged (6), whereas the renal excretion of calcium decreases (1, 5, 6). A rise in sodium excretion has also been noted (5). In the dog, the excretion rates of these electrolytes are not modified (7). Nevertheless, when a large dose is infused into the renal artery of a dog, there is a rise in phosphate excretion (8). A similar effect has been described in the pig (8). In man, we have previously shown that thyrocalcitonin brings about an increase in the excretion rates of phosphate and calcium (9), sodium and chloride (10). The purpose of the present study was to investigate the duration of these changes during and after a prolonged infusion of thyrocalcitonin in man and to compare the results with those previously obtained in laboratory animals by other workers.

Subjects and Methods. Six male subjects were studied: there were four normal, one hyperparathyroid in whom the presence of an adenoma was later surgically confirmed, and one hypoparathyroid whose disease was primary. A diet containing 300 mg of calcium and 1,000 mg of phosphate was given on the day of the experiment and on the 3 days preceding it. Control values for plasma calcium, plasma phosphate and inulin clearance

TABLE I. Plasma Concentrations of Calcium and Phosphate, Inulin Clearances Immediately before the Study.

	Plasma calcium (meq/liter)	Plasma phosphorus (mmoles/liter)	Inulin clearance (ml/min)
Normal subject 1	4.30	0.71	111
2	5.0	0.60	122
3	5.3	0.78	124
4	5.3	0.77	100
Hypoparathyroidism	3.70	1.57	116
Hyperparathyroidism	5.50	0.33	108