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Transport of folates at maternal and fetal sides of the placenta: lack of inhibition by methotrexate

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Folate (pteroylglutamate) and methotrexate rapid (seconds) uptake by the trophoblast was investigated from either the maternal or fetal circulations of the isolated dually-perfused guinea-pig placenta. Tissue uptake was measured by using a single-circulation paired-tracer (^{3}H-test and ^{14}C-extracellular marker) technique [^{3}H]Folate uptakes were 80 and 52% (mean) in perfusates without unlabelled folate, on maternal and fetal sides, respectively. There was negligible ^{3}H-tracer backflux into the circulation up to 6 min probably due to metabolic sequestration. [^{3}H]Methotrexate uptakes were about 85 and 22% on maternal and fetal sides, respectively; however these uptakes were followed by rapid and complete backflux of the label. Specific transplacental transfer of [^{3}H]folate or [^{3}H]methotrexate in either direction was not detectable within 5–6 min. At the brush-border side (maternal) uptake of [^{3}H]folate was highly inhibited by 100 nM unlabelled folate or its reduced form, methyltetrahydrofolate (the main form in plasma); however, equimolar methotrexate (an antifolate chemotherapeutic agent) failed to produce any inhibition of folate uptake. Our findings demonstrate that on both sides of the placenta a high-affinity transport system exists for trophoblast uptake of folate compounds. For methotrexate, either a separate transport system may exist or methotrexate may have a very low affinity for the folate system. These results are distinct from the findings reported in mouse L1210 leukemia cells

Transport of folates across cellular membranes has been a subject of much research given the importance of folate compounds in intracellular intermediary metabolism and also due to the chemotherapeutic value of folate antagonists Transport system for folate compounds have been described in a variety of tissues (see review by Huennekens et al [1]) Early reports in intact mouse L1210 leukemia cells demonstrated that folic acid (pteglu) and methotrexate are pumped out of the cells [2,3] Yang et al [4] showed that folates and methotrexate, a chemotherapeutic folate antagonist, are transported into L1210 plasma membrane vesicles against a concentration gradient In intestinal epithelial cells folate is accumulated via a sodium-dependent transport system as shown in studies with enterocytes [5] and in intact rat jejunum [6] In rat intestinal brush-border vesicles both folate and methotrexate were taken up by a pH-dependent saturable process [7] Recently, Selhub et al [8] showed that rat intestinal loops in vivo transported the reduced folate compound, methyltetrahydrofolate (CH_3THF) by a saturable mechanism and this uptake was completely inhibited by folate and methotrexate suggesting that in the intestine only one transport system serves folate compounds and methotrexate In contrast in L1210 experimental tumor cells the

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Abbreviation pteglu, pteroylglutamate

available evidence [4] (see also Ref 1) indicates that folic acid is not a substrate for the carrier for reduced folates and methotrexate

Total plasma folates are in the range of 14–34 nM, with methyltetrahydrofolate as the major fraction [9] and the fetal plasma total folate concentration is higher than that in the maternal circulation [10] In the placenta there appears to be very little information regarding the transport of folates (and also of other water-soluble vitamins) (see reviews by Munro et al [11], and Truman and Ford [12]) A folate(pteglu)-binding protein has recently been described in a homogenate of human placental villi and it was suggested that this protein could be involved in the transport of folate [13] The placenta would not normally encounter the fully oxidized form of folate (pteroylglutamate) However, due to the unavailability of labelled methyltetrahydrofolate commercially, and due to the [^{3}H]folate being a more stable molecule, we employed the latter in the present study in the intact guinea-pig placenta to investigate (i) rapid uptake of [^{3}H]folate at the maternal and fetal sides of the trophoblast, and (ii) transplacental [^{3}H]folate transfer

Isolated dually-perfused (maternal and fetal circulations) placentae [14,15] were prepared from pentobarbitone (i v 20–25 mg/kg) anaesthetised guinea-pigs The maternal and fetal circulations were perfused with Krebs-Ringer solutions containing 40 g/l dextran (40000) and 1 g/l bovine serum albumin, gassed with 95% O_2/5% CO_2 at 37°C to a pH of 7 35–7 40 Both circulations were perfused successively with control solutions, which do not contain unlabelled substrate and one containing either unlabelled folate, methyltetrahydrofolate or methotrexate All chemicals were purchased from Sigma Chemical Co , U K [3′ 5′,7,9-^{3}H]Folic acid (28 or 39 Ci/mmol) was purchased from Amersham International, U K [L-*glutamyl*-3,4-^{3}H]Methotrexate (47 Ci/mmol) and [^{14}C]sucrose (671 mCi/mmol) were purchased from New England Nuclear Chemicals, Dreieich, F R G

The single-circulation paired-tracer dilution technique used here has been fully described in previous publications by Yudilevich et al see Refs 15–17)• In the dually-perfused placenta successive maternal and fetal tracer injections were made A paired-tracer experiment consisted of a rapid (< 2 s) intra-arterial injection of a bolus (100 μl) containing a mixture of [^{3}H]folate or [^{3}H]methotrexate and [^{14}C]sucrose (an extracellular marker) which was immediately followed by sequential sampling (15 or 30 samples in about 70–80 s) of the venous effluent on the ipsilateral side, a final 4 min accumulative sample was collected [^{3}H]Folate or [^{3}H]methotrexate uptake, U, was calculated for each sample from the normalised (expressed as a percentage of the injected dose) venous tracer concentrations $U = 1 - ([^3H]test/[^{14}C]sucrose)$ Total uptake U_T, was calculated from the integrated test and reference tracer recoveries over the 5–6 min period Transplacental transfer of the tracers was assessed by sampling the venous effluent (a single 6 min accumulative sample) on the contralateral circulation Placental tissue 'retention' of the test tracers was measured from the difference between [^{14}C]sucrose and [^{3}H]folate (or [^{3}H]methotrexate) total recoveries (5–6 min) in the venous effluents from both circulations

The time-course of [^{3}H]folate uptake (Fig 1 upper panels) shows that on both sides of the trophoblast there was a high and rapid (seconds) uptake which was maintained and a maximal unidirectional value, U_{max}, could be estimated Furthermore, over the 5–6 min total sampling time, the net uptake (U_T) did not appear to be reduced (Table I) suggesting cellular trapping of the ^{3}H-label

In contrast to [^{3}H]folate, [^{3}H]methotrexate uptake (Fig 1, lower panels) exhibited an initial maximal plateau of short duration followed by a rapid downslope of the uptake which indicated fast backflux (efflux into the ipsilateral circulation) This [^{3}H]methotrexate efflux is reflected in a negligible U_T value (Table I) These results were observed on both sides of the trophoblast, however, on the fetal side the early maximal uptake was relatively lower (Fig 1) Table I summarises results for [^{3}H]folate and [^{3}H]methotrexate obtained from ten dually-perfused placentae and illustrates the reproducibility of the findings shown in Fig 1 For [^{3}H]folate, there was excellent correlation (by linear regression analysis $r = 0\,97$, slope = 1 00, ordinate-intercept = 4 18 ± 6 00, n = 14 maternal and fetal paired-tracer experiments) between U_{max} and U_T (Table I) In Table I it can

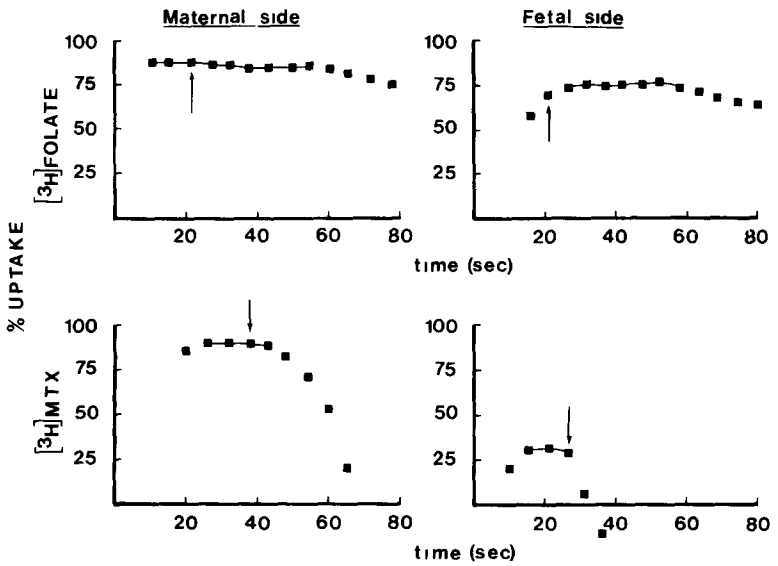

Fig 1 Folate and methotrexate (MTX) rapid uptake and efflux measured by using the paired-tracer technique in the dually-perfused placenta For both [^{3}H]folate (upper panels) and [^{3}H]methotrexate (lower panels) a unidirectional maximal uptake value, U_{max} (joined data points) was estimated and efflux was assessed by comparing this value with the total uptake (U_T) measured over 5–6 min (Table I) U_{max} and U_T parameters for these (Expt Nos 14A and 12A) and other similar experiments are given in Table I In these 'control experiments' the perfusate did not contain unlabelled substrate and the bolus injectate concentrations of folate and methotrexate were 85 and 252 nM respectively Arrows indicate the peak concentration of the venous dilution curves

TABLE I

ESTIMATES OF UNIDIRECTIONAL MAXIMAL UPTAKE (U_{max} see Fig 1) TOTAL UPTAKE OVER 5–6 MINUTES (U_T) AND TISSUE RETENTION OF (A) [^{3}H]FOLATE AND (B) [^{3}H]METHOTREXATE

In each placenta maternal and fetal side tracer injections were successively performed in control perfusates (not containing unlabelled folate) These results are from ten dually-perfused placenta and are shown individually and also averaged The bolus injectate concentration of unlabelled folate was 85 or 291 nM and for MTX, 252 nM

Expt No	U_{max} (%)		U_T (%)		Tissue retention (% dose)	
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
A [^{3}H]Folate						
101A	87	31	82	33	69	28
106A	87	46	84	47	90	43
107D	38	26	33	23	33	26
108E	83	69	85	49	78	69
109E	79	50	75	45	68	39
14A	88	75	87	68	76	59
15A	98	64	87	63	78	63
Mean ±S E	80±7	52±7 *	76±7	47±6 *	70±7	59±6 *
B [^{3}H]Methotrexate						
11A	85	20	−2	−2	−1	−2
12A	89	31	2	3	2	4
17A	82	16	8	−2	9	2
Mean ±S E	85±2	22±4	3±3	0±2	3±3	1±2

* $P < 0.01$ (paired t-test) compared with maternal side

be observed that the retention of [^{3}H]methotrexate was negligible, whereas the retention of [^{3}H]folate was very high This tissue retention correlated with the total uptake (U_T) measurements (Table I) and confirms the absence of a significant release of the labelled-folate which contrasts with the finding with [^{3}H]methotrexate

Transplacental transfer of [^{14}C]sucrose from maternal-to-fetal or fetal-to-maternal circulations ranged between 0 and 65% of the injected dose (Fig 2) As previously reported this is related to the variable degree of leakiness of the isolated perfused placenta [15,17] There was no excess [^{3}H]folate or [^{3}H]methotrexate over [^{14}C]sucrose to indiate specific transplacental transfer of these molecules On the contrary, there was often a larger recovery of the leak pathway marker compared to [^{3}H]folate, particularly in placentae with high leakage (Fig 2)

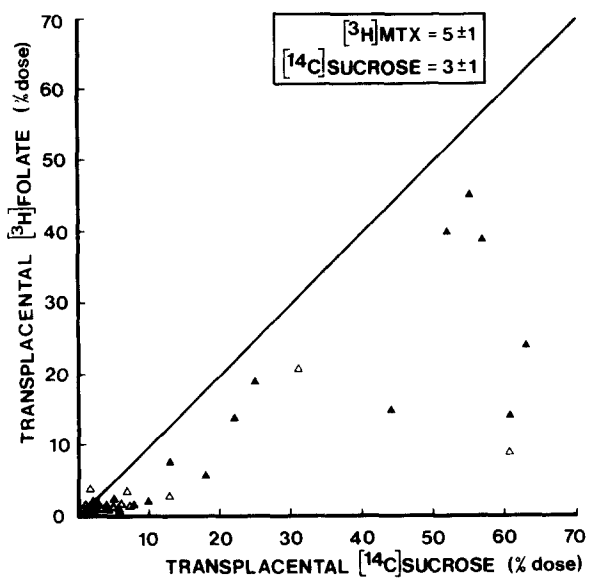

Fig 2 Transplacental transfer of tracers Recoveries of [^{3}H]folate (ordinate) and [^{14}C]sucrose (abscissa) in the contralateral venous outflow (accumulated over 5–6 min) following either a maternal (closed triangles) or fetal (open triangles) tracer injection The results are from control experiments with perfusates not containing unlabelled folate The data represent maternal ($n = 20$) and fetal ($n = 10$) paired-tracer experiments in 13 dually-perfused placentae The inset shows transplacental recoveries with the [^{3}H]methotrexate ([^{3}H]MTX) experiments Results are mean ± S E of six maternal and six fetal paired-tracer experiments obtained in three placentae

At the maternal side we investigated the inhibition of [^{3}H]folate uptake by comparing data from folate-free perfusate (Fig 1 and Table I) with data obtained with perfusates (both circulations) containing 100 nM of either unlabelled folate, methyltetrahydrofolate, or methotrexate (see Ref 1 for the structure of these molecules) While the inhibition (mean ± S D , n = number of placentae) by folate (68 ± 6, $n = 6$) and methyltetrahydrofolate (54 ± 10, $n = 5$) was marked, methotrexate had no significant effect (3 ± 3, $n = 5$) Since mercaptoethanol was added (100 μM) to the perfusate containing methyltetrahydrofolate in order to maintain the molecule in its reduced form, control experiments using this substance were also undertaken it was found that there was no effect on [^{3}H]folate uptake % inhibition was 4 ± 2 (mean ± S E , $n = 4$)

These findings in the intact placenta demonstrate, for the first time, specific and high-affinity carrier-mediated uptake of folate on both maternal and fetal sides of the trophoblast The folate uptake system appears to be shared by the physiological substrate methyltetrahydrofolate [9] since folate uptake was inhibited (at the low concentrations of 100 nM) by this reduced form of folate Since methotrexate failed to inhibit folate uptake at equimolar concentration (100 nM), it is suggested that either separate transport systems exist for folates and methotrexate, or that methotrexate has a relatively low affinity for the system Furthermore, the results obtained with labelled methotrexate demonstrated that essentially there is no long-term binding or transport of this molecule However, the very transient uptake of [^{3}H]methotrexate which was observed (Fig 2), particularly on the maternal side, could in the future allow further characterization of such phenomenon

In contrast to our results, in plasma membrane vesicles prepared from L1210 leukaemia cells both methotrexate and methyltetrahydrofolate are very poor competitive inhibitors of folate uptake [4] However, in L1210 membranes methotrexate is a competitive inhibitor of 5-formyltetrahydrofolate (CHOTHF), which is another reduced form of folate [4] Based on kinetic analyses these authors suggested two transport systems, one specific for folate and another for reduced folates and

methotrexate (see also review [1]) In the present study the placental transport system appears to exhibit unusually high affinity as compared to L1210 tumor cells since potent inhibition of folic acid uptake was observed at 100 nM, whereas the kinetic constants (K_m and K_i values) measured for methotrexate, CH_3THF and CHOTHF in tumor cells are in the range 1 25–4 6 μM [4] This difference in concentration taken together with the specificity data (see results above) supports the conclusion that the folate carrier in our placental preparation is distinct from that in tumor cells In the intestine [7,8] and in the choroid plexus [18] a single carrier-mediated transport system appeared to be shared by folate, its reduced derivatives and methotrexate This again is different from our findings which separate folate transport (and its reduced derivatives) from methotrexate transport

Recently, Green and Ford [19] showed that human placental microvilli contain high-affinity uptake sites for folate Similar to our results, at the single concentration (50 nM) tested methyltetrahydrofolate inhibited folate uptake by 49% whereas there was no inhibition by methotrexate The fact that in their membrane vesicles the uptake of [^{3}H]folate did not change with increasing osmolality of the incubation medium led Green and Ford [19] to suggest that folate was membrane-bound and not transported across the cellular membrane On the contrary, in the same vesicles 2-aminoisobutyric acid (AIB) uptake, as other amino acids (see review by Yudilevich and Sweiry [20]), was sensitive to changes in the osmolality of the medium and this has been used as an index of placental intracellular transfer

The single circulation paired-tracer technique showed placental tissue uptake which was high, while there was no apparent facilitated transfer of folate across the placenta The slow rate at which folate transplacental transfer occurs appears to agree with the findings in humans, where Landon et al [10] injected [^{3}H]folate into the mother and showed that even after 24 h there was only 0 6% of the dose in the fetus, and that most of the activity was found in the placenta Furthermore, we observed relatively less [^{3}H]folate compared to [^{14}C]sucrose in the contralateral circulation (Fig 2) This result could be attributed to the difference in diffusion through a leak pathway of the charged (anionic) folate molecule compared to the neutral extracellular marker

The placental investigations here presented could prove to be valuable for characterizing folate tissue uptake in other organs Moreover, since folate antagonists, for example methotrexate, are employed in cancer chemotherapy (see Refs 1, 21) our methods could contribute to research on the interactions of folate antagonists at the cellular membrane level

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