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Transfer of Riboflavin by the Perfused Human Placenta

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ABSTRACT. The transfer of radioactive riboflavin across the human term placenta has been studied in an *in vitro* perfusion system. The clearance index (clearance riboflavin:clearance antipyrine) toward the fetus averaged 0.69 and the transfer index (clearance riboflavin:clearance L-glucose) averaged 3.40. The respective indices in the reverse direction were 0.25 and 0.87. Stepwise increases in the concentration of riboflavin in the maternal perfusate were associated with parallel increases in transfer rates, expressed as ng/min, up to concentrations approximating 100 ng/ml. Above that concentration, the transfer rates continued to increase at a slower rate. Concurrently, there was a reduction of the transfer index from 2.7 to 1.1 at 1000 ng/ml. With the fetal circulation closed, the placenta established a gradient toward the fetus over a period of 150 min of 1.7. The transferred radioactivity was identified as riboflavin by high-performance liquid chromatography, whereas that retained within the placenta was metabolized to flavin mononucleotide (33–75%). The observations indicate a very effective active transport system directed toward the fetus which is limited in capacity to low concentrations of riboflavin. (*Pediatr Res* 19: 1143–1146, 1985)

Abbreviations

FAD, flavin adenine dinucleotide
FMN, flavin mononucleotide
HPLC, high-performance liquid chromatography

Riboflavin (vitamin B_2) is an essential nutrient which the fetus must derive from the mother. Its two major metabolites, FMN and FAD, are required metabolic cofactors. Riboflavin deficiency causes symptoms in the adult and congenital malformations in the developing experimental animal (1). The vitamin is commonly prescribed for the pregnant woman as a precautionary measure.

There have been no direct studies of placental transfer of riboflavin in the human or experimental animal. Measurements have been made of circulating levels in maternal and cord blood and attempts have been made to deduce from that information the mechanisms of placental transfer (2–4). The many factors that enter into the dynamics of maintaining the concentrations in the blood make this approach very uncertain. We have applied the technique of dual perfusion of human term placenta to the study of riboflavin transfer and have obtained evidence of an active transport system directed toward the fetus.

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MATERIALS AND METHODS

Placental perfusion. Term placentas were obtained following delivery by cesarian or per vaginam. An undamaged lobule was selected and the tributary fetal artery and vein were cannulated, establishing the fetal circulation. The corresponding decidual plate was pierced by four blunt metal cannulas to provide the inflow for the "maternal" circulation. Outflow was along the margin of the isolated lobule and through openings in the decidual plate. Earle's buffered salt solution with added amino acids was used as perfusate. The perfusates were oxygenated by bubbling with O_2–CO_2, 95–5% (5).

Most experiments were conducted with both circuits open, *i.e.* not recirculated. Flow rates averaged 10–12 ml/min for the maternal circulation and 6–8 ml/min for the fetal. In some experiments, the fetal perfusate was recirculated through a reservoir of 70 ml. Losses from the reservoir as the result of bulk flow into the maternal circuit were less than 6 ml during the 2–3 h study which was equivalent to less than 2%/h of the volume in the fetal reservoir and tubing and less than 0.5% of the flow rate.

Each experiment was preceded by a 30-min perfusion to stabilize the placenta and to remove any retained blood. The experiments were perfused in very dim light because of the light sensitivity of riboflavin. Perfusate samples were treated with similar care during analysis. Antipyrine and L-glucose were added to the perfusate in concentrations of 10 mg/dl. Riboflavin was used variously at concentrations of 5 to 1000 ng/ml.

Materials. D-2-^{14}C-riboflavin (52.4 mCi/mmol) was purchased from Amersham and checked for purity by ascending chromatography in *n*-butanol:acetic acid:water (40:10:50). L-1-^{3}H-glucose (10.7 Ci/mmol) was obtained from New England Nuclear (Boston, MA) and checked for purity by ascending chromatography in isopropanol:water (80:20). L-Glucose, riboflavin and antipyrine were purchased from Sigma (St. Louis, MO).

Analytical methods. Riboflavin and its metabolites were separated by reverse phase HPLC on a Beckman model 421 instrument using an Altex C18 column (Altex Scientific, Ann Arbor, MI). The column dimensions were 4.6 mm ID by 25 cm and the particle size was 5 μ. The instrument was programed to deliver a linear gradient starting with 95% solvent 1, 5% solvent 2 to 100% solvent 2 over a 15-min period. Flow rate was 1.5 ml/min. Solvent I was potassium acetate buffer, 0.1 M, adjusted to pH 4.7 with formic acid. Solvent 2 was 50% acetonitrile, 50% water. In this system FAD, FMN, and riboflavin were sharply separated, eluting at 8.3, 9.3, and 10.3 min, respectively (Fig. 1). The separated peaks were analyzed fluorimetrically in an Aminco-Bowman spectrofluorometer using wavelengths of 463 and 520 nm for excitation and emission.

Transfer calculations toward the fetus:

$$\text{clearance} = \frac{(Fv - Fa)\ Qf}{Ma.}$$

F_v, fetal vein concentration; F_a, fetal artery concentration; M_a, maternal artery concentration; Q_f, fetal flow rate

$$\text{clearance index} = \frac{\text{clearance of substrate}}{\text{clearance of antipyrine}}$$

Antipyrine is transferred as a flow-limited molecule providing an indication of the effectiveness of the placental perfusion. By correcting for this technical variability among placental experiments a more consistent measure of transfer rate is obtained.

$$\text{transfer index} = \frac{\text{clearance of substrate}}{\text{clearance of L-glucose}}$$

L-Glucose is transferred as a membrane-limited molecule. It provides a measure of transfer rate by simple diffusion of a small (mol wt 180) water-soluble, uncharged molecule. In these experiments as in our previous studies (6), L-glucose was added to the perfusate in a concentration of 10 mg/dl. Antipyrine was measured colorimetrically (7). Radioactivity was assayed in a Packard tricarb scintillation counter. Transferred radioactivity approximated 10× background, minimally, with a calculated counting error of less than 5%.

RESULTS

Transfer rate of riboflavin. Riboflavin, 5 ng/ml, L-glucose-^{3}H, 10 mg/dl and antipyrine, 10 mg/dl were added to the maternal or fetal perfusate. D-2-^{14}C-riboflavin was added in an amount which, by calculation, brought the total concentration to approximately 10 ng/ml. To be noted in Table 1 is that the clearance index of riboflavin toward the fetus averaged 0.69 as compared to 0.25 in the reverse direction. This difference in transfer rates is reflected in the transfer indices which were, respectively, 3.40 and 0.87.

At the end of the experiments, the maternal and fetal perfusates and the placenta were analyzed by HPLC. Only radioactive riboflavin was found in the perfusates. In the placenta, riboflavin had been converted to FMN in amounts ranging from 33 to 75%. There was no detectable FAD.

Transfer rates at increasing concentrations of riboflavin. In these experiments, the transfer rates of riboflavin toward the fetus were measured during stepwise increases in the concentration in the maternal circulation every 30 min. There were four steps in each experiment. L-Glucose concentration was kept constant at 10 mg/dl. In the first experiment, there was a straight-line increase in transfer rates up to the maximum concentration of 100 ng/ml used in the experiment (Fig. 2). In the subsequent three experiments, the rate of increase notably slowed at concentrations above 100 ng/ml.

Similar information can be derived by inspection of the transfer indices (Table 2). The clearance of riboflavin greatly exceeds that of L-glucose at 50 ng/ml and falls with increasing concentrations of riboflavin above 100 ng/ml.

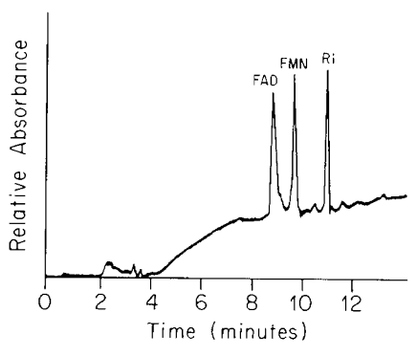

Fig. 1. Separation by HPLC of FMN, FAD, and riboflavin. For details see "Materials and methods."

Table 1. *Placental transfer of riboflavin**

Experiment	Antipyrine clearance	Riboflavin		L-Glucose		Transfer index
		Cl†	CI	Cl	CI	
Maternal to fetal transfer						
1	2.8	2.0	0.70			
2	2.5	1.3	0.53	0.53	0.22	2.4
3	1.9	1.1	0.52	0.30	0.16	3.2
4	2.7	2.0	0.71	0.42	0.15	4.7
5	2.2	2.2	1.0	0.68	0.30	3.3
Mean	2.4	1.7	0.69	0.48	0.21	3.4
Fetal to maternal transfer						
6	2.1	0.49	0.23	0.74	0.35	0.66
7	2.5	0.42	0.17	0.50	0.21	0.78
8	2.8	0.94	0.33	0.76	0.28	1.2
9	2.5	0.74	0.28	0.89	0.34	0.83
Mean	2.5	0.65	0.25	0.72	0.30	0.87

* Riboflavin-^{14}C, *ca* 10 ng/ml, L-glucose-^{3}H, 10 mg/dl, antipyrine, 10 mg/dl were added to either the maternal or fetal perfusate (see "Results" for details). Weight of perfused lobule ranged from 20–34 g except for experiment 4 (14 g).

† Cl, clearance; CI, clearance index (see "Materials and Methods" for definitions).

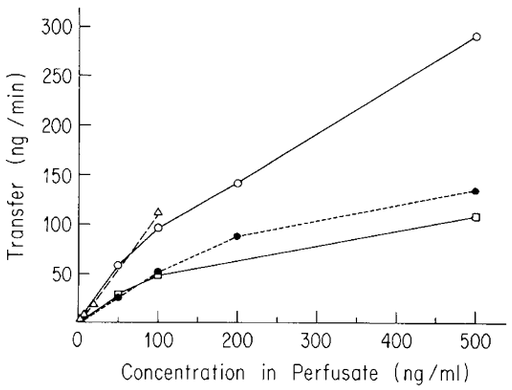

Fig. 2. The effect of increasing concentrations of riboflavin in the perfusate on the transfer rate. The perfusates were not recirculated and transfer was measured from the maternal to fetal circuit. Each *curve* represents observations on one placental lobule during the course of a perfusion study.

Table 2. *Transfer index of riboflavin at several substrate concentrations**

Experiment	Substrate concentration (ng/ml)				
	50	100	200	500	1000
1	3.2	2.6		1.3	1.1
2	3.0	3.0	2.2	1.5	
3	2.8	2.6	1.8	1.3	
Mean	3.0	2.7	2.0	1.4	

* Transfer index = clearance riboflavin/clearance L-glucose.

Transplacental gradient. After stabilizing the placenta by perfusing with buffer with both circuits open, the perfusates were changed so that the maternal and fetal perfusates contained identical concentrations of radioactive riboflavin (50 ng/ml). The fetal perfusate was recirculated and the maternal was not. Antipyrine, 10 mg/dl, was added only to the maternal perfusate.

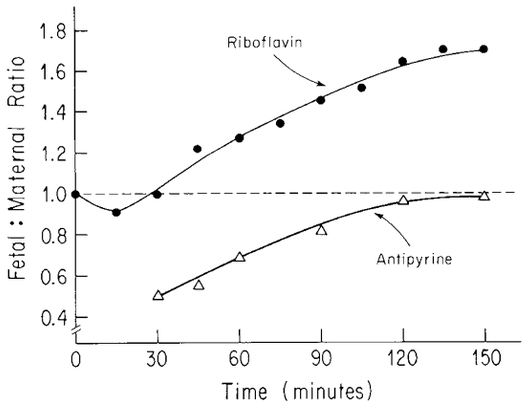

Fig. 3. Transplacental gradient of riboflavin. Maternal and fetal perfusates contained, initially, equal concentrations of riboflavin. The maternal circuit was kept open (single-pass) and the fetal perfusate was recirculated.

The first experiment lasted 105 min. At the end of the experiment, the antipyrine concentration in the fetal circuit approached that in the maternal inflow (ratio of 0.95) whereas the fetal riboflavin concentrations clearly exceeded the maternal (ratio of 1.24). The study period was prolonged to 150 min in the second experiment permitting the fetal concentrations of antipyrine and riboflavin to more closely approach equilibration with the maternal (Fig. 3). The brief drop in riboflavin concentration at the beginning of the experiment was caused by dilution with retained perfusate in the fetal vasculature and tubing following the stabilization period. Riboflavin concentration then rapidly increased establishing a striking transplacental gradient (fetal concentration:maternal concentration = 1.7). In contrast, antipyrine plateaued at a ratio that approximated 1.0.

DISCUSSION

The reported blood levels of riboflavin in maternal and cord blood vary over a broad range (2–4) reflecting different assay techniques and the problems inherent in measuring substances in very low concentrations. The analytical techniques did not sharply differentiate riboflavin from its two major metabolites, FMN and FAD. There is a general agreement in the literature that riboflavin and/or its metabolites are present in higher concentration in fetal than maternal blood. In one report, it was suggested that the placenta was actually impermeable to riboflavin (2). The speculation was that the placenta actively accumulated FAD from the mother and hydrolyzed it to riboflavin which was released to the fetus. Riboflavin accumulated in the fetal circuit establishing a gradient.

We have approached the question directly using an *in vitro* perfusion technique of a placental lobule. Three experimental designs have been used all of which provide clear evidence of an efficient transport mechanism for riboflavin directed toward the fetus. Two reference materials have been used in the studies. The transfer rate of antipyrine was concurrently measured in all experiments and of L-glucose in many experiments. Antipyrine has been classified as a flow-limited substrate. Its clearance is determined by the flow rate and the relative arrangements of the maternal and fetal circulations at the area of exchange. It is lipid-soluble, diffusing freely across membranes. L-Glucose is a water-soluble molecule, which is not metabolized and is small in size (mol wt 180). The diffusion route is limited to the water-filled channels that are presumed to traverse membranes. By comparing riboflavin transfer to the reference materials in each placental preparation, convincing interpretations can be made after relatively few experiments.

The first series of experiments demonstrated that, contrary to previous speculation, riboflavin was readily transferred across the placenta toward the fetus (Table 1). HPLC analysis of the radioactivity in the fetal perfusate confirmed the fact that riboflavin had been transferred without conversion to either of its major metabolites, FMN or FAD.

The rate of transfer of riboflavin toward the fetus was considerably greater than for L-glucose, even though its molecular weight (mol wt 376.4) is greater (Table 1). This observation was not consistent with a process of simple diffusion suggesting some type of mediated transport, presumably by a carrier system.

The demonstration of saturation of transport with increasing concentrations of substrate was also consistent with mediated transport (Table 2, Fig. 2). No attempt was made to define accurately the level at which saturation of riboflavin transport occurred. The important conclusion was that saturation of the transport system did occur and that it occurred at very low concentrations of riboflavin, in the range of 100 ng/ml in this experimental model.

Facilitated diffusion and active transport are examples of mediated transport. The former maintains the essential characteristic of diffusion in that transfer is only from higher to lower concentrations of the substance under study. The transfer rate is, however, accelerated above that to be expected from simple diffusion. In active transport, there is the capability of transfer against a concentration gradient.

The higher clearance index toward the fetus than in the reverse direction is inconsistent with a diffusion process. The critical experiments, however, that demonstrated that an active transport system was available to riboflavin were those in which the fetal perfusate was recirculated and the maternal perfusate was not. In this experimental design, the riboflavin concentration in the fetal perfusate is permitted to equilibrate against a constant concentration in the maternal circuit. A substantially higher concentration of riboflavin was established in the fetal circulation indicating active transfer across the placenta toward the fetus (Fig. 3). Analysis by HPLC identified the radioactive material in the fetal perfusate as riboflavin. In contrast antipyrine, which is transferred by diffusion, achieved equivalent concentrations in the two circuits.

Comparison of riboflavin transport to that of D-glucose and L-amino acids, which has been previously studied in the same perfusion system, places the present observations in broader perspective. The transfer index of D-glucose also approximates 3, very similar to that of riboflavin. These two nutrients are the most rapidly transferred that have been studied so far in this system. The capacity of the glucose transport system, however, far exceeds that of riboflavin by approximately a million-fold. Whereas the former transfers milligrams of substrate, the latter is equipped for nanograms. This difference is appropriate to the differences in circulating maternal concentrations of the two substrates as well as to the fetal requirements.

Another major difference is that D-glucose is transferred by facilitated diffusion. Transfer across the placenta is equally rapid in both directions and, as a corollary, a gradient is not established. In this respect, the placental transfer of riboflavin is more comparable to the active transport of amino acids (8). The capacity of the transport system for amino acids is also considerably greater than for riboflavin but the transfer rate is slower. The clearance indices vary among the several amino acids, ranging from approximately one-third to one-half that of riboflavin (9).

In summary, riboflavin is actively transferred across the perfused human placenta. The transfer rate toward the fetus greatly exceeds that in the reverse direction and a gradient is established with the concentration in the fetal circulation higher than in the maternal. The capacity for active transport is very small. It will be of interest to extend these observations to other water-soluble vitamins.

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Announcements

American Pediatric Society and Society for Pediatric Research Schedule of Annual Meetings

1986—Washington, D. C.—May 5–9

1987—Anaheim, CA—April 27–May 1

1988—Washington, D. C.—May 2–6

1989—Washington, D. C.—May 1–5

1986 Abstract Receipt Deadline: December 2, 1985.

For meeting registration or abstract information, contact: Audrey Brown, M.D., Secretary-Treasurer, American Pediatric Society, Downstate Medical Center, Department of Pediatrics, 450 Clarkson Avenue, Box 49, Brooklyn, NY 11203 (718) 270-1692 or William Berman, Jr., M.D., Secretary-Treasurer, Society for Pediatric Research, Department of Pediatrics, University of New Mexico School of Medicine, Albuquerque, NM 87131 (505) 277-6628.

Annual Meeting of the Society for Adolescent Medicine

The Annual Meeting of the Society for Adolescent Medicine will be held in Boston, MA, March 13–16, 1986. We will be holding our annual J. Roswell Gallagher Lectures with expert speakers from the fields of medicine and the behavioral and social sciences. The theme for our Gallagher Lectures will be Growth and Development. The Society will also offer workshops on relevant topics and present clinical and experimental research papers and posters.

For further information please contact: Ms. Edie Moore, Administrative Director, The Society for Adolescent Medicine, P.O. Box 3462, Granada Hills, CA 91344 (818) 368-5996.

Histiocytosis-X

The Food and Drug Administration is sponsoring a nationwide clinical study of Suppressin A, a thymic hormone preparation, in the treatment of histiocytosis-X (N Engl J Med 304: 146–153, 1981 and Thymic Factor Therapy, Academic Press, 1984, pp 391–398).

For referral of patients and/or additional information please contact the Principal Investigator: Michael E. Osband, M.D., Division of Pediatric Hematology-Oncology, Boston University School of Medicine, 80 E. Concord Street, Boston, MA 02118 (617) 638-4182.