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THE TRANSPORT OF RIBOFLAVIN BY HUMAN PLACENTA [1]

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The human placenta has been shown to have the ability to transfer thiamin (1), ascorbic acid (2–4), fructose (5), organic and inorganic phosphate (6), and, early in pregnancy, glucose (6) from maternal to fetal blood. No data were available concerning the concentration of riboflavin in normal maternal and fetal bloods. Although some analyses had been performed of the riboflavin content of tissues from fetal and stillborn infants (cf. Smith [7]), the riboflavin content of the placenta was unknown. In this paper are presented the results of riboflavin analyses of ten normal placentas and of maternal and cord blood obtained during ten normal deliveries. These analyses suggest that an active metabolic process is involved in the transfer of riboflavin from maternal to fetal blood by the placenta.

METHODS

Samples of maternal blood were collected by venepuncture during full term delivery and samples of cord blood were obtained immediately after the expulsion of the placenta. The blood samples were permitted to clot normally and then centrifuged to obtain the serum. The serums were analyzed by the method of Burch, Bessey, and Lowry (8), in which separate estimations of free riboflavin (FR), riboflavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) are made. The serums were diluted ten-fold with 5 per cent trichloroacetic acid for analysis. The placentas from these deliveries were also analyzed for free riboflavin, riboflavin mononucleotide and flavin adenine dinucleotide by the method of Bessey, Lowry, and Love (9). One Gm. of placenta was minced in a Waring blendor with 50 ml. of redistilled water and then diluted with an equal volume of 20 per cent trichloroacetic acid.

The determinations of riboflavin were made using a Farrand photofluorometer [2]; fluorescein standards of 2.5 and 10 millimicrograms per ml. were used. The analyses were carried out in the dark at 5° C. and were made in duplicate or triplicate.

RESULTS

The results of the analyses of serum are given in Table I. Fetal (cord) blood at term has a significantly higher concentration of total riboflavin than maternal blood. This is due to the fact that fetal blood has about four times as much free riboflavin as maternal blood yet only half as much flavin adenine dinucleotide. The amounts of riboflavin mononucleotide (FMN) in both maternal and fetal bloods are very small. The analyses of maternal blood are comparable to those found by Burch, Bessey, and Lowry (8) for normal human blood.

Analyses of placenta showed that it had an average of 214 micrograms total riboflavin per 100 Gm. (wet weight) tissue. Of this 205 micrograms were FAD and only 9 were FMN plus free riboflavin. This is comparable to the amounts found in rat brain and skeletal muscle by Bessey, Lowry, and Love (9). These investigators found much higher concentrations of riboflavin in rat liver, kidney and heart, but in all tissues, 80 per cent or more of the riboflavin was present as FAD.

The results of the analyses of serum suggested that neither free riboflavin nor FAD can diffuse readily across the placenta and that the placenta maintains the higher concentration of riboflavin in fetal blood by splitting FAD to free riboflavin. To determine whether the placenta had enzymes for splitting FAD, minced placenta was incubated in a modified Ringer's solution (90 mM per L. Na^+, 50 mM per L. K^+, 10 mM per L. Mg^{++}, and 40 mM per L. phosphate), to which was added enough FAD so that the initial FAD concentration was 300 micrograms per 100 Gm. placenta. One Gm. of placenta was minced and incubated at 37° C. for one hour with shaking in 100 ml. of solution contained in 250 ml. Erlenmeyer flasks.

The results of analyses for FAD, FMN, and free riboflavin before and after incubation are given in Table II. It is clear that the placenta has a mechanism, presumably enzymatic, for

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[2] We are greatly indebted to Dr. Clement A. Smith for his kindness in permitting us to use his Farrand photofluorometer.

TABLE I

Riboflavin concentrations in maternal and fetal serum expressed in micrograms per 100 ml. of serum

Sample	Free riboflavin		Flavin adenine dinucleotide		Riboflavin mononucleotide		Total riboflavin	
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
1	1.36	3.17	3.00	2.20	0	0.1	4.10	5.40
2	0.68	2.25	2.31	1.74	0.3	0.25	3.29	4.25
3	0.26	2.19	2.70	0.85	0.04	0.6	3.00	3.65
4	0.67	2.49	3.18	1.99	0	0	3.87	4.47
5	0.25	2.24	2.08	0.83	0.04	0.5	2.37	3.53
6	0.64	2.77	3.33	0.95	0	0.45	3.80	4.15
7	0.18	1.76	1.86	1.09	0.31	0.17	2.35	3.02
8	0.56	1.46	2.25	1.11	0	0.26	2.75	2.83
9	0.50	1.42	1.70	0.96	0.09	0.17	2.29	2.55
10	0.35	1.63	2.34	1.27	0.16	0.28	2.85	3.18
Mean	0.55	2.14	2.48	1.29	0.09	0.28	3.07	3.70
Standard error	±0.12	±0.18*	±0.18	±0.16*	±0.10	+0.15	±0.20	±0.29†

* p 0.01 and † p 0.05 that the fetal-maternal differences are not significant, calculated by paired t test.

splitting FAD to FMN and FR and that the amount of FMN and FR formed accounts for the amount of FAD that disappeared.

DISCUSSION

These experiments show that there is significantly more free riboflavin and significantly less FAD in fetal blood than in maternal blood. The total amount of riboflavin (FAD + FMN + FR) is greater in fetal blood than in maternal blood.

These analyses also show that placenta contains large amounts of riboflavin and that, as in other tissues, most of this riboflavin is present as FAD. Minced placenta incubated *in vitro* will split FAD to FMN and FR.

These data suggest that the placenta transfers riboflavin from maternal to fetal blood by taking in riboflavin from the maternal blood as FAD, splitting the FAD and secreting the free riboflavin into the fetal blood. An alternative explanation, that fetal tissues can synthesize the isoalloxazine ring and thus increase the concentration of free riboflavin in fetal blood, seems unlikely. Presumably, fetal tissues need FAD, not free riboflavin, and synthesize their own FAD from the free riboflavin taken in from the fetal blood. In the course of these experiments one sample of 20-week fetal liver became available. It was found to contain 670 micrograms of total riboflavin per 100 Gm. wet tissue, almost all of which was FAD. This finding is in accordance with the hypothesis that fetal tissues synthesize their own FAD from riboflavin of the blood.

TABLE II

*The conversion of flavin adenine dinucleotide to flavin mononucleotide and free riboflavin by minced human placenta**

	Initial	Final	Change
Total riboflavin	362	383	+ 21 ± 7
Flavin adenine dinucleotide	292	169	−123 ± 13
Free riboflavin	8	56	+ 48 ± 3
Flavin mononucleotide	62	161	+ 99 ± 14

* Values are the averages of three experiments, expressed as micrograms per 100 Gm. (wet weight) placenta, ± the standard error of the mean change.

SUMMARY

1. Photofluorometric analyses of maternal and fetal serum demonstrated that the concentration of free riboflavin is about four times as great in fetal blood as in maternal blood; in contrast, the concentration of flavin adenine dinucleotide is about twice as great in maternal blood as in fetal blood. The total concentration of all forms of riboflavin, free, mononucleotide and dinucleotide, is greater in fetal than in maternal blood.

2. Minced placenta incubated *in vitro* will split FAD to FMN and free riboflavin.

3. The data suggest that the placenta transfers riboflavin from maternal to fetal blood by taking in FAD from the maternal blood, splitting this to free riboflavin, and secreting the free riboflavin into fetal blood.

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