

# Placental Transport of Vitamin B<sub>12</sub> in the Pregnant Rat

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**ABSTRACT** Placental transport of vitamin B<sub>12</sub> was studied in the pregnant rat in two series of experiments. In the first series animals were given cyanocobalamin-<sup>57</sup>Co intravenously at various stages of gestation. High specific activity tracer was used and doses of B<sub>12</sub> were 1–2 ng per animal. The rats were killed from 15 min to 24 hr after injection and the fetuses, placentas, and serum were assayed for radioactivity. In the second series using uninjected animals, absolute amounts of vitamin B<sub>12</sub> in fetuses and placentas were measured at stages of gestation from day 12 through day 20.

There was a progressive increase in B<sub>12</sub> transferred to the fetus during gestation. Although the quantity of vitamin B<sub>12</sub> transported per 24 hr was proportional to fetal weight, the amount transported per gram of placenta increased tenfold from day 10 through day 19. Uptake of tracer B<sub>12</sub> by placenta was initially rapid; however, no radioactivity appeared in the fetus until 2 hr after injection. The actual amount of B<sub>12</sub> in placenta increased throughout gestation, and the placental concentration of B<sub>12</sub> was greater than maternal plasma and fetal tissue concentrations at all times measured.

These data suggest that the ability of placenta to transport B<sub>12</sub> increased throughout gestation, and that the rate-limiting step in the transport process was either the passage of B<sub>12</sub> from the maternal to the fetal side of placenta or the transfer from placenta into fetal plasma.

## INTRODUCTION

Several investigators have shown that radioactive cyanocobalamin<sup>1</sup> crosses placenta from mother to fetus

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<sup>1</sup> The terms cyanocobalamin, vitamin B<sub>12</sub> and B<sub>12</sub> are used interchangeably throughout the text and refer to cyanocobalamin.

(1–3). This transfer can occur against a concentration gradient, since maternal plasma vitamin B<sub>12</sub> levels late in pregnancy are much lower than corresponding fetal levels (4, 5). Luhby, Cooperman, Stone, and Slobody have suggested that labeled vitamin B<sub>12</sub> accumulates in the human placenta before passing to the fetus (6). In their work, substantial amounts of radioactivity were not transferred to the fetus until 24 hr after administration (7). Ullberg, Kristoffersson, Flodh, and Hanggren (8) injected mice with supra-physiologic doses of cyanocobalamin-<sup>58</sup>Co and also showed a buildup of radioactivity in placenta with relatively slow passage to fetus. Other than the above, little is known about the transport process. The experiments reported here were undertaken to characterize further the kinetics and mechanism of vitamin B<sub>12</sub> placental transfer.

## METHODS

*Animal experiments.* Pregnant Sprague-Dawley descendant rats<sup>2</sup> were used as an animal model. Total gestation in this species is 21 days and the number of fetuses per litter ranges from 8 to 16. Onset of pregnancy was timed by the observation of a copulation plug. All animals were fed a standard commercial laboratory rat diet.<sup>3</sup> Two separate methods of study were employed. In the first method, each rat was given 1–2 ng of high specific activity (165  $\mu$ Ci/ $\mu$ g) cyanocobalamin-<sup>57</sup>Co<sup>4</sup> intravenously through a tail vein. At selected time intervals the rats were killed and the fetuses, placentas, and maternal serum were assayed for <sup>57</sup>Co radioactivity. Animals from 10 to 19 days of gestation were studied 24 hr after injection, and 16- and 18-day pregnant rats were studied from 15 min to 24 hr postinjection. In several other animals, whole body and urine radioactivity was monitored for a period of 48 hr after injection. During this time whole body radioactivity did not change and less than 1% of the injected dose appeared in the urine.

In the second method, rats which had not been given tracer vitamin B<sub>12</sub> were sacrificed at days 12, 14, 16, 18, and 20 of gestation. The placentas and fetuses from each animal were separated and pooled and the vitamin B<sub>12</sub> was extracted and measured by a competitive binding assay.

*Tissue extraction of vitamin B<sub>12</sub>.* Vitamin B<sub>12</sub> was extracted from fetuses and placentas by a modification of the

<sup>2</sup> Charles River Breeding Laboratories, Wilmington, Mass.

<sup>3</sup> Ralston Purina Company, St. Louis, Mo.

<sup>4</sup> Amersham-Searle Corp., Arlington Heights, Ill.

method of Bacher, Boley, and Shonk (9). Tissues were homogenized in deionized water and transferred quantitatively to a boiling flask. A known amount of tracer cyanocobalamin- $^{57}\text{Co}$  was then added. Sufficient volumes of 2% sodium nitrite and 0.8% potassium cyanide were placed in the flask to give a final concentration of 0.5 and 0.2% respectively. The pH was adjusted to 4.5 with concentrated acetic acid and the mixture was boiled vigorously for 7-8 min. After cooling, the slurry was filtered through a Büchner funnel. A 10 ml sample of this filtrate was passed through a millipore filter (pore diameter  $0.45\ \mu$ ) and then placed on an ion exchange column consisting of 10 ml of Amberlite<sup>5</sup> IR 120 overlaid with 10 ml of IRA 400. The first 10 ml of eluate was discarded (void volume) and the second 10 ml containing most of the radioactivity was collected. The  $^{57}\text{Co}$  radioactivity in 1.0 ml of this material was measured and compared to the total counts added to determine the recovery of vitamin B<sub>12</sub>. A sample of this column eluate was then used in the competitive binding assay of B<sub>12</sub>.

*Assay of nonradioactive vitamin B<sub>12</sub>.* Vitamin B<sub>12</sub> was measured by a modification of the competitive binding assay described by Friedner, Josephson, and Levin (10). Saliva (11) was used as the B<sub>12</sub> binder and cyanocobalamin- $^{57}\text{Co}$  as the assay tracer. This B<sub>12</sub> binder has an estimated molecular weight of 60,000 (11), while cyanocobalamin's molecular weight is 1357. Free B<sub>12</sub> was separated from bound B<sub>12</sub> by ultrafiltration through Centrifo<sup>6</sup> membrane cones, which retain molecules above 50,000 molecular weight and have essentially no retention for molecules below 5000. The  $^{57}\text{Co}$  radioactivity in an aliquot of the ultrafiltrate was used as a measure of free B<sub>12</sub>. For each run a standard curve was constructed by treating tubes containing from 0 to 1500 pg of cyanocobalamin exactly as the unknowns. The standard curves were sigmoid in shape and most sensitive in the middle two-thirds. Therefore, a dilution of the column eluates of the unknowns which gave values within this portion of the curve was used.

*Radioactivity measurement.* All specimens were measured for radioactivity in a well-type NaI(Tl) scintillation detector coupled to a standard spectrometer. Sufficient counts were obtained to ensure counting errors of less than 2%. Dual isotope counting for separation of  $^{57}\text{Co}$  from  $^{58}\text{Co}$  activity was performed by the two window method (12). The per cent injected dose or per cent recovery was determined by comparison with the appropriate standard prepared in an equal volume for identical counting geometry.

## RESULTS

Fig. 1 shows the radioactivity in fetuses and placentas at various times during gestation in animals killed 24 hr after injection of vitamin B<sub>12</sub>- $^{57}\text{Co}$ . The rate of transfer of label to the fetuses per 24 hr period was found to increase throughout pregnancy from 0.5% of the injected dose at day 10 to 55% at day 19. This increase was particularly rapid during the latter part of gestation, rising from 33% on day 18 to 55% on day 19. Accumulation of label in placentas followed a similar pattern to fetuses through day 14, then plateaued at about 12% of the dose, a level considerably less than the corresponding fetal values. In Fig. 2, the same fetal

<sup>5</sup> Mallinckrodt Chemical Works, St. Louis, Mo.

<sup>6</sup> Amicon Corporation, Lexington, Mass.

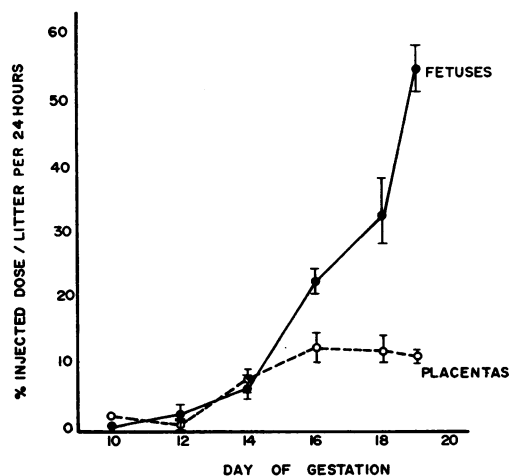


FIGURE 1 Fetal and placental vitamin B<sub>12</sub>- $^{57}\text{Co}$  radioactivity 24 hr after injection at various stages of gestation. Values equal the mean of three or four animals  $\pm 1$  SD except the 10-day points, which represent a single animal.

data shown in Fig. 1, are plotted on a per gram basis. The concentration of tracer in the fetus did not change appreciably during gestation. However, the amount of label transported to the fetuses per gram of placenta per 24 hr period steadily increased from 0.5% on day 10 to 5.4% on day 19.

Fig. 3 shows the accumulation of labeled B<sub>12</sub> in fetuses and placentas of 16-day animals at various times after injection. Initially there was very rapid uptake of tracer by placenta, which increased until 2 hr after injection. The radioactivity then leveled off at 17% of the injected dose and finally began to slowly decrease after 16 hr.

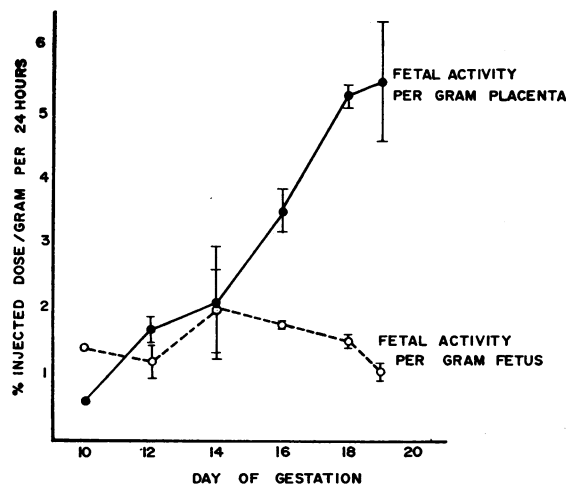


FIGURE 2 Fetal data from Fig. 1 expressed as radioactivity per gram fetus and per gram placenta. Values equal the mean of three or four animals  $\pm 1$  SD except the 10-day points, which represent a single animal.

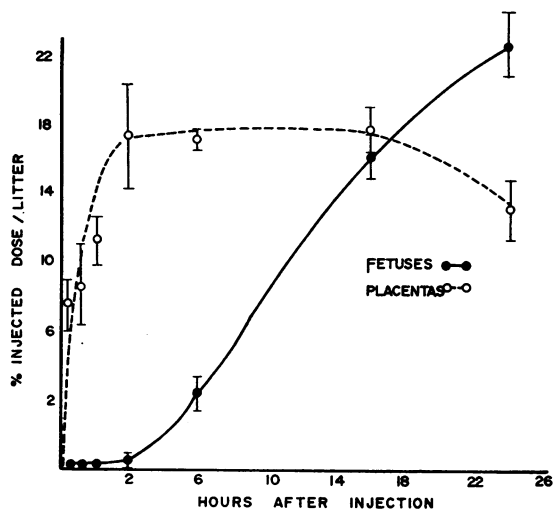


FIGURE 3 Fetal and placental vitamin B<sub>12</sub>-<sup>57</sup>Co radioactivity 15 min to 24 hr after injection in the 16th day of pregnancy. Values equal the mean of three or four animals  $\pm 1$  SD.

In contrast, no activity was seen in the fetuses until 2 hr postinjection when only 0.3% of the injected dose was present. Subsequently, there was progressive fetal uptake of label reaching 22.5% of the dose at 24 hr. This same type of data obtained in 18-day rats is shown in Fig. 4. Total uptake by the placentas and fetuses was significantly greater than in 16-day animals, but the pattern of uptake was similar. In both the 16- and 18-day animals the plasma clearance of radioactive B<sub>12</sub> was initially very rapid, with less than 0.55% of the dose per ml of serum present 15 min after injection at both

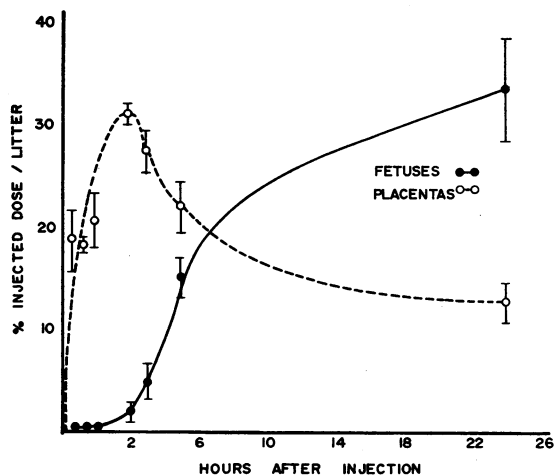


FIGURE 4 Fetal and placental vitamin B<sub>12</sub>-<sup>57</sup>Co radioactivity from 15 min to 24 hr after injection in the 18th day of pregnancy. Values equal the mean of three or four animals  $\pm 1$  SD.

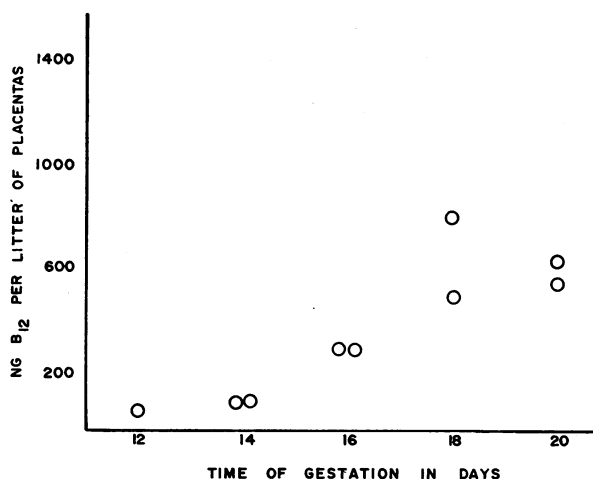


FIGURE 5 Vitamin B<sub>12</sub> content of placentas on various days of gestation. Each point represents the pooled placentas from a single animal.

stages of gestation. The serum activity then decreased slowly over the next 24 hr to 0.043 and 0.039% per ml.

Figs. 5 and 6 show the total amount of actual vitamin B<sub>12</sub> in placenta and fetus at various stages of gestation from day 12 through day 20. The quantity of vitamin in the placenta appeared to increase throughout gestation, as the total content per litter rose from 75 ng on day 12 to over 550 ng on day 20. The quantity of B<sub>12</sub> in the fetuses also increased progressively, rising from 40 ng on day 12 to 1400 ng on day 20. The amount of vitamin B<sub>12</sub> per gram of fetus or placenta at the various stages of gestation is plotted in Fig. 7. The fetal concentration was relatively constant throughout pregnancy ranging from 22 to 39 ng per gram of tissue, but the

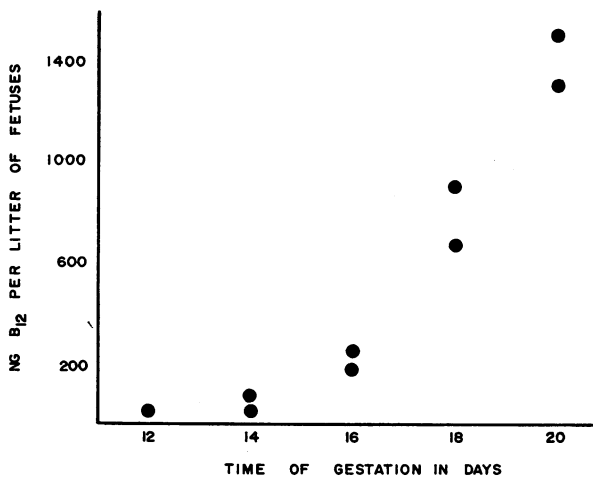


FIGURE 6 Vitamin B<sub>12</sub> content of fetuses on various days of gestation. Each point represents the pooled fetuses from a single animal.

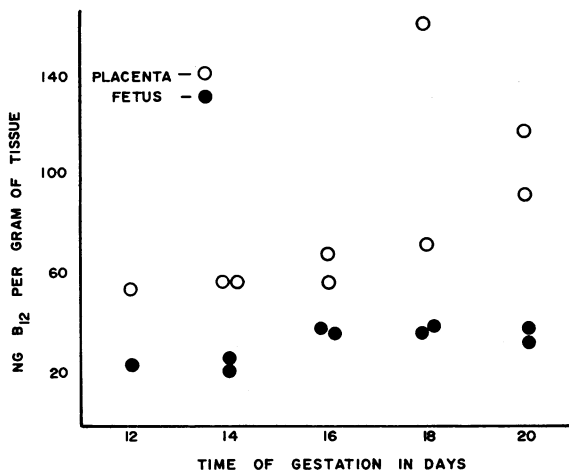


FIGURE 7 Vitamin B<sub>12</sub> content per gram fetus and per gram placenta on various days of gestation. Each point represents the pooled fetuses or placentas from a single animal.

placental concentration increased during the latter 4 days of pregnancy, and was much greater than the fetal concentration at all times measured during gestation.

## DISCUSSION

Previous investigators (6-8) have shown that late in pregnancy, radioactive cyanocobalamin accumulates in the placenta and only slowly passes to the fetus. Our work has confirmed these findings. In addition, we have used very small doses of radioactive B<sub>12</sub> to study the rate of transfer of tracer to the fetus throughout gestation, and have measured the total vitamin B<sub>12</sub> content of the fetuses and placentas periodically during pregnancy.

In our experiments the 24 hr rate of placental transfer of tracer B<sub>12</sub> increased progressively throughout gestation, as did the fetal content of vitamin B<sub>12</sub>. These increases were most rapid during the latter 3-4 days of gestation, and appeared to be proportional to fetal growth. The concentration per gram of fetus of radioactivity and of total B<sub>12</sub> remained relatively constant throughout pregnancy. On the other hand the amount of tracer vitamin B<sub>12</sub> transferred daily to fetus per gram of placenta steadily rose some ten-fold from day 10 to day 19. This suggests that either the ability of placenta to transport B<sub>12</sub> increased during gestation or alternatively, that fetal growth was associated with removal of some block in the transport process. Possibly a feedback mechanism exists which is related to fetal tissue vitamin B<sub>12</sub> concentration.

The rat chorioallantoic placenta consists of three layers of trophoblast, a basement membrane, and one layer of fetal endothelium (13). It is perfused on one side by maternal blood and on the other by fetal blood.

Transport of vitamin B<sub>12</sub> from mother to fetus can thus be divided arbitrarily into three stages: (a) transfer of vitamin from maternal plasma across the maternal-placenta interface into placenta, (b) passage from the maternal to the fetal side of placenta, and (c) transfer across the placental-fetal interface into the fetal plasma. In the tracer experiments in which rats at one stage of gestation were killed at various times after injection, there was rapid uptake of labeled vitamin B<sub>12</sub> by placenta and approximately 2 hr elapsed before label was found in the fetus. These results are in agreement with those of Ullberg et al. (8), who used much larger doses of vitamin B<sub>12</sub>, and indicate that stage (a) of the transport process is rapid and not rate limiting. Furthermore, since the concentration of B<sub>12</sub> in placenta, which ranged from 55 to 163 ng per g, was much greater than maternal plasma levels (4, 5, 14), this initial uptake of vitamin B<sub>12</sub> by placenta may represent an active transport process. Alternatively, placental tissue might contain a binder with a much higher affinity for vitamin B<sub>12</sub> than maternal plasma. The 2 hr lag before appearance of radioactivity in the fetus and the accumulation of B<sub>12</sub> in the placentas during gestation suggest that either stage (b) or (c) represents the rate-limiting step of the transport process. This concept would be in keeping with the observed downhill concentration gradient from placenta to fetus throughout pregnancy, which was greatest during the latter part of gestation when B<sub>12</sub> transport was increasing rapidly.

The 1-2 ng of tracer cyanocobalamin given each animal apparently did not exceed the capacity of its plasma B<sub>12</sub>-binding proteins, since virtually no label appeared in the urine, and whole body radioactivity was unchanged for the 48 hr following injection. Rosenblum, Reizenstein, Cronkite, and Meriwether have shown identical tissue distribution of oral and intravenously administered radioactive cyanocobalamin (15). The small amounts of tracer B<sub>12</sub> given in our studies thus presumably behaved in a similar fashion to vitamin B<sub>12</sub> absorbed from the mother's gastrointestinal tract.

The time pattern of the placental transport of B<sub>12</sub> may be analogous to the transfer of intrinsic factor bound B<sub>12</sub> across ileal mucosa into blood. In this case in man there is slow passage of labeled B<sub>12</sub> across the mucosal cell and blood levels do not peak until 6-10 hr after oral ingestion (16). Booth, Chanarin, Anderson, and Mollin used 2.5 ng oral doses of B<sub>12</sub>-<sup>58</sup>Co and demonstrated in the rat that 40% of the ingested B<sub>12</sub> was bound to the wall of the small intestine within 15 min (17). No radioactivity was found in the blood until 1.5 hr after ingestion and peak levels were not seen until 4 hr after ingestion.

In contrast, the pattern of placental transport of vitamin B<sub>12</sub> appears to be distinctly different from that of

iron, a nutrient which like vitamin B<sub>12</sub> is necessary for normal erythropoiesis, is bound to a specific plasma protein, and is present in lower concentrations in maternal than fetal plasma (18). When tracer <sup>59</sup>Fe was bound to transferrin and injected into 18-day pregnant rats, significant differences in placental transport from tracer B<sub>12</sub> were observed (19, 20). Transfer of <sup>59</sup>Fe to the fetus began immediately and rapidly reached a plateau, while B<sub>12</sub>-<sup>57</sup>Co did not appear in the fetus until 2 hr after injection and continued to increase for the period of observation. In addition, significant placental accumulation of radioactivity was not observed when <sup>59</sup>Fe was injected, but did occur after injection of B<sub>12</sub>-<sup>57</sup>Co.

#### ACKNOWLEDGMENTS

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