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Transport of Orally Administered Vitamin B_{12} to Embryo and Placenta *in vivo* and Intrinsic Factor Dependent Vitamin B_{12} Adsorption to Placenta *in vitro* at Various Stages of Gestation in Rats

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Summary

Pregnant rats were administered with vitamin B_{12} by mouth. Transplacental transfer of vitamin B_{12} to embryo increased toward the last stage of gestation. But when expressed by the amount of vitamin B_{12} per g body weight of embryo, a peak was shown on the sixteenth day of gestation. Vitamin B_{12} per individual placenta or per g tissue of placenta were also highest on the sixteenth day. The intrinsic factor-mediated vitamin B_{12} adsorption capacity of placenta *in vitro* per g tissue was lowest on the eighteenth day of gestation. The amount of vitamin B_{12} adsorbed with the aid of the intrinsic factor to total placentas in all *in vitro* increased toward the last stage of gestation in parallel with the transplacental transfer of vitamin B_{12} to embryo *in vivo*.

In the previous paper (1), we have reported that much of the vitamin B_{12} which had been administered to pregnant rats accumulated in the placenta and the embryo, and that the intrinsic factor source caused an increased adsorption of vitamin B_{12} to the placenta homogenate *in vitro*. The present report details the change of trasplacental transfer of vitamin B_{12} during the gestation process. Also, the fluctuation of the intrinsic factor-mediated vitamin B_{12} adsorption to the placenta obtained from rats at various stages of gestation is described.

Materials and Methods

Wistar strain albino rats fed on a commercial diet (F-1; product of Funabashi Farmstead Co. Ltd.) were used throughout the study. Female rats weighing 200–250 g were mated with male rats and a smear test was applied to know the first day of pregnancy. It was prescribed arbitrarily that the zero day of gestation means the day on which sperm was found in the vagina. Every daily administra-

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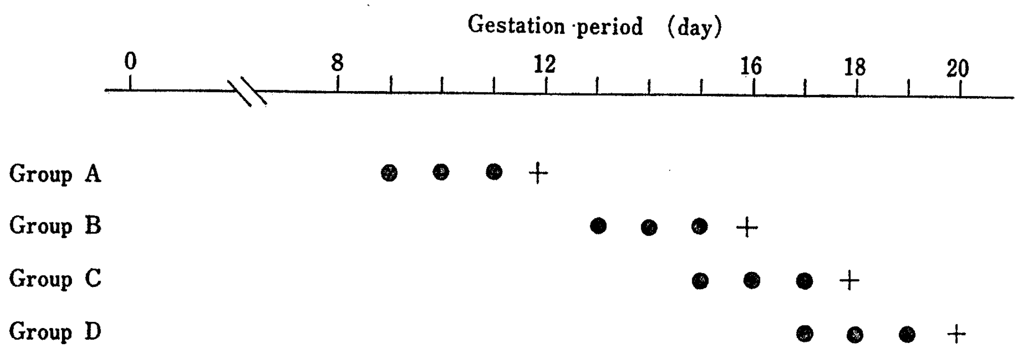

FIG. 1. Schedule for experiment to investigate the transport of orally administered vitamin B_{12} to the placenta and the embryo at various stages of gestation.
●; vitamin B_{12} administration, +; sacrifice. Pregnant rats were administered with daily doses of 5 mμg of vitamin B_{12} for three days by stomach tube beginning on the appropriate day, and were sacrificed 24 hours after the last administration. For example, rats in Group A were administered with vitamin B_{12} on the 9th, 10th and 11th day and then sacrificed on the 12th day of gestation. The amounts of vitamin B_{12} transported to the placenta and embryo were measured.

tion of 5 mμg of ^{57}Co labelled vitamin B_{12} was performed by stomach tube in 0.6 ml saline solution for three days beginning on the appropriate day. Twentyfour hours after the last administration, animals were sacrificed (see Fig. 1). Placentas and embryos were resected, washed with saline, blotted on filter paper, weighed and then placed in counting tubes to count the radioactivity for ^{57}Co-vitamin B_{12} with a well type scintillation counter. The results were reculculated in μμg of vitamin B_{12}. The intrinsic factor-mediated vitamin B_{12} adsorption capacity was measured as previously described (2). As the intrinsic factor source, the gastric juice collected by the method of Shay *et al* (3, 4) was used. The amount of intrinsic factor source employed was that which caused the maximal adsorption of vitamin B_{12} within a range in which the amount of vitamin B_{12} adsorbed by the placenta was in proportion to the amount of intrinsic factor source added. The intrinsic factor-mediated vitamin B_{12} adsorption capacity was calculated by subtracting the amount of vitamin B_{12} adsorbed in the absence of the intrinsic factor source, which was generally constant, from the total amount of vitamin B_{12} adsorbed in the presence of the intrinsic factor source.

Results

1. *Weight Change of Placentas and Embryos.* In Fig. 2 is shown the weight changes of placentas and embryos during the gestation period from 12th to 20th day. The rate of increase of placenta weight was relatively steady with some slowness toward the last stage of gestation. Contrastively, the weight of the embryo increased gradually until the 18th day and then showed a sharp rise between the 18th and 20th day.

2. *Transport of Vitamin B_{12} to Embryo and Placenta.* The ^{57}Co-vitamin B_{12} which had been administered to pregnant rats was found in the placenta and

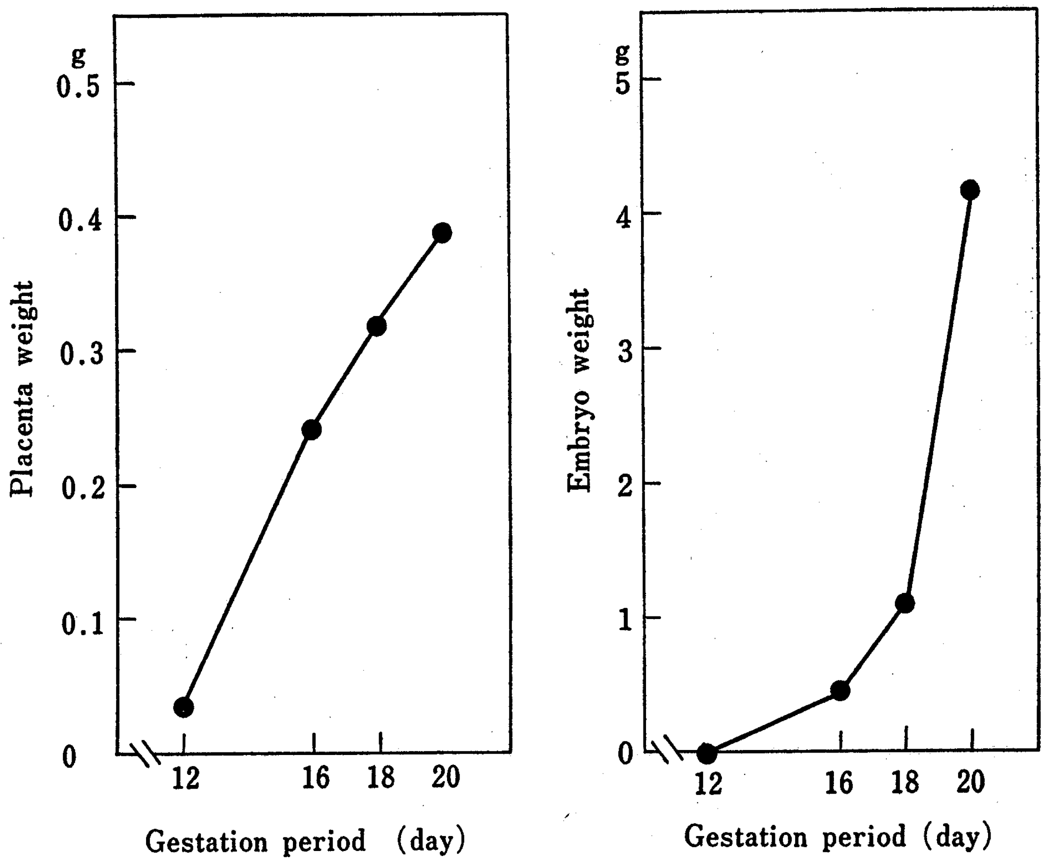

FIG. 2. Weight changes of the placentas and embryos during the gestation period from the 12th to 20th day.
Pregnant rats were sacrificed after the duration of pregnancy as indicated on the abscissa, and the weights of the placentas and embryos were measured.

embryo in various amounts according to the stage of gestation as shown in Fig. 3. The amount acquired by the individual embryo increased rapidly toward the last stage. The increase was not necessarily in proportion to the increase of the body weight of the embryo. When the amount of vitamin B_{12} was expressed by $\mu\mu g$ per g body weight of embryo, the maximum was shown on the 16th day and the minimum on the 20th day (Fig. 3-a). The vitamin B_{12} in placenta was the highest on the 16th day and was low on the 20th and 12th day whether the amount was calculated in $\mu\mu g$ of vitamin B_{12} per individual placenta or per g tissue of placenta (Fig. 3-b).

3. *Intrinsic Factor-mediated Vitamin B_{12} Adsorption to Placenta.*

The amount of vitamin B_{12} adsorbed with the aid of the intrinsic factor to placenta from various stages of gestation is shown in Fig. 4. The intrinsic factor-mediated vitamin B_{12} adsorption capacity of the placenta per g tissue varied inversely to the transport of vitamin B_{12} to the placenta *in vivo*, i.e. on the 16th and 18th day, when the latter was great, the former was small and on the 12th and

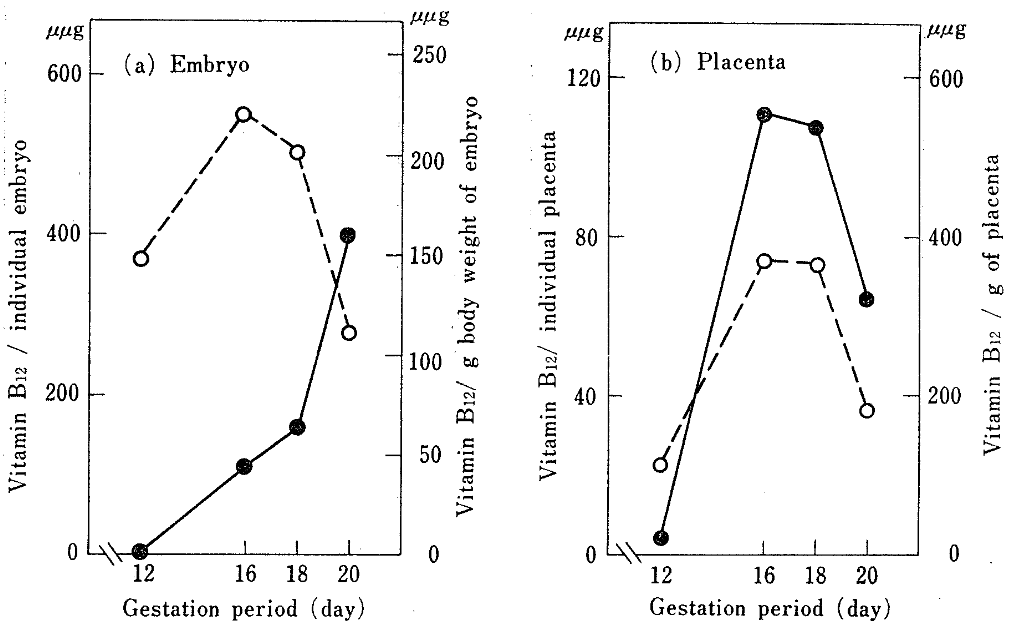

FIG. 3. Transport of orally administered vitamin B_{12} to the embryo and placenta. (a) Transport to embryo. ●——●; vitamin B_{12}/individual embryo, ○- - -○; vitamin B_{12}/g body weight of embryo. (b) Transport to placenta. ●——●; vitamin B_{12}/individual placenta, ○- - -○; vitamin B_{12}/g of placenta. Pregnant rats were administered daily with 5 mμg of vitamin B_{12} for three days and then sacrificed on the day indicated on abscissa. The amounts of vitamin B_{12} in the embryo and placenta were measured.

20th day, *vice versa* (see Fig. 3-a). The intrinsic factor-mediated vitamin B_{12} adsorption capacity to the total placenta, that is the capacity cited above multiplied by the weight of all the placenta, increased toward the last stage of gestation. The trend of increase simulated the amount of vitamin B_{12} acquired by the individual embryo but not that of the placenta weight nor that of the embryo body weight.

Discussion

Data obtained here suggest that the capacity of the placenta to take up vitamin B_{12}, which presumably is bound to a carrier protein in the blood, from dam's blood and send it into the embryo changes with the lapse of time during the gestation period. A correlation was not observed between the capacity and the weight of the placenta. It seems that the development of the part of the placenta which is concerned in the transport of vitamin B_{12} is not in proportion to the development of the whole placenta.

On the 12th and 20th day circumstances seem somewhat similar, namely, on both days the transport of vitamin B_{12} to placenta *in vivo* was small and the intrinsic factor-mediated vitamin B_{12} adsorption to placenta *in vitro* was great. But physiological significances implied therein should not be seen as identical.

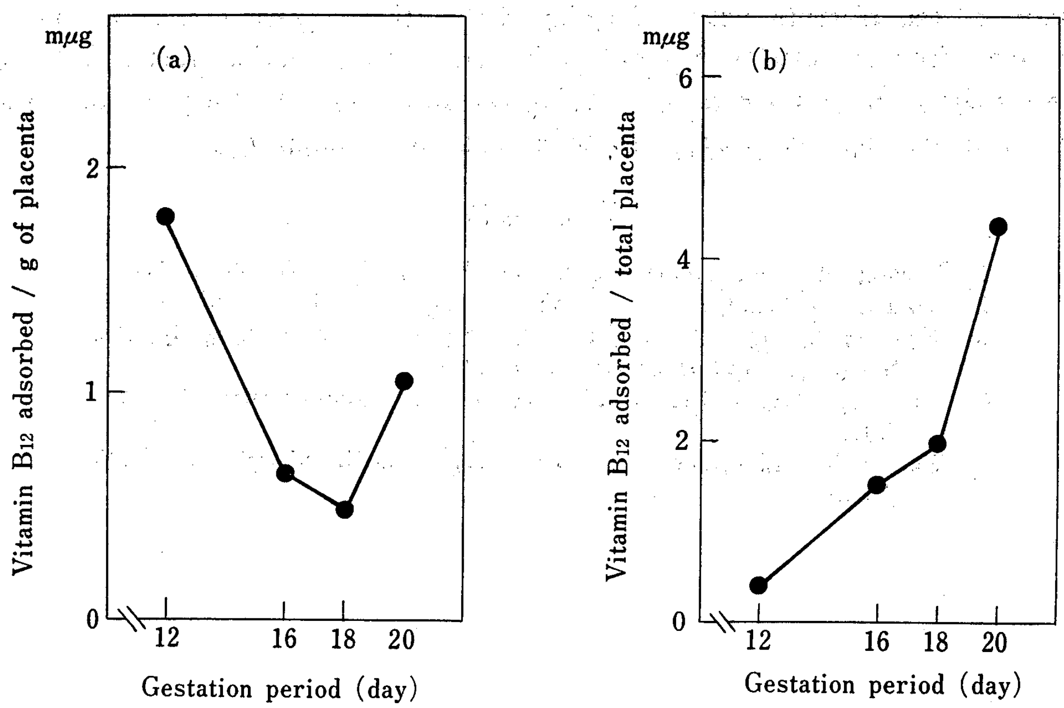

FIG. 4. Intrinsic factor-mediated vitamin B_{12} adsorption capacity of placenta. (a) Vitamin B_{12} adsorbed with the aid of intrinsic factor by 1 g of placenta.
(b) Vitamin B_{12} adsorbed with the aid of intrinsic factor by total placentas in all in the pregnant rat. The intrinsic factor-mediated vitamin B_{12} adsorption capacity was calculated by subtracting the amount of vitamin B_{12} adsorbed in the absence of intrinsic factor from total amount of vitamin B_{12} adsorbed in the presence of intrinsic factor.

We cannot difinitely conclude this point at present, but the following possibilities may be indicated. On the 12th day, although the capacity of the placenta to take up carrier protein- or intrinsic factor-bound vitamin B_{12} was great, and the major portion of the vitamin B_{12} ingested was taken up by other organs because the embryo which receives the vitamin was still undeveloped, or because the metabolism needed for pregnancy was small related to the entire metabolism of the dam. On the 20th day the placenta took up vitamin B_{12} and sent it into the embryo very actively. The vitamin B_{12} taken up by the placenta was so promptly sent out into embryo that it accumulated very little in the placenta. The fact that the intrinsic factor-mediated vitamin B_{12} adsorption capacity of total placentas in all *in vitro* was in proportion to the amount of vitamin B_{12} transported to the embryo *in vivo* seems to prove the above cited possibilities more than anything.

Karlin and Dumont (5) reported that in human subjects the concentration of vitamin B_{12} in the blood serum of the umbilical cord was as much as twice that of the mother's blood. They also stated that the vitamin B_{12} content in the placenta which lies in the region between the umbilical cord blood and the mother's blood was low when compared with that in the liver. This may be due to the peculiar mission of the placenta which, differing from other organs, exclusively sends substances into the embryo without utilization or accumulation them. Further investigations will be necessary concerning the roles played by the placenta in the metabolism of carrier-requiring substances such as vitamin B_{12}.

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