Placental Transfer and Biological Half-life of Radioactive Vit. B_{12} in the Dog.* (24576)

A. Leonard Luhby, Jack M. Cooperman and Alvin M. Donnenfeld
Depts. of Pediatrics and Obstetrics, N. Y. Medical College, Flower-Fifth Avenue Hospitals, N. Y. City

Little is known about placental transfer of Vit. B_{12} in humans. In the rat, Chow *et al.* studied placental transfer using radioactive $Co^{60}B_{12}$ as tracer. They found that after subcutaneous injection into pregnant rats, 12% of 200 mμg dose(1) and in later experiments, 60% of 10 $\mu\mu$g dose(2) was transferred to the litter. Rice *et al.*(3) also reported on placental transfer of $Co^{60}B_{12}$ in the rat, showing average of 2.3% of 3 μg orally administered dose given to pregnant rats, was transferred to the fetuses. We investigated the placental transfer and biological half-life of $Co^{60}B_{12}$ in the dog, during studies of the physiology of Vit. B_{12} in human maternal and fetal nutrition. The dog was chosen in preliminary investigation of certain technical problems anticipated in the study of human maternal-fetal Vit. B_{12} metabolism. Some of these results are reported herein.

Methods and materials. A 16 lb. bitch of mixed breed was given subcutaneous dose of 9.6 μc Vit. $Co^{60}B_{12}$ equivalent to 9.6 μg Vit. B_{12}, 5 months prior to mating and approximately 7 months prior to delivery of her litter. She nursed the litter of 4 pups until weaned at 4 weeks of age. Uptake and retention of radioactivity was studied by external scanning over the hepatic area. A scintillation probe-type counter employing 1″ x 1″ NaI thallium-activated crystal was used for external radioactivity scanning. Hepatic surface radioactivity in counts/min. (CPM) was determined 3 times a week on each dog. "Shielded" background radioactivity was determined by scanning the left lower leg. Absolute radioactivity (body cpm—shielded background cpm) were corrected for physical decay of Co^{60}. The mother and pup No. 1 were followed by surface scanning of radioactivity for 6 weeks post partum. At this time they were sacrificed and certain organs obtained. The tissues were cleaned, homogenized in Waring blender with measured amounts of water, and 3 weighed aliquots of each homogenate counted in duplicate for radioactivity in well-type NaI scintillation counter. Radioactivity counts were equated to microcuries of Vit. $Co^{60}B_{12}$ by comparison with known standard $Co^{60}B_{12}$ solution counted under identical conditions. Pups No. 2 and 3 were given small parenteral "calibration" doses of $Co^{60}B_{12}$, 4 weeks after birth. By determining increase in external hepatic area counts due to "calibration" dose, it was possible to estimate(4) the amount of Vit. $Co^{60}B_{12}$ transferred placentally. Pup No. 4 was continued on routine diet until all externally detectable radioactivity disappeared.

Results. Placental transfer of $Co^{60}B_{12}$ estimated from hepatic radioactivity. Data for calculation of amount of Vit. B_{12} transferred placentally as determined by external hepatic area radioactivity scanning technic, are presented in Table I. The 2 small physiologic doses of 0.0385 and 0.116 μc $Co^{60}B_{12}$ (μc = μg) were assumed to be completely retained. From the increment in cpm over the hepatic area after the calibration dose, it was calculated that millicuries/cpm were 0.208 and 0.172 for pup Nos. 2 and 3, respectively. Therefore, counts of 390 and 400 at birth represent 0.081 and 0.069 μg $Co^{60}B_{12}$ transferred placentally. Thus 3-4% of initial subcutaneous dose given to the bitch was transferred to the 4 pups.

Tissue distribution of placentally transferred $Co^{60}B_{12}$. Distribution of $Co^{60}B_{12}$ in certain major organs of the bitch and pup No. 1 are presented in Fig. 1. In tissues studied, concentration ($\mu\mu$g/g) in the bitch was highest in the kidney followed in order by spleen, pancreas, liver and small intestine. However, by far the greatest total amount of radioactivity resided in the liver because of its much greater weight. Similarly, in the pup, the kidney contained the highest concentration of $Co^{60}B_{12}$ although the liver con-

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TABLE I. Placental Transfer of $Co^{60}B_{12}$ in the Dog. Calculated from external hepatic area. Radioactivity (EHAR) scanning.

	EHAR at birth (avg cpm)	"Calibration" dose (μc $Co^{60}B_{12}$)	Increment of EHAR (avg cpm)	$m\mu c$/cpm	μg $Co^{60}B_{12}$ placentally transferred
Pup 2	390	.0385	185	.208	.081
3	400	.116	670	.172	.069

tained the largest amount of this vitamin. The proportional distribution of $Co^{60}B_{12}$ among liver, kidneys and pancreas for both dogs was similar.

Placental transfer from tissue distribution of $Co^{60}B_{12}$. The above characteristic permits calculation of placentally transferred $Co^{60}B_{12}$ by comparison of individual organ content of $Co^{60}B_{12}$ in the mother dog with that in her pups. Such data are presented in Table II, from which it is evident that an average of 5.1% of radioactive $Co^{60}B_{12}$ *retained* by the mother was placentally transferred to the pup. Since externally detectable radioactivity

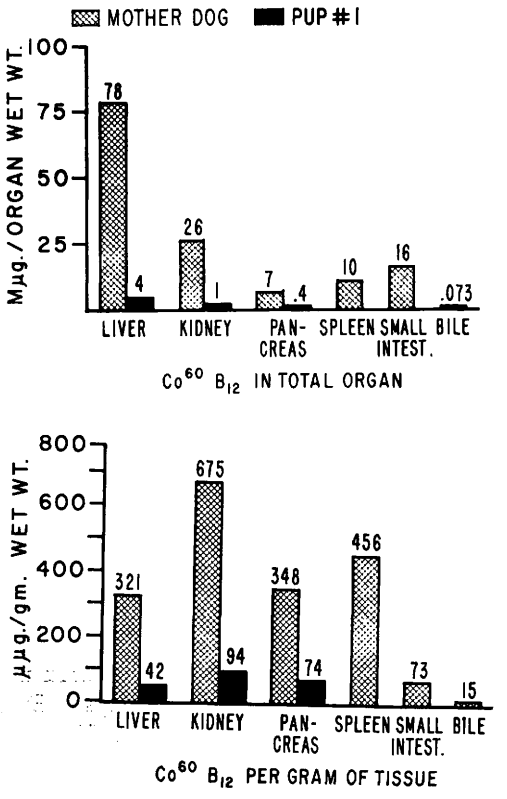

FIG. 1. Tissue distribution of $Co^{60}B_{12}$ in the dog: Comparison of mother dog with that placentally transferred to her pup.

over the hepatic area at birth was similar for the other 3 pups, it is reasonable to assume that approximately 20% of mothers B_{12} goes to the entire litter.

Biological half-life of $Co^{60}B_{12}$. Biological elimination of radioactive $Co^{60}B_{12}$ was studied in all dogs, by following disappearance rate of externally detectable radioactivity over the hepatic area, measured in carefully standardized manner so that the relative geometry was always essentially the same. The percentage of counts over the hepatic area decreased in all pups and the bitch at a similar rate, regardless of number of counts in the particular animal. The time for radioactivity to decrease by half varied from 48 to 70 days (Fig. 2). This indicates a biological half-life for Vit. B_{12} of 1.6 to 2.3 months in the post partum adult dog and pups.

Discussion. Two technics were employed for study of placental transfer of $Co^{60}B_{12}$ in the dog: (a) calibration of externally detectable hepatic radioactivity in the newborn animal, and (b) comparison of organ content of $Co^{60}B_{12}$ in mother and pup. The dog lends itself well to the first method because of its relatively large size. In this respect, the technical situation is akin to that in human newborns.

Determination of extent of placental transfer by comparison of $Co^{60}B_{12}$ content of similar organs in mother and newborn is a novel method, capable of great accuracy, and is

TABLE II. Placental Transfer of $Co^{60}B_{12}$ in Dog. Calculated from comparison of individual organ tissue content of $Co^{60}B_{12}$ in mother dog and her pup.

Organ	$m\mu g$ $Co^{60}B_{12}$ Mother	Pup	Total	% of total $Co^{60}B_{12}$ transferred to pup
Liver	77.8	4.3	82.1	5.23
Kidney	26	1.25	27.3	4.6
Pancreas	7.3	.4	7.7	5.2
				5.1 Avg

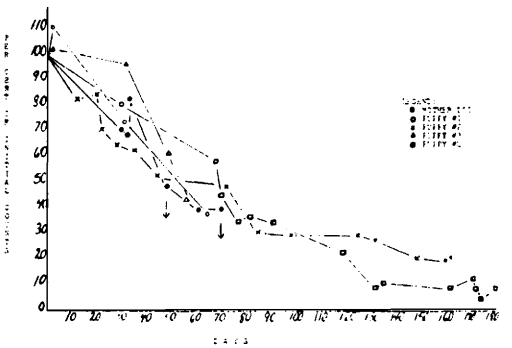

FIG. 2. Biological half-life of $Co^{60}B_{12}$ in post partum dog and puppies.

especially useful in large animal species where there is difficulty in determining B_{12} content of the entire carcass. For this comparison to be a true reflection of physiologic placental transfer, it is essential that Vit. B_{12} be administered and distributed in the mother's organs before gestation begins. From our data in humans(5,6) it appears that administration of B_{12} during pregnancy floods the blood stream and a considerable portion of these newly introduced B_{12} molecules are taken up by the placenta and fetus.

It is significant that concentration of Co^{60} B_{12} in liver, kidney and pancreas of the newborn pup was 1/5 to 1/8 that of corresponding maternal tissue (Fig. 1).

From data of Ross and Mollin(7) on total Vit. B_{12} content of liver in human infants, it is apparent that concentration of B_{12} in the liver of human newborns is in the same proportional range to adult liver as we found in the dog. The finding that maternal plasma Vit. B_{12} level falls progressively throughout pregnancy, reaching subnormal levels at term, and plasma Vit. B_{12} level of the newborn is very high, approaching 2 to 5 times that of the corresponding maternal value(8) has been explained by many workers(2,3,9) as indicating that the fetus parasitizes and depletes the maternal stores of Vit. B_{12}. The recently reported increased gastrointestinal absorption of Vit. B_{12} during normal pregnancy(2,3) also supports the latter inference. However, huge unphysiologic doses of Vit. B_{12} were used in some of the latter experiments, and it is questionable whether such data reflect the status of absorption of dietary amounts of B_{12}.

An alternate explanation for the physiology of maternal-fetal Vit. B_{12} interrelationships may be suggested from our data. The progressively decreasing Vit. B_{12} plasma level during pregnancy may be explained by our previously published finding(5,6) that (a) gastrointestinal absorption of dietary amounts of Vit. B_{12} is diminished in the last trimester of pregnancy, and that (b) a large part of Vit. B_{12} introduced into the maternal blood stream during latter part of pregnancy (whether after gastrointestinal absorption or following parenteral administration) is selectively distributed to the placenta and fetus in addition to the maternal tissues. The fetal parasitization of the mother may thus be limited to Vit. B_{12} in the mother's "mobilizable stores" which in turn is possibly highly dependent upon her dietary intake rather than her tissue stores which may be tightly bound.

The high Vit. B_{12} levels in the newborn, on the other hand, do not appear to reflect increased accumulation of Vit. B_{12} in fetal tissues. An explanation of these high fetal levels is not forthcoming from currently available data, but it is probable that this phenomenon is due to a characteristic of placental B_{12} transfer system. Such a mechanism explains the maintenance of a similar maternal fetal plasma gradient for riboflavin(10).

The biological half-life of Vit. B_{12} in the dog is surprisingly short in comparison to that in humans. The dog may thus be a good experimental subject for study of factors involved in biological turnover of Vit. B_{12}.

Summary. Placental transfer of radioactive Vit. B_{12} in the dog following pregestational administration, was found in the entire litter to be 20% of the residual radioactive vitamin in the bitch at time of delivery. Because the proportional distribution of radioactive B_{12} among major organs of the mother and pups was found to be similar, it was possible to calculate placental transfer from this characteristic as well as by postpartum "calibration" of the hepatic radioactivity in pups. Biological half-life of the radioactive Vit. B_{12} averaged 2 months in pups and bitch during postpartum period. A new explanation is suggested for the low maternal and high fetal plasma levels of Vit. B_{12}.

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Total, Phospholipid and Labile Phosphorus in Serum and Tissue Employing Chloric Acid and N-Phenyl-P-Phenylenediamine.* (24577)

JESSE F. GOODWIN (Introduced by Alfred Jay Bollet)

Dept. of Medicine, Wayne State University College of Medicine, and Receiving Hospital, Detroit, Mich.

The advantages of chloric acid as a reagent suitable for digestion of serum and serum extracts in the colorimetric estimation of phosphorus have been pointed out(1). Its rapidity, ease of digestion and of handling has resulted in considerable reduction of digestion time. Our purpose is to report an improvement in the chloric acid procedure which provides a less tedious method for color development thus rendering the method more desirable for routine determinations. In most procedures the inorganic phosphate present after digestion is treated with molybdate forming a phosphomolybdate complex. Addition of a suitable reducing agent selectively reduces this complex to yield a blue color. Many reducing agents have been investigated in an effort to find one which will give a stable color in the presence of the neutralized decomposition products of chloric acid digestion. Stannous chloride(2,3,4), 1,2,4-aminonaptholsulfonic acid(5) 2,4-diaminophenol(6), p-methyl aminophenol sulfate(7,8) and ferrous sulfate (9) all proved to be unsatisfactory with chloric acid digests. Hydrazine sulfate(1) has proved a sensitive reductant, but requires heating for color development. Use of p-semidine hydrochloride(10) eliminates this step.

Procedure for phospholipid phosphorus in serum. Chloric acid reagent. 500 g of potassium chlorate in a 3 liter beaker with 900 ml of distilled water was heated until solution was effected. After cooling, 375 ml of 72% perchloric acid was added with constant stirring. Some potassium perchlorate precipitated; the covered beaker was kept in a refrigerator for 12-24 hours, then filtered with suction through Whatman 41H filter paper. The filtrate was an approximately 28% solution of chloric acid. *Standard phosphorus solution stock.* Pure dry mono potassium phosphate, 0.351 g was dissolved in distilled water. Ten ml of 10N sulfuric acid was added and diluted to 1 liter with water. This solution contained 0.08 ml of phosphorus per ml. *Working standard phosphorus solution.* 50 ml of the standard phosphorus stock solution was diluted to 200 ml with water. Each ml contained 0.02 mg of phosphorus. *Molybdate reagent.* 0.024 M ammonium molybdate in

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