[Chem. Pharm. Bull. 20(12)2561—2569(1972)]

UDC 615.356.015.4.076.9

Metabolism of ^{57}Co-Methylcobalamin in Rat and Guinea Pig

Kensaku Kinoshita and Takeshi Fujita

Department of Pharmacology, Eisai Research Laboratories[1]

(Received March 21, 1972)

The metabolic fate of ^{57}Co-methylcobalamin after oral or intraperitoneal administration to rats and guinea pigs (25 μg/kg) have been studied. From fecal excretion test, the intestinal absorption of ^{57}Co-methylcobalamin seems to be less than 5% of dose and the time course of fecal excretion was differed in both species. The fecal excretion in guinea pig was slower and more continuous than in rat. The urinary excretion of radioactivity after intraperitoneal administration was lower in rat (41% of dose during the first 24 hr) than in guinea pig (68%) and the main excretion form in urine was ^{57}Co-methylcobalamin itself in both species. ^{57}Co-methylcobalamin might be stable in intestinal tract and transfered through intestinal wall in unchanged form. Tissue distribution of radioactivity after intraperitoneal administration was higher in rat than in guinea pig generally and the highest uptake in rat was found in kidney, while the trend of higher uptake in guinea pig was found in liver. The biological conversion of ^{57}Co-methylcobalamin incorporated in liver and kidney into other cobamide analogues has been studied and it has been evident that ^{57}Co-methylcobalamin was transformed to 5,6-dimethylbenzimidazolylcobamide coenzyme and hydroxocobalamin in both species.

It has been established in animals as well as in microorganisms that methylcobalamin (CH_3-B_{12}) operates as a cofactor in biological transmethylation such as methionine biosynthesis.[2] CH_3-B_{12} has been detected in calf liver and human serum.[3] The metabolic fate of ^{57}Co-labeled methylcobalamin (^{57}Co-CH_3-B_{12}) at the physiological doses has been studied by Okuda, *et al.*[4] but the biological roles and metabolic fate of CH_3-B_{12} at a large dose have not been fully elucidated. The authors have studied the metabolic fate of CH_3-B_{12} after oral or intraperitoneal administration of a large dose of ^{57}Co-CH_3-B_{12} to rats and guinea pigs. This paper describes absorption, excretion, tissue distribution, excretion forms in urine and conversion rate into other cobamide analogues after administration of ^{57}Co-CH_3-B_{12}.

Experimental

Materials——^{57}Co-CH_3-B_{12} was synthesized according to the method of Johnson[5] using ^{57}Co-hydroxocobalamin (The Radiochemical Center, U.K.) and methyl iodide. The radiochemical purity of ^{57}Co-CH_3-B_{12} was found to be above 90% on a thin-layer chromatogram of MN cellulose (0.25 mm in thickness) using a mixture of *sec*-BuOH, H_2O and conc. NH_4OH (100: 36: 14) as developing solvent. Cellulose powder MN 300 (Machery, Nagel Co., Germany) and phosphate cellulose (Seikagaku Kogyo Co., Japan) were used. Prior to use, phosphate cellulose was washed repeatedly with large amount of mixture of acetone and 2N NaCl (1:1) until the filtrates became clear. Then it was washed thoroughly with distilled water.

Experimental Animals and Administration of ^{57}Co-CH_3-B_{12}——Male albino rats of Wistar strain and male albino guinea pigs weighing 200±10 g were maintained on commercial chow (Nihon Clea Co., for rats and Oriental Yeast Industry Co., for guinea pigs). Prior to use, the animals were fasted for about 20 hr, but water was available ad libitum. Animals were given an aqueous solution of ^{57}Co-CH_3-B_{12} orally or intraperitoneally at single dosage of 25 μg/kg. The specific radioactivity of ^{57}Co-CH_3-B_{12} was 3 μCi/μg in the case of rat and 1.5 μCi/μg in guinea pig.

1) Location: *Bunkyo-ku, Tokyo.*
2) R.H. Taylor and H. Weissbach, *Arch. Biochem. Biophys.*, **123**, 109 (1968).
3) *a*) K. Lindstrand, *Acta Chem. Scand.*, **19**, 1785 (1965); *b*) K. Lindstrand, *Nature*, **204**, 188 (1964).
4) *a*) K. Okuda, K. Tashima, I. Takara, T. Kitasaki, M. Kurashige, and M. Takamatsu, *Vitamin*, **40**, 224 (1969); *b*) K. Okuda, K. Yashima, T. Fujii, I. Takara, T. Kitasaki, and M. Takamatsu, *ibid.*, **41**, 399 (1970).
5) A.W. Johnson, L. Mervyn, W. Shaw, and E.L. Smith, *J. Chem. Soc.*, **1963**, 4146.

Measurement of Radioactivity in Blood——Fifty μl of blood was obtained from caudal vein of rat at various time and 0.1 ml by cardiac puncture in guinea pig. Each sample was added to 5 ml of distilled water and the radioactivity was measured with Aloka JDC-207 type scintillation counter (Nihon Musen Irigaku Co., Japan). Each value of radioactivity was calculated as equivalent to mμg of ^{57}Co-CH_3-B_{12}.

Measurement of Urinary, Fecal and Biliary Excretion——Each animal was kept in an individual metabolic cage. Each urine sample was adjusted to 100 ml with distilled water and the radioactivity in 5 ml of the diluted urine was measured. After the mixture of collected feces and small amount of water was homogenized completely and adjusted to 100 ml with water, the radioactivity in 5 ml of diluted suspension of feces was determined. Bile was collected from a cannula inserted into common bile duct of rat under anesthesia with pentobarbital sodium (30 mg/kg *i.p.*).

Tissue Distribution——Animals were sacrificed by decapitation at appropriate time interval after administration and various tissues were dissected out. These tissues were rinsed with saline and blotted with filter paper. The appropriate amount of organs were weighed and sujected to digestion with 5 ml of 30% KOH in boiling water. The concentration of radioactivity was calculated as equivalent to mμg of ^{57}Co-CH_3-B_{12}/g wet weight of tissue.

Intestinal Absorption of ^{57}Co-CH_3-B_{12} *in Vitro*——According to Crane and Wilson's method[6] the everted intestinal sac of rat was inserted into the tube containing 7 mg of glucose and 10 μCi of ^{57}Co-CH_3-B_{12} in 7 ml of Krebs Ringer bicarbonate solution. About 1 ml of Krebs Ringer bicarbonate was introduced into serosal side. Incubation was made for 2 hr at 37° under passing the gas mixture (95% O_2 and 5% CO_2) at 10 ml/min of flow rate. Two hr later, each 500 μg of 5,6-dimethylbenzimidazolylcobamide coenzyme (DBCC), CH_3-B_{12} and hydroxocobalamin (OH-B_{12}) was added as carrier to the serosal fluid collected after incubation.

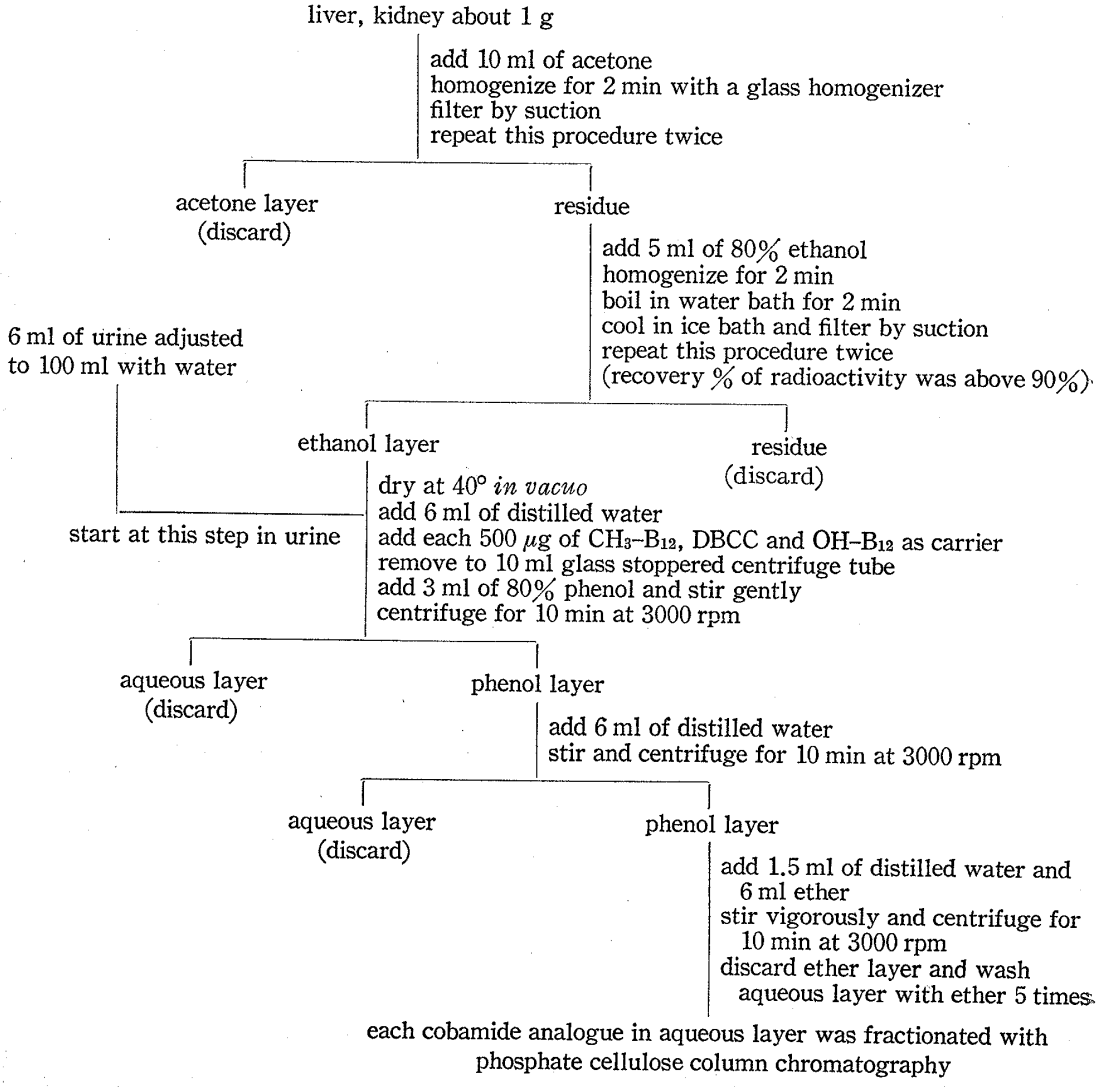

Chart 1. Extraction Method of Cobamide Analogues from Tissue and Urine

6) R.K. Crane and T.H. Wilson, *J. Appl. Physiol.*, 12, 145 (1958).

These samples were subjected to thin-layer chromatography (TLC) on MN cellulose (0.25 mm in thickness) using the mixture of *sec*-butanol, water and conc. ammonium hydroxide (100: 36:14) as developing solvent. After developing about 10 cm, the region corresponding to CH_3-B_{12} was scraped off. The ratio of radioactivity of ^{57}Co-CH_3-B_{12} to the spotted total radioactivity was measured.

In addition, the conversion of ^{57}Co-CH_3-B_{12} in the homogenate of liver or small intestine of male Wistar rat was examined. Liver or small intestine was homogenized with 9 times volume of Krebs Ringer phosphate. After 1 ml of the homogenate containing 1 μCi of ^{57}Co-CH_3-B_{12} was incubated for 2 hr at 37°, each 0.5 ml of 5% $Ba(OH)_2$ and 5% $ZnSO_4$ was added to the incubation mixture and protein was eliminated by centrifugation. The supernatant was examined by TLC as described above. The experiments were carried out under subdued red light for avoiding the decomposition of CH_3-B_{12}.

Analysis of Cobamide Analogues in Liver, Kidney and Urine——Extraction and fractionation of cobamide analogues from tissue or urine were made according to the modified method reported by Yagiri[7] as described in Chart 1. Phosphate cellulose was packed in a glass column (1×10 cm) and bufferized with 0.1N acetate buffer (pH 3.5). CH_3-B_{12}, OH-B_{12} and DBCC charged on this column were eluted with 25 ml of distilled water, 0.05M acetate buffer (pH 3.0) and 0.1M ammonium acetate (pH 6.9), respectively. Flow rate was about 1 ml/min for all fractionations. The recovery of each cobamide analogue in chromatographic procedure was 100%. A typical elution pattern of ^{57}Co-cobamide analogues of rat liver extract, which was obtained after intraperitoneal administration of 25 μg/kg of ^{57}Co-CH_3-B_{12}, is shown in Fig. 1.

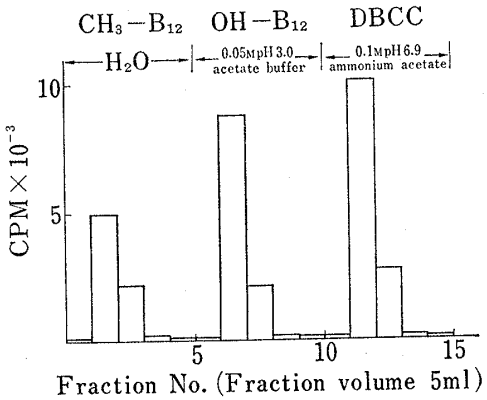

Fig. 1. Phosphate Cellulose Column Chromatogram of Cobamide Analogues in Liver Extract

Result

Blood Levels of Radioactivity after Administration of ^{57}Co-CH_3-B_{12}

Blood levels of radioactivity in rats and guinea pigs are shown in Fig. 2 and Fig. 3.

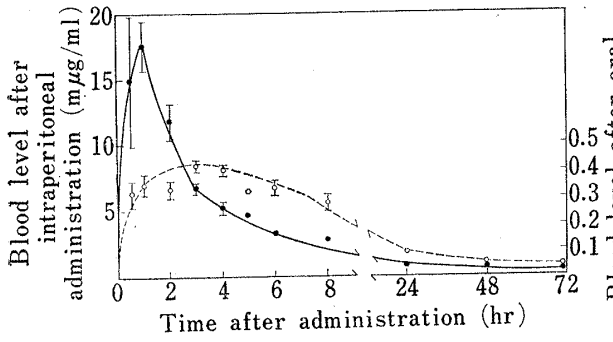

Fig. 2. Blood Level of Radioactivity after Oral or Intraperitoneal Administration of ^{57}Co-CH_3-B_{12} in Rats

Vertical lines indicate S.E. in 3 animals.
—●—: *i.p.* ---○---: *p.o.*

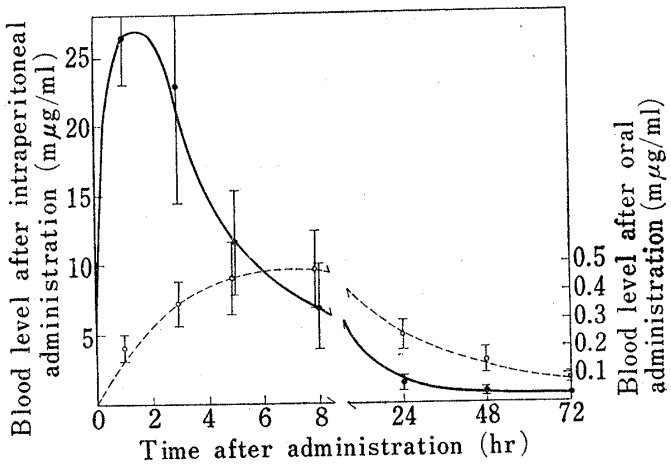

Fig. 3. Blood Level of Radioactivity after Oral or Intraperitoneal Administration of ^{57}Co-CH_3-B_{12} in Guinea Pigs

Vertical lines indicate S.E. in 3 animals.
—●—: *i.p.* ---○---: *p.o.*

Blood levels of radioactivity in rats reached the maximum of 18 mμg/ml blood at about 60 min after intraperitoneal injection and thereafter decreased rapidly. After oral admin-

7) Y. Yagiri, *J. Vitaminol.*, 13, 228 (1967).

istration to rats, a broad peak of 0.45 mμg/ml blood within 1—8 hr was observed in time course of blood levels.

Following the intraperitoneal application of ^{57}Co-CH_3-B_{12} to guinea pig, as indicated in Fig. 3, the time course of blood levels appeared to be similar to that in rats. But the maximum level in guinea pigs was higher than in rats. The blood level in guinea pig after oral administration reached a broad peak at 8 hr, whereas in rat the maximum was observed at about 3 hr.

Urinary and Fecal Excretion of Radioactivity

Cumulative urinary and fecal excretion of radioactivity in rats and guinea pigs after oral or intraperitoneal administration of ^{57}Co-CH_3-B_{12} are shown in Fig. 4 and Fig. 5.

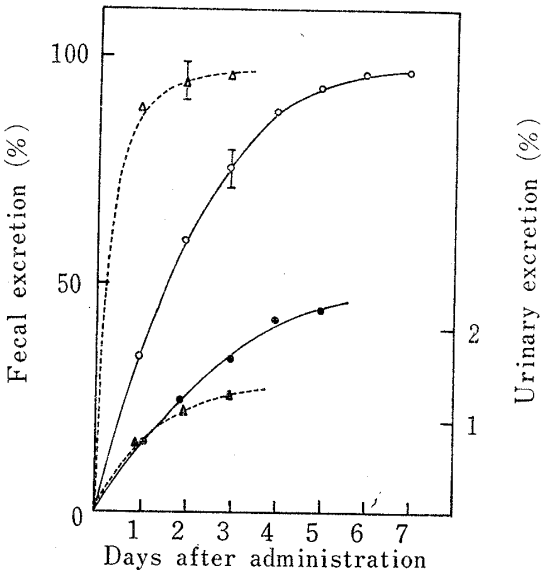

Fig. 4. Cumulative Fecal and Urinary Excretion of Radioactivity after Oral Administration of ^{57}Co-CH_3-B_{12} in Rats and Guinea Pigs

Vertical lines represent 95% confidential limit of mean values. $n=3$.
---△---: rat feces ---▲---: rat urine
—○—: guinea pig feces —●—: guinea pig urine

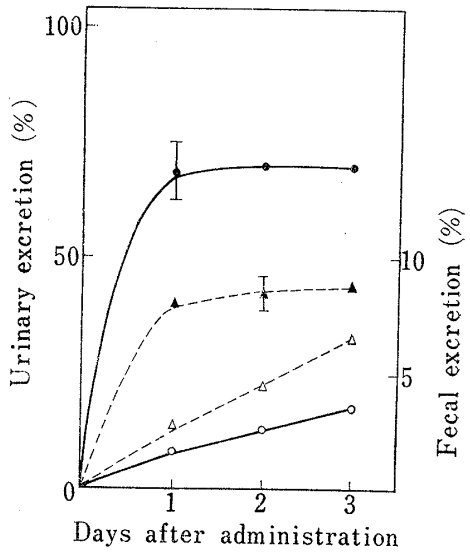

Fig. 5. Cumulative Fecal and Urinary Excretion of Radioactivity after Intraperitoneal Administration of ^{57}Co-CH_3-B_{12} in Rats and Guinea Pigs

Vertical lines indicate 95% confidential limit of mean values. $n=3$.
---△---: rat feces ---▲---: rat urine
—○—: guinea pig feces —●—: guinea pig urine

The fecal excretion % of dose during the first 24 hr after oral administration to rats and guinea pigs was approximately 88% and 34%, respectively. Thus the fecal excretion rate in guinea pigs given orally was rather slow.

The urinary excretion in guinea pigs after intraperitoneal administration was about 1.5 times higher than in rats.

Tissue Distribution of Radioactivity

Tissue distribution of radioactivity at 24 hr, 72 hr and 2 weeks after oral or intraperitoneal administration of ^{57}Co-CH_3-B_{12} to rats and guinea pigs are shown in Fig. 6 and Fig. 7.

In general, the highest tissue concentration after oral administration was achieved at 24 hr in rats and 72 hr in guinea pigs. Thereafter, gradual decline in the tissue levels of radioactivity occurred in both animals. The radioactivity remaining in the tissue of rats was generally much higher than that of guinea pigs after intraperitoneal administration. The tissue distribution of radioactivity in rats during the first 2 weeks was found especially much high in kidney, then followed in adrenal gland, pancreas, liver and stomach. The concentration of radioactivity in liver of guinea pig was higher than in other tissues, but uptake of

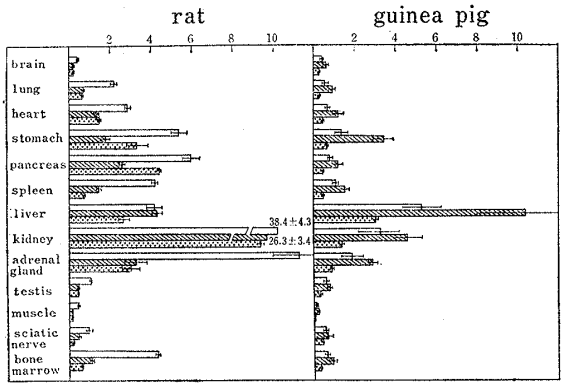

Fig. 6. Tissue Distribution of Radioactivity after Oral Administration of ^{57}Co-CH_3-B_{12} in Rats and Guinea Pigs

Each column represents the mean concentration in 3 animals with S.E. as a bar.
□: 24 hr after administration
▨: 72 hr
▩: 2 weeks

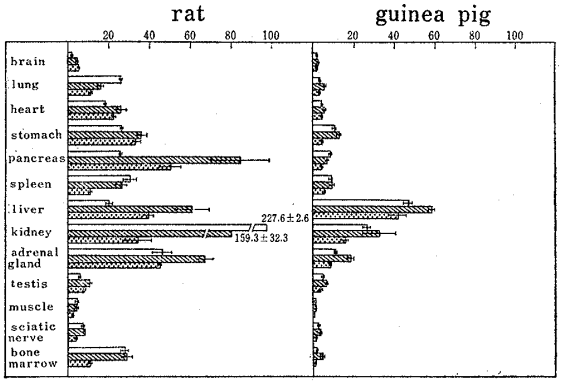

Fig. 7. Tissue Distribution of Radioactivity after Intraperitoneal Administration of ^{57}Co-CH_3-B_{12} in Rats and Guinea Pigs

Each column represents the mean concentration in 3 animals with S.E. as a bar.
□: 24 hr after administration
▨: 72 hr
▩: 2 weeks

radioactivity by pancreas was not so high in contrast with that of rat. The uptake of radioactivity by muscle, brain and sciatic nerve was observed to be very low.

The Biliary Excretion of Radioactivity

Table I shows the biliary excretion of radioactivity after intramuscular or intraperitoneal injection.

Approximately 0.3% of the dose was found in bile within 4 hr and 0.1% of radioactivity administered was excreted in bile in 24 to 29 hr after intraperitoneal injection.

The Intestinal Absorption of ^{57}Co-CH_3-B_{12} *in Vitro*

The serosal fluid was collected after incubation and subjected to TLC on MN cellulose.

TABLE I. The Biliary Excretion of Radioactivity after Intramuscular or Intraperitoneal Administration of ^{57}Co-CH_3-B_{12} in Bile-Duct Fistulated Rat

Time (hr)	Route	
	Intramuscular mμg/hr (%)	Intraperitoneal mμg/hr (%)
1	1.70 (0.034)	
2	6.04 (0.121)	
3	4.81 (0.096)	
4	1.47 (0.029)	
25		0.84 (0.017)
26		0.97 (0.018)
27		1.13 (0.023)
28		1.08 (0.022)
29		1.32 (0.026)

Values show the biliary excretion of radioactivity calculated as equivalent to mμg of ^{57}Co-CH_3-B_{12}/hr and those in parenthesis express the excretion % of dose/hr. 2 animals were used.

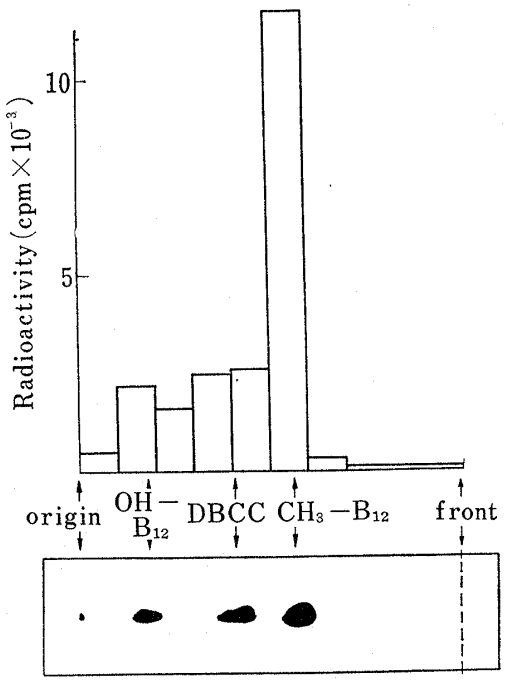

Fig. 8. Thin-Layer Chromatogram of Serosal Fluid

As shown in Fig. 8 and Table II, the radioactivity recognized in the serosal fluid was almost identified as CH_3-B_{12} on chromatogram. This result suggests that ^{57}Co-CH_3-B_{12} was transfered through intestinal wall in almost unchanged form. This suggestion is also supported by the finding that the conversion of ^{57}Co-CH_3-B_{12} into other analogues by liver and intestinal homogenate was scarcely observed (Table III).

Table II. The Ratio of ^{57}Co-CH_3-B_{12} to Total Radioactivity in Serosal Fluid

No. of experiment	^{57}Co-CH_3-B_{12} (%)
1	89
2	86
3	90
Mean	88

Table III. Recovery % of ^{57}Co-CH_3-B_{12} from Liver or Ileal Homogenate

No. of experiment	Homogenate		
	Control[a)]	Liver	Ileum
1	78	77	76
2	76	77	77
3	61	69	65
4	71	70	62
5	62	74	77
6	71	76	71
Mean±S.E.	68±3	74±2	71±2

a) 1 μCi of ^{57}Co-CH_3-B_{12} was added to Krebs Ringer phosphate and incubated for 2 hr at 37°.

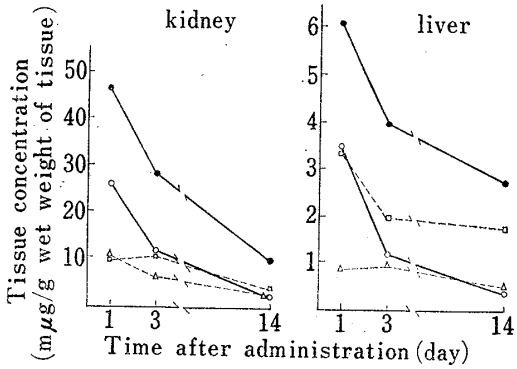

Fig. 9. The Biological Conversion Rate of ^{57}Co-CH_3-B_{12} into Other Cobamide Analogues in Liver and Kidney of Rat after Oral Administration

Each point represents mean of 2 experiments.
—●—: total radioactivity
—○—: ^{57}Co-CH_3-B_{12}
---△---: ^{57}Co-OH-B_{12}
---□---: ^{57}Co-DBCC

The Biological Conversion of ^{57}Co-CH_3-B_{12} into Other Cobamide Analogues in Liver and Kidney

The biological conversion rates of ^{57}Co-CH_3-B_{12} into other cobamide analogues in liver and kidney after oral or intraperitoneal administration are shown in Fig. 9, Fig. 10, Fig. 11, and Fig. 12.

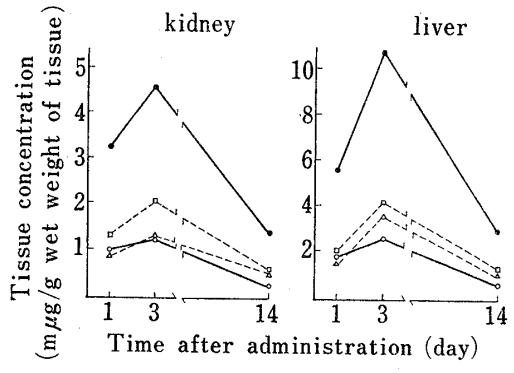

Fig. 10. The Biological Conversion Rate of ^{57}Co-CH_3-B_{12} into Other Cobamide Analogues in Liver and Kidney of Guinea Pig after Oral Administration

Each point represents mean of 2 experiments.
—●—: total radioactivity
—○—: ^{57}Co-CH_3-B_{12}
—△—: ^{57}Co-OH-B_{12}
---□---: ^{57}Co-DBCC

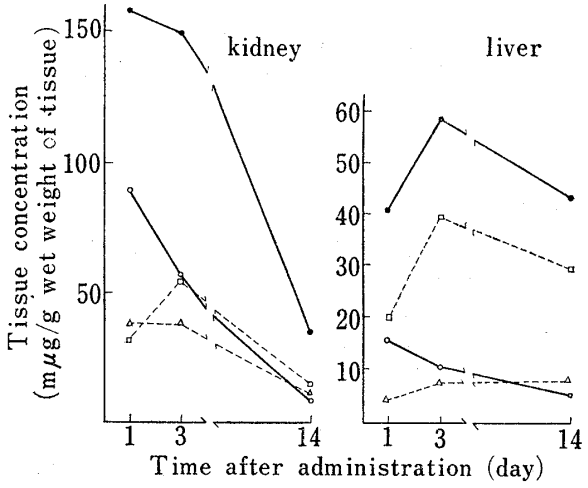

Fig. 11. The Biological Conversion Rate of ^{57}Co-CH_3-B_{12} into Other Cobamide Analogues in Liver and Kidney of Rat after Intraperitoneal Administration

Each point represents mean of 2 experiments.
—●—: total radioactivity —○—: ^{57}Co-CH_3-B_{12}
---△---: ^{57}Co-OH-B_{12} ---□---: ^{57}Co-DBCC

During the first 24 hr after administration of $^{57}Co\text{-}CH_3\text{-}B_{12}$ by both route, the total distribution of radioactivity in rat kidney was markedly higher than that of guinea pig. On the other hand, the total distribution in rat liver seemed to be slightly lower than that in guinea pig. In regard to the time course of each cobamide analogue in tissue, the content of radioactivity identified as $^{57}Co\text{-}CH_3\text{-}B_{12}$ in rat liver and kidney decreased rapidly after both administration. Similar tendency was also observed in guinea pig after intraperitoneal injection, but after oral administration the concentration of $^{57}Co\text{-}CH_3\text{-}B_{12}$ in liver and kidney reached the maximum at 72 hr and thereafter declined gradually.

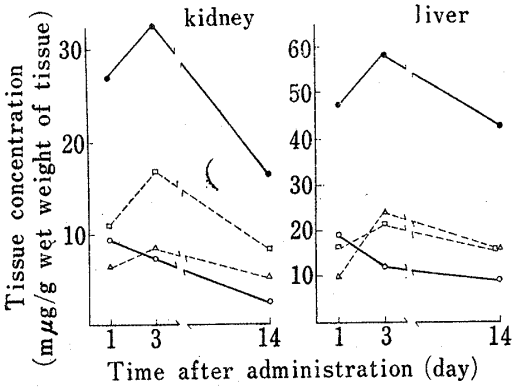

Fig. 12. The Biological Conversion Rate of $^{57}Co\text{-}CH_3\text{-}B_{12}$ into Other Cobamide Analogues in Liver and Kidney of Guinea Pig after Intraperitoneal Administration

Each point represents mean of 2 experiments.
—●—: total radioactivity
—○—: $^{57}Co\text{-}CH_3\text{-}B_{12}$
---△---: $^{57}Co\text{-}OH\text{-}B_{12}$
---□---: $^{57}Co\text{-}DBCC$

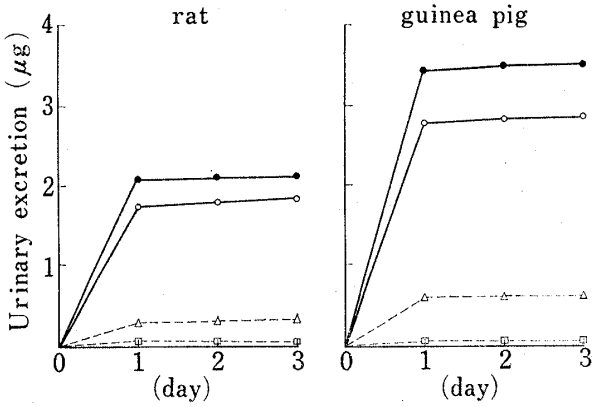

Fig. 13. The Urinary Excretion Form of $^{57}Co\text{-}CH_3\text{-}B_{12}$ after Intraperitoneal Administration to Rat and Guinea Pig

Each point represents mean of cumulative excretion in 2 experiments.
—●—: total radioactivity —○—: $^{57}Co\text{-}CH_3\text{-}B_{12}$
---△---: $^{57}Co\text{-}OH\text{-}B_{12}$ ---□---: $^{57}Co\text{-}DBCC$

The concentration of $^{57}Co\text{-}DBCC$ converted from $^{57}Co\text{-}CH_3\text{-}B_{12}$ at 24 hr, 72 hr, and 2 weeks after intraperitoneal administration of $^{57}Co\text{-}CH_3\text{-}B_{12}$ was 20.5, 39.8, and 29.8 mμg/g wet tissue in rat liver and 16.8, 21.8, and 16.8 mμg/g wet tissue in guinea pig liver, respectively. It is evident that the content of $^{57}Co\text{-}DBCC$ in liver after intraperitoneal administration was higher in rat than in guinea pig on the whole.

Following oral administration, the conversion rate of $^{57}Co\text{-}CH_3\text{-}B_{12}$ into $^{57}Co\text{-}DBCC$ was higher in rat liver than in guinea pig, while total radioactivity incorporated into rat liver was slightly lower.

The concentration of $^{57}Co\text{-}DBCC$ in kidney at 24 hr, 72 hr and 2 weeks after intraperitoneal administration of $^{57}Co\text{-}CH_3\text{-}B_{12}$ was 32.1, 54.9, and 15.0 mμg/g wet tissue in rat and 11.0, 17.0, and 8.4 mμg/g wet tissue in guinea pig, respectively. After oral administration, the concentration of $^{57}Co\text{-}DBCC$ in kidney was also higher in rat than in guinea pig. In rat, the ratio of $^{57}Co\text{-}DBCC$ to total radioactivity in liver was higher than in kidney, while this tendency was reversed in guinea pig.

The Urinary Excretion Form of $^{57}Co\text{-}CH_3\text{-}B_{12}$

The urinary excretion form of $^{57}Co\text{-}CH_3\text{-}B_{12}$ after intraperitoneal administration to rats and guinea pigs was shown in Fig. 13.

As shown in Fig. 13, the greater part of urinary radioactivity was consisted of $^{57}Co\text{-}CH_3\text{-}B_{12}$ itself. $^{57}Co\text{-}DBCC$ was less than 2% and other was $^{57}Co\text{-}OH\text{-}B_{12}$.

Discussion

From the experimental data *in vitro* studies using isolated intestine or liver and intestinal homogenate of rat, as shown in Table II and Table III, it is expected that $^{57}Co\text{-}CH_3\text{-}B_{12}$ given

orally is mostly transported in unchanged form across intestinal wall. Okuda, *et al.*[8] have reported that large quantities of $^{14}CO_2$ was quickly expired following oral administration of photolyzed $^{14}CH_3$-B_{12} but only a trace of $^{14}CO_2$ during the first 3 hr after oral administration of non-photolyzed $^{14}CH_3$-B_{12}. They suggested from this findings that methyl group of CH_3-B_{12} would be stable in intestinal tract. This suggestion is compatible with our results.

The data in Fig. 4 and Fig. 5 demonstrate that the intestinal absorption of ^{57}Co-CH_3-B_{12} given orally at a dose of 25 μg/kg seems to range within a few percent in both animals used. Okuda, *et al.*[8] reported that the absorption percentage calculated from the fecal recovery of radioactivity during the first 6 days after oral administration of ^{57}Co-CH_3-B_{12} in rat were 68.9, 43.2, and 7.8% at the doses of 67 mμg/kg, 1.3 μg/kg, and 13 μg/kg, respectively. It has been observed in the oral ingestion of CN-B_{12},[4a] OH-B_{12},[8] and DBCC[9] that their absorption percentages became lower with increasing dose. There might be no significant differences among intestinal absorption of cobamide analogues at high dose.

In blood levels of radioactivity after oral administration, the species difference was observed between rat and guinea pig (Fig. 2 and Fig. 3). Namely, the maximum level in rat was arrived at 2 to 4 hr after administration, but in guinea pig it was observed at 6 to 8 hr. The blood level at 24 hr in guinea pig after oral ingestion was higher than in rat. These results are in good agreement with the finding that fecal excretion in rat was about 90% within 24 hr, while in guinea pig it continued for 5 days after administration. At present, it can not be explained in details, but species difference in blood levels of radioactivity in both animals seems to be attributable to the period of ^{57}Co-CH_3-B_{12} existence in intestinal canal. Although the maximum blood level of radioactivity was arrived at 1 hr after intraperitoneal administration of ^{57}Co-CH_3-B_{12} and thereafter decreased rapidly in both species, it seemed that the blood levels in guinea pig were somewhat higher than in rat on the whole. This fact might be attributable to the low uptake of ^{57}Co-CH_3-B_{12} to tissues of guinea pig as compared with that of rat, as shown in Fig. 6 and Fig. 7.

The main route for excretion after oral administration of ^{57}Co-CH_3-B_{12} was in feces, while the main excretory organ after intraperitoneal injection was kidney in both species. There was species difference between both animals in the pattern of fecal excretion after oral ingestion and in urinary excretion after intraperitoneal administration. The urinary excretion of radioactivity after intraperitoneal administration was about 40% in rat within 24 hr but it was about 70% in guinea pig. Suzuki, *et al.*[10] have reported that 35 to 43% of dose was excreted in urine after intramuscular administration of 1000 μg of CH_3-B_{12} to man. The urinary excretion in man seems to be nearly equal to that in rat. Although fecal excretion after intraperitoneal administration was about 6% during the first 72 hr in both species, all of this could not be derived from biliary excretion, as cleared from Table III. Okuda, *et al.*[4b] have shown that 6% of ^{57}Co-CH_3-B_{12} given to rat intramuscularly was accumulated in intestinal mucosa at 72 hr after. Then they have considered that cobamide excreted in feces after parenteral administration might be mainly derived from exfoliation of intestinal mucosa rather than biliary excretion.[11] The possibility of exfoliation of intestinal mucosa should also be considered in the present studies. Furthermore, fecal excretion (2.2%) of radioactivity in rats during 48 to 72 hr after intraperitoneal administration became somewhat higher than urinary excretion (0.8%), as shown in Fig. 4 and Fig. 5. Since it has been reported that the main excretory pathway after parenteral administration of a large dose of cobamide is *via* urine but physiological dose of cobamide is mainly excreted *via* feces,[11] it

8) K. Okuda, K. Yashima, I. Takara, T. Kitasaki, K. Morokuma, A. Kinoshita, and M. Takamatsu, *Vitamin*, **40**, 232 (1969).
9) H. Uchino, S. Ukyo, Y. Yagiri, and G. Wakisaka, *Vitamin*, **25**, 190 (1962).
10) K. Suzuki, T. Ikeda, M. Tohda, N. Tanaka, and H. Uchino, *Vitamin*, **42**, 198 (1970).
11) K. Okuda, R. Grasbeck, and B.F. Chow, *J. Lab. Clin. Med.*, **51**, 17 (1958).

might be considered that ^{57}Co-CH_3-B_{12} after being incorporated into tissues is excreted *via* feces.

As shown in Fig. 13, the most predominant radioactivity in urine of rat and guinea pig after intraperitoneal administration was composed of ^{57}Co-CH_3-B_{12}. No species difference was observed at this point. Suzuki, *et al.*[10] have obtained similar result in urine of human subjects after intramuscular administration of 1000 μg of CH_3-B_{12}. The main form excreted in urine of rat, guinea pig and human subject seems to be not different significantly.

The highest uptake of radioactivity at 24 hr after oral administration of ^{57}Co-CH_3-B_{12} to rat was found in kidney, followed by adrenal gland, pancreas, stomach, bone marrow, spleen and liver. On the other hand, the highest concentration of radioactivity in guinea pig was found in liver, followed by kidney, adrenal gland, stomach and spleen. Furthermore, it appeared that the highest tissue concentration was achieved within 24 hr in rat, while in guinea pig it was found at 72 hr after. This difference in distribution rate might be caused by the difference in fecal excretion rate between both species.

The tendency in tissue distribution of radioactivity after intraperitoneal administration to rat and guinea pig was almost similar to the result in oral administration (Fig. 6 and Fig. 7). The uptakes by pancreas and bone marrow were markedly different between rat and guinea pig. The remaining radioactivity 2 weeks after in rat was higher than in guinea pig. As described before, the urinary excretion, which is the main excretory pathway after intraperitoneal administration, was about 70% of ^{57}Co-CH_3-B_{12} given to guinea pig as compared with 43% in rat. These findings suggested that the affinity of ^{57}Co-CH_3-B_{12} to rat tissue was greater than to guinea pig. It has been reported that the biological half life of cobamide is more than 1 year in man[12] and about 50 days in rat.[13] It can also be clearly deduced from our data that the disappearance of ^{57}Co-CH_3-B_{12} incorporated into tissue is very slow.

From our present results, it is evident that ^{57}Co-CH_3-B_{12} is converted to DBCC in liver and kidney. The conversion rate of ^{57}Co-CH_3-B_{12} to DBCC in rat is markedly high in comparison with guinea pig liver, namely the ratio of ^{57}Co-DBCC to total radioactivity at 72 hr after intraperitoneal administration was about 70% in rat liver, while it was about 40% in guinea pig liver. Okuda, *et al.*[4a] and Suzuki, *et al.*[14] have also observed the results agreeable with our data in the conversion of CH_3-B_{12} to DBCC. The biological roles of CH_3-B_{12} have not been fully elucidated. But it has been reported that methyl group of $^{14}CH_3$-B_{12} was transfered to DNA and phospholipid fraction.[15] It has been evident that CH_3-B_{12} is concerned in various transmethylation reactions such as biological synthesis of methionine.[2] From the present data, it is expected that CH_3-B_{12} plays important roles in these reactions and thereafter converted to DBCC.

Acknowledgement The authors are indebted to Dr. K. Miyao, Director of Institute of Pharmacology, for valuable discussion in this investigation and also Dr. K. Satoh, Vice President, for support of this research.

12) P. Reizenstein, E.P. Cronkite, and S.A. Cohn, *J. Lab. Clin. Med.*, **62**, 255 (1963).
13) H.C. Heinrich and E.E. Gabbe, *Ann. N.Y. Acad. Sci.*, **112**, 871 (1964).
14) K. Suzuki, M. Tohda, N. Kuwana, K. Ohmae, and H. Uchino, *Vitamin*, **40**, 358 (1969).
15) U. Misra and K. Lindstradt, *Indian J. Biochem.*, **4**, 132 (1967).