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# Metabolic Fate of $^{57}$Co-Labeled Hydroxocobalamin and $^{57}$Co-Labeled 5,6-Dimethylbenzimidazolylcobamide Coenzyme in Rats

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The metabolic fate of $^{57}$Co-hydroxocobalamin ($^{57}$Co-OH-$B_{12}$) and $^{57}$Co-5,6-dimethylbenzimidazolylcobamide coenzyme ($^{57}$Co-DBCC) has been studied after intraperitoneal administration at the dosage of 25 μg/kg in rats. About 60% of radioactivity administered was recovered in urine during the first 24 hr after administration of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC. The highest tissue uptake was found in kidney, especially after injection of $^{57}$Co-DBCC, followed by adrenal gland, pancreas, stomach and liver. The distribution in brain, muscle and adipose tissue was much lower. It is evident that $^{57}$Co-OH-$B_{12}$ was converted to $^{57}$Co-DBCC and reverse conversion of $^{57}$Co-DBCC occured in liver and kidney. Furthermore, $^{57}$Co-methylcobalamin ($^{57}$Co-$CH_3$-$B_{12}$) was found to be yielded from both the cobamides. The biological interconversion among $^{57}$Co-$CH_3$-$B_{12}$, $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC was established.

The metabolism of hydroxocobalamin (OH-$B_{12}$) or 5,6-dimethylbenzimidazolylcobamide coenzyme (DBCC) at a large dose was not fully elucidated and especially the biological conversion of OH-$B_{12}$ or DBCC into methylcobalamin ($CH_3$-$B_{12}$) has not been established. The authors have reported the metabolic fate of $CH_3$-$B_{12}$ at a large dose in this Bulletin.[2)] In the present study the metabolic fate of other cobamide analogues was examined so as to compare with that of $CH_3$-$B_{12}$. This paper describes tissue distribution, urinary excretion and biological interconversion of $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC after intraperitoneal administration at the dosage of 25 μg/kg in rats.

## Experimental

**Chemicals**——The specific radioactivity of $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC (The Radiochemical Center, Amersham, U.K.) was 1.5 μCi/μg and 0.7 μCi/μg, respectively. The radiochemical purity of both the labeled cobamides was found to be above 90% on thin-layer chromatogram of MN cellulose (Machery, Nagel Co., Germany, 0.25 mm in thickness) using a mixture of sec-butanol, water and conc. $NH_4OH$ (100 : 36 : 14) as a developing solvent.

**Experimental Animals and Administration**——Male albino rats of Wistar strain weighing about 200 g were maintained on commercial chow (Nihon Clea Co., Japan.). Twenty five μg/kg of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC was injected intraperitoneally. Animals were killed by cervical dislocation at appropriate time after administration and organs were dissected out. These organs were rinsed with physiological saline and blotted with filter paper. The appropriate amounts of organs were subjected to digestion with 5 ml of 30% KOH in boiling water bath and their radioactivities were measured with Aloka JDC-207 type scintillation counter (Nihon Musen Irigaku Co., Japan).

**Urinary Excretion**——Each animal was kept in an individual metabolic cage and urine collected for every 24 hr was adjusted to 100 ml with distilled water and the radioactivity in 5 ml of the diluted urine was measured.

**Analysis of Cobamide Analogues in Liver and Kidney**——Extraction and fractionation of cobamide analogues from tissues were made by modification[2)] of the method of Yagiri.[3)] In order to identify $CH_3$-$B_{12}$ more precisely, reversed dilution analysis was carried out as follows. The $^{57}$Co-$CH_3$-$B_{12}$ fraction isolated according to previous report[2)] was evaporated to dryness. Six ml of acetone was added to a mixture of

1) Location: *Bunkyo-ku, Tokyo.*
2) K. Kinoshita and T. Fujita, *Chem. Pharm. Bull.* (Tokyo), **20**, 2561 (1972).
3) Y. Yagiri, *J. Vitaminol.*, **13**, 228 (1967).

the fraction and 10 mg of carrier $CH_3$-$B_{12}$ dissolved in 1 ml of distilled water. After standing overnight at 4°, the specific radioactivity of precipitate collected by filtration was determined by measurement of radioactivity against absorbancy at 522 mμ in aqueous solution. Recrystallization was repeated until specific radioactivity became constant.

## Result and Discussion

### Tissue Distribution

Tissue distribution of radioactivity at 24 hr, 72 hr and 2 weeks after intraperitoneal administration of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC is shown in Fig. 1. As shown in Fig. 1, the highest uptake of radioactivity was found in kidney, especially after injection of $^{57}$Co-DBCC, followed by adrenal gland, pancreas, stomach and liver. The distribution of radioactivity in brain, muscle, adipose tissue and testis was much lower. The uptake of $^{57}$Co-OH-$B_{12}$ by tissues with the exception of kidney appeared to be somewhat higher than that of $^{57}$Co-DBCC.

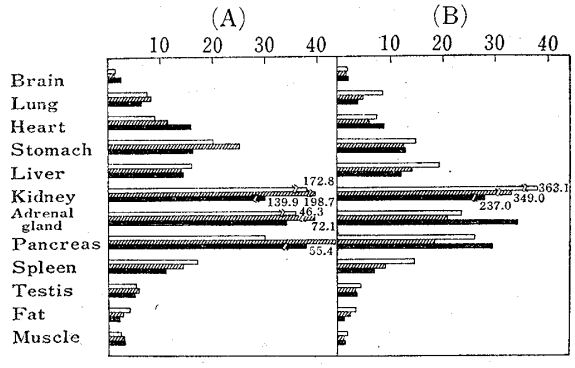


Fig. 1. Tissue Distribution of Radioactivity after Intraperitoneal Administration of $^{57}$Co-OH-$B_{12}$ (A) or $^{57}$Co-DBCC (B) to Rats

mean of 2 experiments
Radioactivity is calculated as equivalent to $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC mμg/g wet weight of tissue.
□: 24 hr after; ▨: 72 hr after; ■: 2 weeks after

Table I. The Urinary Excretion of Radioactivity after Intraperitoneal Administration of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC to Rats

| Time (hr) | $^{57}$Co-OH-$B_{12}$ | $^{57}$Co-DBCC |
|---|---|---|
| 0—24 | 61.5 | 57.4 |
| 24—48 | 1.0 | 0.04 |
| 48—72 | 0.8 | 0.03 |

Values express the excretion % of dose.

### Urinary Excretion

About 60% of radioactivity administered was recovered in urine during the first 24 hr after intraperitoneal administration of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC and a small amount of radioactivity was found thereafter (Table I). There was no difference between $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC in urinary excretion profile.

### Biological Conversion of $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC into Other Cobamide Analogues

As shown in Fig. 2 and Fig. 3, it is evident that $^{57}$Co-OH-$B_{12}$ was converted to $^{57}$Co-DBCC and reverse conversion of $^{57}$Co-DBCC occurred in liver and kidney. Furthermore, $^{57}$Co-$CH_3$-$B_{12}$ was found to be yielded from both the cobamides.

No significant difference in total hepatic radioactivity was observed between intraperitoneal administration of $^{57}$Co-OH-$B_{12}$ and that of $^{57}$Co-DBCC but hepatic concentration of $^{57}$Co-$CH_3$-$B_{12}$ converted from $^{57}$Co-DBCC was somewhat higher than that from $^{57}$Co-OH-$B_{12}$. In contrast, the renal concentration of total radioactivity after administration of $^{57}$Co-DBCC was markedly higher than that of $^{57}$Co-OH-$B_{12}$ on the whole and $^{57}$Co-$CH_3$-$B_{12}$ converted from $^{57}$Co-DBCC was much higher than from $^{57}$Co-OH-$B_{12}$.

Cyanocobalamin is also eluted together with $CH_3$-$B_{12}$ in our chromatographic procedure. In order to confirm that the radioactivity in $^{57}$Co-$CH_3$-$B_{12}$ fraction is originated from $^{57}$Co-$CH_3$-$B_{12}$ itself, reversed dilution analysis was carried out and no decrease of specific radioactivity was found after repeated recrystallization in all cases.

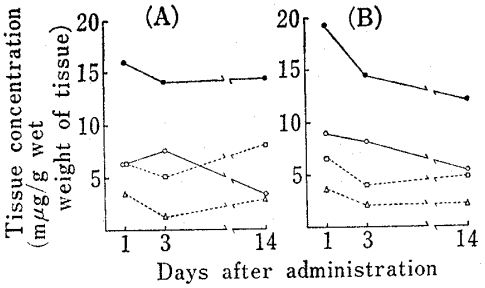


Fig. 2. Biological Conversion Rate of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC into Other Cobamide Analogues in Rat Liver after Intraperitoneal Administration

(A): after administration of $^{57}$Co-OH-$B_{12}$
(B): after administration of $^{57}$Co-DBCC
—●—: total radioactivity
—○—: $CH_3$-$B_{12}$
---△---: OH-$B_{12}$
---□---: DBCC

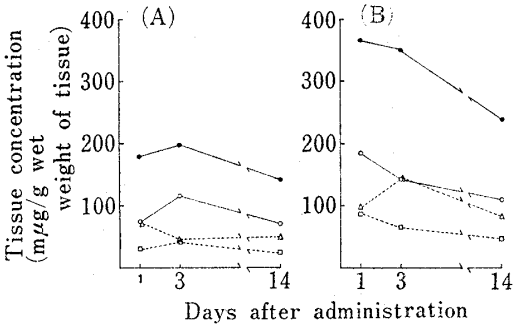


Fig. 3. Biological Conversion Rate of $^{57}$Co-OH-$B_{12}$ or $^{57}$Co-DBCC into Other Cobamide Analogues in Rat Kidney after Intraperitoneal Administration

(A): after administration of $^{57}$Co-OH-$B_{12}$
(B): after administration of $^{57}$Co-DBCC
—●—: total radioactivity
—○—: $CH_3$-$B_{12}$
---△---: OH-$B_{12}$
---□---: DBCC

The urinary excretion profile of radioactivity after intraperitoneal administration of $^{57}$Co-OH-$B_{12}$ in rats was almost similar to that of $^{57}$Co-DBCC. As reported in our previous paper,[2] the urinary excretion of radioactivity after intraperitoneal administration of $^{57}$Co-$CH_3$-$B_{12}$ at the same dosage in rats was about 41% of dose during the first 24 hr and it is much lower as compared with the excretion in the injection of $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC. Hiratsuka[4] has reported that the urinary excretion of $^{57}$Co-OH-$B_{12}$ after subcutaneous administration of 5 μg/rat was 52%. Although this value seems to be somewhat lower than our result, it might be attributable to difference in administration route.

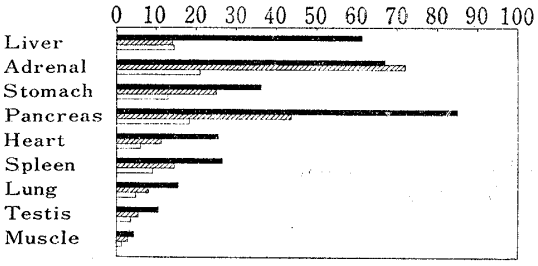


Fig. 4. Comparison of Tissue Radioactivity 72 hr after Intraperitoneal Administration of $^{57}$Co-labeled $B_{12}$ Vitamers to Rats

Radioactivity is calculated as equivalent to each $^{57}$Co-labeled $B_{12}$ vitamer administered (mμg/g wet tissue).
■: $^{57}$Co-$CH_3$-$B_{12}$; ▨: $^{57}$Co-OH-$B_{12}$; □: $^{57}$Co-DBCC

The tissue distribution of $^{57}$Co-OH-$B_{12}$ except kidney seems to be somewhat higher than that of $^{57}$Co-DBCC. Since the uptake of $^{57}$Co-DBCC by kidney was markedly high in comparison with that of $^{57}$Co-OH-$B_{12}$, the retention of $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC in whole body appeared to be nearly equal. As compared with our previous paper, tissue uptake of $^{57}$Co-$CH_3$-$B_{12}$ was the highest among these three vitamers (Fig. 4).

Concerning the uptakes of the three vitamers by each organ, the highest uptake was observed in kidney followed by adrenal gland and pancreas and the lowest in brain, muscle and adipose tissue. The time courses in renal concentration of radioactivity were interestingly differed among these three vitamers. The renal content of radioactivity after administration of $^{57}$Co-DBCC was the highest followed by $^{57}$Co-$CH_3$-$B_{12}$[2] and $^{57}$Co-OH-$B_{12}$. The radioactivity in kidney after administration of $^{57}$Co-$CH_3$-$B_{12}$ decreased rapidly with time,[2] while that after administration of $^{57}$Co-DBCC or $^{57}$Co-OH-$B_{12}$ was changed gradually. Although Okuda, *et al.*[5] have reported that the kidney is a kind of storage organ of vitamin $B_{12}$ and the uptake of vitamin $B_{12}$ by kidney reflects the preceding intake of vitamin $B_{12}$,

4) H. Hiratsuka, *Bitamin*, 33, 474 (1966).
5) K. Okuda and H. Takedatsu, *Proc. Soc. Exptl. Biol. Med.*, 123, 504 (1966).

the difference in time course of renal content of $B_{12}$ vitamers might be attributed to differences in renal affinities of these vitamers, since in our experiments animals were maintained on the same conditions and administered with very large amount of vitamin $B_{12}$ and it is, therefore, expected that animals would be in saturated state with respect to vitamin $B_{12}$. Yagiri[3] reported that $^{57}$Co-CN-$B_{12}$ and $^{57}$Co-OH-$B_{12}$ were converted to $^{57}$Co-DBCC in liver and kidney. The conversion of $^{57}$Co-OH-$B_{12}$ to $^{57}$Co-DBCC or $^{57}$Co-$CH_3$-$B_{12}$ and that of $^{57}$Co-DBCC to $^{57}$Co-$CH_3$-$B_{12}$ or $^{57}$Co-OH-$B_{12}$ were demonstrated in the present paper. The biological interconversion among $^{57}$Co-$CH_3$-$B_{12}$, $^{57}$Co-OH-$B_{12}$ and $^{57}$Co-DBCC was established from our previous[2] and present reports.