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The Metabolic Fate of ^{57}Co-Methylcobalamin in the Rat with a Crushed Sciatic Nerve——Especially on the Uptake by the Nerve Tissue and by Muscles innervated by Sciatic Nerves

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The metabolic fate of ^{57}Co-labeled methylcobalamin (^{57}Co–CH_3–B_{12}) at various doses (5, 50 and 500 μg/kg/day) after consecutive, intraperitoneal administration within 7 days to rats, of which the right sciatic nerve was degenerated by crushing, was studied. The uptake of radioactivity by sciatic nerve in both sides was increased with time and doses but the radioactivities in sciatic nerve and muscles innervated by this nerve such as *musculus gastrocnemius* were significantly higher in crushed side than in intact one at all doses investigated. The distribution of radioactivity in tissues other than nerve and muscles was also increased with time and doses. The highest uptake was observed in kidney, followed by adrenal gland, pancreas and liver and the lowest in brain and testis. The urinary excretion of radioactivity in each dosage within 24 hr after every administration of ^{57}Co–CH_3–B_{12} was found to be nearly constant at each dose during 7 days, namely, about 25, 70 and 75%/day at 5, 50 and 500 μg/kg/day, respectively.

It has been widely known that pernicious anemia is frequently accompanied with neurological derangements. Its characteristic clinical and pathological features were studied in detail by Russel, *et al.*[2] and designated as subacute combined degeneration of the spinal cord. The primary relationship between severity of pernicious anemia and neural degeneration has been not yet recognized clearly. However, a great number of clinical and fundamental papers concerning the neurological dysfunction due to vitamin B_{12} deficiency have shown that vitamin B_{12} treatment prevents or improves the neurological syndrome in pernicious anemia.[3–7] Furthermore, the therapeutic effectiveness of a large dose of vitamin B_{12} on vitamin B_{12} independent neuropathies was also recognized by some investigators,[8,9] although its mechanism has not been fully elucidated.

On the other hand, Lindstrand and Stahlberg[10] found a "fourth factor" of vitamin B_{12} besides coenzyme B_{12} in human serum and calf liver, and thereafter identified it to be methylcobalamin (CH_3–B_{12}).[11] It has been shown that CH_3–B_{12} participates in enzymatic transmethylation in various microorganisms[12,13] and mammals.[14] Furthermore, very recently

1) Location: *Koishikawa-4, Bunkyo-ku, Tokyo.*
2) J.S.R. Russel, F.E. Batten and J. Collier, *J. Brain*, **23**, 39 (1900).
3) I. Torres N., W.T. Smith and C. Oxnard, *Experientia*, **25**, 273 (1969).
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it was decided by double blind test that a large dose treatment of CH_3–B_{12} was clinically effective on peripheral neuropathies.[15]

Previously, the authors reported the metabolic fates of ^{57}Co–labeled vitamin B_{12} analogues at a large dose in normal rats and guinea pigs,[16,17] but the uptake of vitamin B_{12} by nerve tissue has not been known except for Otaguro's report[18] concerning the distribution of ^{60}Co–labeled cyanocobalamin and ^{57}Co–labeled hydroxocobalamin to optic nerve. The purpose of this paper was to study the influence of degeneration of sciatic nerve on the uptake of ^{57}Co–labeled CH_3–B_{12} (^{57}Co–CH_3–B_{12}) by the sciatic nerve and by the muscles innervated by this nerve. Furthermore, this paper describes the metabolic profiles of ^{57}Co–CH_3–B_{12} after consecutive administration at various doses in rats.

Experimental

Materials——^{57}Co–CH_3–B_{12} was synthesized from ^{57}Co-labeled hydroxocobalamin (The Radiochemical Center, Amersham, U.K.) according to Johnson's method.[19] Its specific radioactivity was 3.43 μCi/μg and the radiochemical purity was 100% on a 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——A total of 36 male Wistar rats weighing approximately 150 g, 6—7 weeks old, were randomly divided into 4 groups of 9 rats each. The right sciatic nerve of rats in 3 groups was crushed by the procedure as described below. The remaining group served as control. Animals were maintained on the laboratory chow (Oriental Yeast Industry Co., Ltd.) and tap water *ad libitum* in air-conditioned room. Sciatic nerve of right thigh was exposed under sodium pentobarbital anesthesia (40 mg/kg, *i.p.*) and was crushed at a point 5 mm upper from branching site of tibial and peroneal nerves by means of locking for 30 seconds with a special Pean's forceps of which edge was smoothed. Dissected site of the thigh was sutured immediately after crushing and 20000 units of penicillin G potassium was injected subcutaneously to prevent infections.

Administration of ^{57}Co-CH_3-B_{12} and Measurement of Radioactivity——Immediately after the operation of crushing, ^{57}Co–CH_3–B_{12} dissolved in distilled water at the doses of 5 μg/kg/day (specific activity 3.43 μCi/μg), 50 μg/kg/day (specific activity 0.343 μCi/μg) or 500 μg/kg/day (specific activity 0.0343 μCi/μg) was injected intraperitoneally to the operated rats every 24 hr. The concentrations of the solutions were adjusted so that the volume administered was 1 ml/200 g. Fifty μg/kg/day of ^{57}Co–CH_3–B_{12} was given intraperitoneally to 9 intact rats in a control group. At 24 hr after the 1st, 3rd and 7th administration, 3 rats randomly selected in each group were sacrificed by cervical dislocation. Sciatic nerve, *musculus gastrocnemius*, *musculus tibialis anterior* and *musculus soleus* of both sides and various organs were removed out, rinsed with 0.9% saline and then blotted with filter paper. The appropriate amounts of these tissues were weighed and subjected to digestion with 5 ml of 30% KOH in boiling water. Urine collected in individual metabolic cage was adjusted to a fixed volume with distilled water. The radioactivity of these specimens was measured with Aloka JDC-207 type scintillation counter and was calculated as equivalent to mμg of ^{57}Co–CH_3–B_{12} administered.

Results

Uptake of ^{57}Co-CH_3-B_{12} by Sciatic Nerve

The uptake of radioactivity by intact sciatic nerve was compared with that by crushed one after consecutive, intraperitoneal administrations of ^{57}Co–CH_3–B_{12} at various doses and the results are shown in Fig. 1. As cleared from Fig. 1, the uptake of radioactivity by sciatic nerve was increased with doses and time intervals regardless of crushing and the incorporation of radioactivity by the nerve of crushed side was significantly higher than that by the nerve of intact side ($p<0.01$, by variance analysis).

15) M. Kameyama, K. Abe, M. Iida, T. Iguchi, N. Ohta, E. Ohtomo, T. Okada, S. Okamoto, S. Kitoh, F. Sakamoto, R. Shiozawa, K. Takahashi, K. Hirayama, T. Matsui, M. Miyazaki, T. Muro and H. Watanabe, *Jap. J. Clin. Exptl. Med.*, **49**, 241 (1972).
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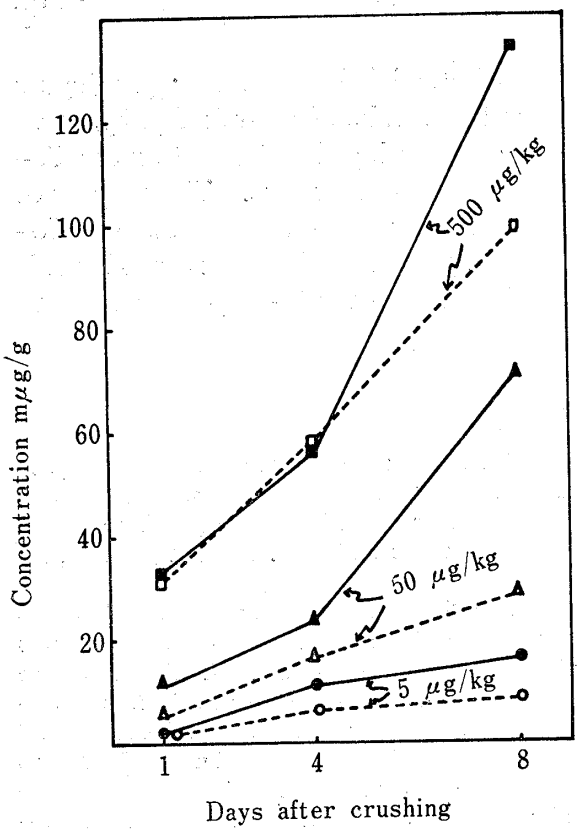

Fig. 1. Uptake of Radioactivity by Sciatic Nerve after Consecutive Administration of ^{57}Co–CH_3–B_{12} to Rats with a Crushed Sciatic Nerve at Various Dosages

——: crushed side ------: normal side

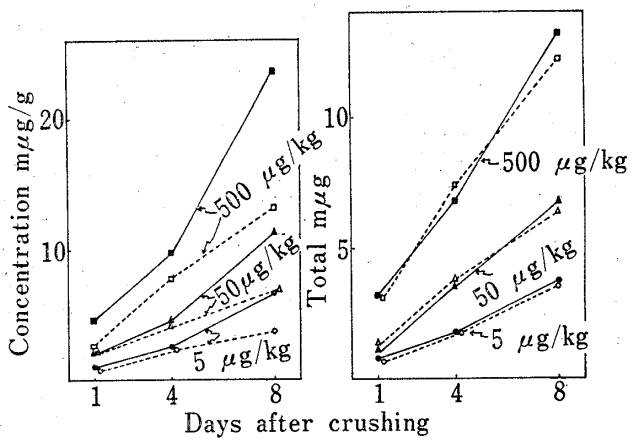

Fig. 2. Uptake of Radioactivity by *M. gastrocnemius* after Consecutive Administration of ^{57}Co–CH_3–B_{12} to Rats with a Crushed Sciatic Nerve at Various Dosages

——: crushed side ------: normal side

Uptake of ^{57}Co-CH_3-B_{12} by Muscles innervated by Sciatic Nerve

The uptake of radioactivity by *M. gastrocnemius*, *M. tibialis anterior* and *M. soleus*, which are innervated by sciatic nerve, was determined in the intact and crushed sides after consecutive, intraperitoneal administration of ^{57}Co–CH_3–B_{12} at various doses. As shown in Fig. 2, 3 and 4, the tissue concentration of radioactivity in the muscle of the crushed side on day 8 was significantly higher than that in the corresponding muscle of the intact side. Regarding total uptake of radioactivity, different phenomena were observed among these three muscles. Namely, in *M. gastrocnemius* it was almost equal in both sides (Fig. 2), but in *M. tibialis anterior* it

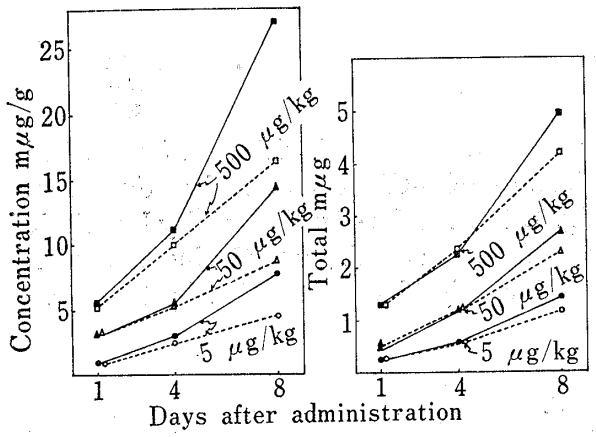

Fig. 3. Uptake of Radioactivity by *M. tibialis anterior* after Consecutive Administration of ^{57}Co–CH_3–B_{12} to Rats with a Crushed Sciatic Nerve at Various Dosage

——: crushed side ------: normal side

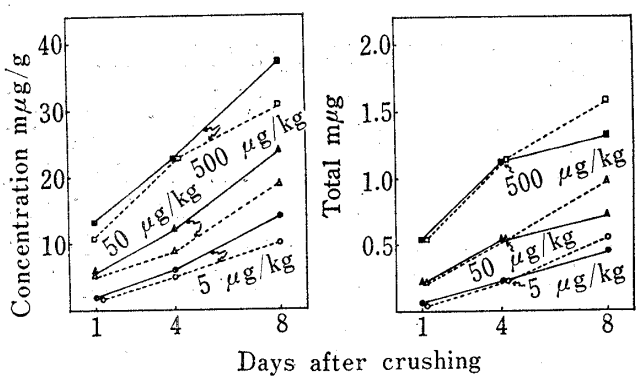

Fig. 4. Uptake of Radioactivity by *M. soleus* after Consecutive Administration of ^{57}Co–CH_3–B_{12} to Rats with a Crushed Sciatic Nerve at Various Dosages

——: crushed side ------: normal side

was significantly higher in crushed side (Fig. 3) and the reverse result was obtained in *M. soleus* (Fig. 4).

In order to confirm whether or not the difference in the uptake of radioactivity in both sides is actually due to nerve degeneration, similar experiments were carried out in intact rats. The tissue concentration and total uptake of radioactivity in the sciatic nerve and in the muscles innervated by sciatic nerve after consecutive administration of ^{57}Co–CH_3–B_{12} at a dosage of 50 μg/kg in intact rats, were nearly equal in both sides (Fig. 5).

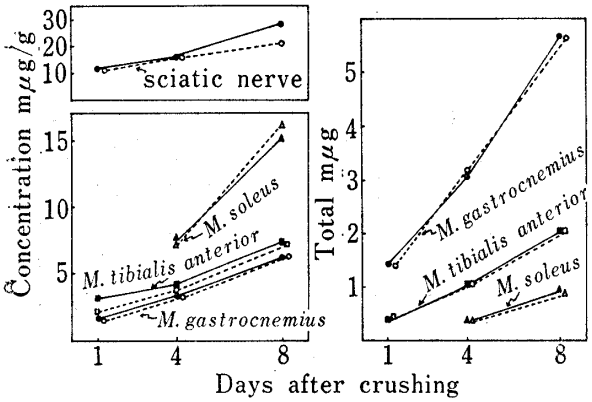

Fig. 5. Uptake of Radioactivity by Muscles and Sciatic Nerves after Consecutive Admin istration of ^{57}Co–CH_3–B_{12} to Intact Rats at a Dosage of 50 μg/kg

——: right ------: left

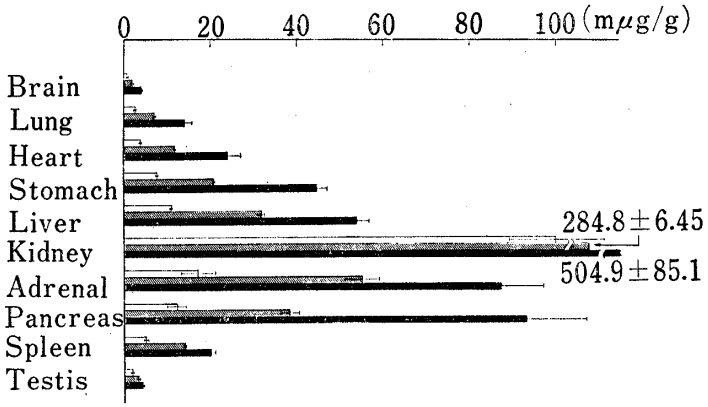

Fig. 6. Tissue Distribution of Radioactivity after Consecutive Administration of ^{57}Co–CH_3–B_{12} to the Rats with a Crushed Sciatic Nerve at the Dosage of 5 μg/kg

□: 1 day after ▨: 4 days after ■: 8 days after

Other Tissue Distribution of Radioactivity

The distributions of radioactivity in organs other than sciatic nerve and muscles both in the rat with a crushed sciatic nerve after consecutive administration of ^{57}Co–CH_3–B_{12} at various doses and in intact rats with 50 μg/kg are shown in Fig. 6, 7, 8 and 9. As clearly recognized, the highest uptake of radioactivity was observed in kidney in all cases, followed by adrenal gland, pancreas and liver, and the lowest uptake in brain and testis. The tissue distribution of radioactivity in rats with a crushed sciatic nerve was found to be almost comparable to that of intact rats after consecutive administration of ^{57}Co–CH_3–B_{12} at a dosage of 50 μg/kg (Fig. 7, 9): that is, no effect attributable to crushing on distribution of radioactivity in organs other than sciatic nerve and muscles was observed. The uptakes of radioactivity by various tissues were increased with time and increasing dosages, and no saturation in uptake of radioactivity by all tissues examined was found.

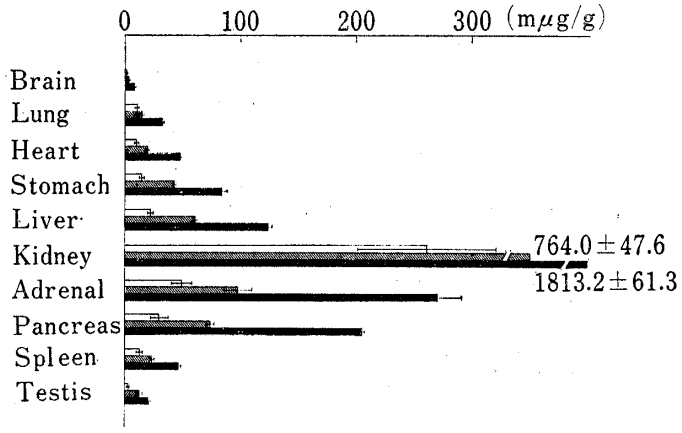

Fig. 7. Tissue Distribution of Radioactivity after Consecutive Administration of ^{57}Co–CH_3–B_{12} to Rats with a Crushed Sciatic Nerve at the dosage of 50 μg/kg

□: 1 day after ▨: 4 days after ■: 8 days after

Urinary Excretion of Radioactivity

Fig. 10 shows the cumulative urinary excretion of radioactivity after consecutive administration of ^{57}Co–CH_3–B_{12} at various doses. As indicated in Fig. 10, the urinary excretion of radioactivity within every 24 hr in each dosage was found to be nearly constant during 7 days, that is, excretion % in urine

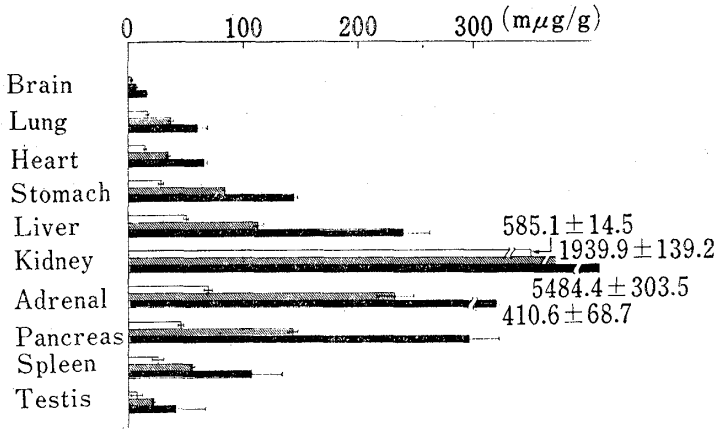

Fig. 8. Tissue Distribution of Radioactivity after Consecutive Administration of ^{57}Co–CH_3–B_{12} to Rats with a Crushed Sciatic Nerve at the Dosage of 500 μg/kg

□: 1 day after ▨: 4 days after ■: 8 days after

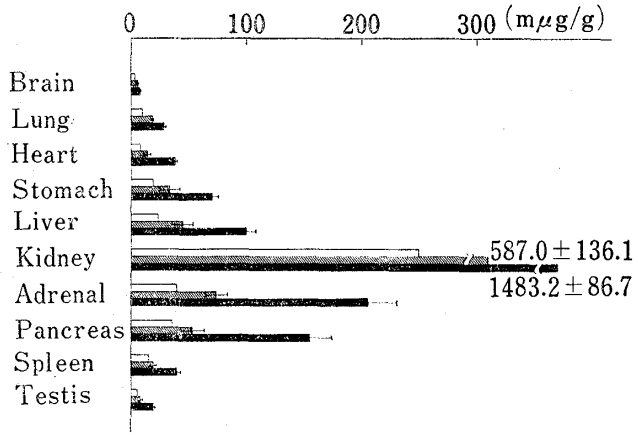

Fig. 9. Tissue Distribution of Radioactivity after Consecutive Administration of ^{57}Co–CH_3–B_{12} to intact Rats at the Dosage of 50 μg/kg

□: 1 day after ▨: 4 days after ■: 8 days after

was about 25 and 75%/day at the dose level of 5 and 500 μg/kg/day in rats with a crushed sciatic nerve, and 70%/day at 50 μg/kg/day in both the intact and nerve-crushed rats.

Discussion

As cleared from Fig. 1, the uptake of radioactivity by sciatic nerve was increased with time and it was always significantly higher in crushed side than in intact one at all doses used. Since the nature of experimental nerve degeneration might be dissimilar to that of

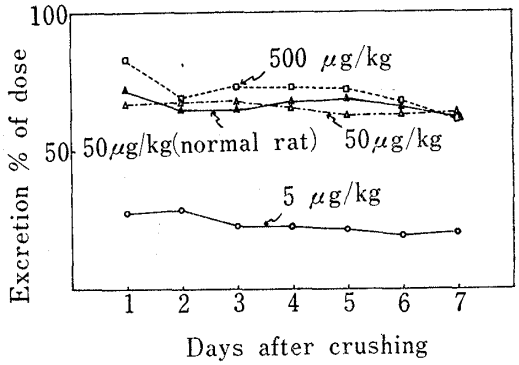

Fig. 10. Cumulative Urinary Excretion of Radioactivity after Consecutive Administration of ^{57}Co–CH_3–B_{12} at Various Dosages

human neuropathies, the present results would not always account for the therapeutic effectiveness of CH_3–B_{12} in human neuropathies. But the fact that the tissue concentration of radioactivity in crushed nerve was distinctly higher than that in intact one, might suggest a reasonable possibility that CH_3–B_{12} plays a role in regeneration process of degenerated nerve. It appears to be necessary to investigate the cellular localization of CH_3–B_{12} in nerve and to establish the regeneration mechanism of nerve.

In regard to the uptake of radioactivity by the muscles such as *M. gastrocnemius*, *M. tibialis anterior* and *M. soleus*, which are innervated by sciatic nerve, the tissue concentration of radioactivity in crushed side was always significantly higher than that in intact side as shown in Fig. 2, 3 and 4. These differences appear to result from nerve degeneration, because the tissue concentration of radioactivity in the muscles of intact rats was nearly equal in both sides as shown in Fig. 5. However, since the turnover rate of CH_3–B_{12} is very slow as reported in our previous paper,[16] the high tissue concentration of radioactivity observed in the muscles of crushed side might be comprehended on the basis of an assumption that ^{57}Co–CH_3–B_{12} incorporated into the muscles was concentrated with the progress of muscular atrophy accompanied with nerve degeneration and the tissue concentration consequently showed apparently

high values. Furthermore, it seems to be due to the differences of muscular function and vascular distribution that the concentration of radioactivity in *M. soleus*, a typical red muscle, was the highest, followed by *M. tibialis anterior* and the lowest in *M. gastrocnemius*, a typical white muscle. Although the tissue concentration of radioactivity in muscles was higher in crushed side than in intact one, no unified inclination was observed in the difference of the total uptake of radioactivity in both sides. This reason has not been clarified yet. Yamatsu, *et al.*[20] studied the electrophysiological and biochemical changes of sciatic nerve and muscles innervated by this nerve using the same experimental system as the present paper and no difference in the extent of muscular atrophy 1 week after crushing was found among these 3 kinds of muscles in the rat with a crushed sciatic nerve. From this finding, the fact that the total muscular content of radioactivity in both sides was varied in accordance with the kind of muscles may be due to the difference with the rate of progress of muscular atrophy at earlier stage within 1 week after crushing.

As shown in Fig. 10, the urinary excretion of radioactivity within 24 hr after every administration of ^{57}Co–CH_3–B_{12} was nearly constant at each dose. On the other hand, as clarified from Fig. 6, 7, 8 and 9, the tissue distribution of radioactivity was increased with an increment of dose, but this increase was not proportional to the doses. When comparing the tissue distribution and urinary excretion of radioactivity in 5 μg/kg group with those in 50 μg/kg group, the urinary excretion % of radioactivity in the latter (approximately 70%/day) was 2.8 times higher than that in the former (approximately 25%/day). If the radioactivity other than that excreted in urine remained in body, it can be expected that the retention of radioactivity in animal body of 50 μg/kg group would be about 3.6 times higher than that in 5 μg/kg one, but the tissue radioactivity in 50 μg/kg group actually showed somewhat lower values than the expected ones. Such difference became more distinct between the retention of radioactivity in 50 μg/kg group and that in 500 μg/kg. The tissue distribution of radioactivity in 500 μg/kg group was considerably less than the values expected, while the urinary excretion of radioactivity in 500 μg/kg group was slightly higher than that in 50 μg/kg. This result might suggest that the excretion route of radioactivity dosed other than *via* kidney, *e.g. via* biliary duct *etc.*, plays an important role in the consecutive administration of large amounts of ^{57}Co–CH_3–B_{12}.

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