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Studies on Lymphatic Absorption of 1′,2′-(^{3}H)-Coenzyme Q_{10} in Rats

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The intestinal absorption of 1′,2′-(^{3}H)-coenzyme Q_{10} (^{3}H-Q-10) was studied in rats with cannulated thoracic duct and the effect of surface-active agents on the lymphatic absorption of ^{3}H-Q-10 was determined.

The amount of radioactivity absorbed *via* lymphatics during the first 48 hr was 1% of the dose after oral administration of ^{3}H-Q-10 dissolved in sesame oil or 20 mM sodium taurocholate, and was 1.5% of the dose of ^{3}H-Q-10 dissolved by HCO-60. Assuming that the amount of radioactivity recovered from urine (0.481%) and liver (0.004%) might come from the radioactivity absorbed *via* portal vein, the total amount of the radioactivity absorbed *via* portal vein and lymphatics was approximately 2% of the dose of ^{3}H-Q-10 dissolved by HCO-60 and the main route in absorption of ^{3}H-Q-10 was lymphatics.

The model for lymphatic absorption of ^{3}H-Q-10 was proposed by kinetic analysis of the data.

It has been recognized that fat-soluble vitamins are absorbed from intestine primarily *via* lymphatic route.[2] Aso, *et al.*[3] have shown that the main route for absorption of coenzyme Q_{10} in rats was the lymphatic pathway and most of absorbed coenzyme Q_{10} appeared in chylomicrons of thoracic duct lymph.

The previous reports[4] on the metabolism of 1′,2′-(^{3}H)-coenzyme Q_{10} have revealed that the blood level of coenzyme Q_{10} reached a maximum 1 hr after oral administration in rat and coenzyme Q_{10} absorbed was mainly incorporated into liver. However, the details on the intestinal absorption of coenzyme Q_{10} have remained unknown. The present study with the absorption of coenzyme Q_{10} was carried out primarily to clarify the effect of surface-active agents on the lymphatic transport rate of coenzyme Q_{10}.

Experimental

Materials——1′,2′-(^{3}H)-coenzyme Q_{10} (abbreviated as ^{3}H-Q-10) was supplied by Mr. K. Hamamura, Department of Organic Chemistry of our research laboratories. The specific radioactivity was 194 μCi/mg and the radiochemical purity measured by thin-layer chromatography was 96—98%. Sodium taurocholate (BDH Chemicals), Nikkol HCO-60, polyoxylethylated derivative of hydrogenated castor oil (Nikko Chemical) and sesame oil (Sanko Pharmaceutical) were used.

Procedure for Absorption Experiments——Male rats of Wistar strain weighing 220—250 g were used. Cannulation into the thoracic duct was carried out under light ethylether anesthesia based on the method of Bollman, *et al.*[5] After operation, animals that were individually placed in restraining cages[6] received

1) Location: *Koishikawa, Bunkyo-ku, Tokyo.*
2) *a*) G.R. Thompson, R.K. Ockner, and K.J. Isselbacher, *J. Clin. Invest.*, **48**, 87 (1969); *b*) H.E. Gallo-Torres, *Internat. J. Vit. Res.*, **40**, 505 (1970); *c*) R. Blomstrand and J. Gürtler, *Internat. J. Vit. Nutr. Res.*, **41**, 189 (1971); *d*) T. Fujita, S. Tanayama, Y. Shirakawa, and Z. Suzuoki, *J. Biochem.*, **69**, 53 (1971).
3) Y. Aso, K. Mishima, T. Arisaka, and H. Kitagawa, Abstracts of Papers, 1sth Symposium on Drug Metabolism and Action, Chiba, November, 1969, p. 4-(1).
4) T. Fujita, T. Matuura, T. Takamatsu, J. Tsutsumi, K. Kinoshita, K. Katayama, K. Miyao, K. Hamamura, S. Kijima, M. Shirato, and S. Baba, *Pharmacometrics*, **6**, 695 (1972); *idem*, *ibid.*, **6**, 707 (1972).
5) J.L. Bollman, J.C. Cain, and J.H. Grindlay, *J. Lab. Clin. Med.*, **33**, 1349 (1948).
6) J.L. Bollman, *J. Lab. Clin. Med.*, **33**, 1348 (1948).

0.9% NaCl solution *ad libitum*. Prior to use, animals were allowed to fast and stabilize overngiht. ^{3}H-Q-10 was dissolved in taurocholate and HCO-60 micelles or sesame oil for oral administration by stomach tube. Details of dosing are shown in each figure and table. Lymph was collected at predetermined time intervals during the first 48 hr following the administration of ^{3}H-Q-10.

Measurement of Radioactivity——The radioactivity was measured by liquid scintillation counter, Aloka model LSC-502 (Nihon Musen). 0.1 ml of aliquots of lymph or urine was taken in vial containing 15 ml of a scintillation solution consisting of 4 g of 2,5-diphenyloxazole (PPO), 200 mg of 1,4-bis-2-(methyl-5-phenyloxazolyl)benzene (Me_2POPOP), 100 g of naphthalene, 750 ml of dioxane, 150 ml of toluene and 100 ml of ethyl cellosolve. The radioactivity in liver was determined by the combustion method using Tri-Carb Sample Oxidizer model 300 (Packard Instrument).

Thin-Layer Chromatography——50 μl of aliquots of lymph were placed on 0.25 mm thin layer plates of Kieselgel G and developed with the following solvents. I) 40% ethylether in *n*-hexane, II) benzene, III) chloroform.

Ultracentrifugation——Analytical ultracentrifugation of 4 ml of lymph was carried out using a Hitachi swinging bucket rotor based on the method of Ockner, *et al.*[7]

Result

Absorption of ^{3}H-Q-10

In order to determine the relationship of the transport of radioactivity between *via* lymphatics and *via* portal vein, 0.6 mg/kg body weight of ^{3}H-Q-10 dissolved in 7.5% HCO-60 was orally administered to thoracic duct fistula rats. Table I shows the recovery of radioactivity in lymph and urine during the first 48 hr and that in liver 48 hr after administration. From the preliminary experiments, the lymphatic absorption was almost completed within 48 hr after the administration.

Table I. Lymphatic Absorption, Urinary and Fecal Excretion, and Liver Contents of Radioactivity after Oral Administration of ^{3}H–Q–10

Sample	Time (hr)	% of dose
Lymph	0—24	0.860±0.140
	0—48	1.538±0.100
Urine	0—24	0.354±0.020
	0—48	0.481±0.031
Feces	0—48	55.03±4.61
Liver	48	0.004±0.001

dose: 0.6 mg/4 ml of 7.5% HCO-60/kg of body weight. Data are expressed in mean ± S.E. (n=3).

The amount of absorbed radioactivity *via* lymphatics during the first 48 hr attained 1.538% of the dose. In contrast, less than 0.5% of the dosed radioactivity was excreted into urine and only 0.004% was recovered in liver. The amount of radioactivity in urine and liver determined as an index of the absorption *via* portal vein was extremely small in comparison with that in lymph, and therefore, it is suggested that orally administered ^{3}H-Q-10 is absorbed primarily *via* lymphatics.

Effect of Different Dose Levels of ^{3}H-Q-10 on Lymphatic Absorption

In order to determine the effect of dosages of ^{3}H-Q-10 on the lymphatic absorption, ^{3}H-Q-10 dissolved in 0.75% HCO-60 at the concentration of 0.15 mg/ml was administered at three dose levels of 0.15 mg/kg, 0.6 mg/kg, and 2.4 mg/kg. As shown in Table II, the amount of absorbed radioactivity *via* lymphatics during the first 48 hr was 1.5—1.6% of the dose in every dose level. This indicates that the percentages of the dose absorbed *via*

7) R.K. Ockner, F.B. Hughes, and K.J. Isselbacher, *J. Clin. Invest.*, **48**, 2079 (1969).

lymphatics was independent of the dosage at the dose level of 0.15—2.4 mg ^{3}H-Q-10/kg body weight.

Table II. Absorption *via* Lymphatics after Oral Administration of ^{3}H-Q-10 in Different Dose Levels

Dose (mg/kg)	Percent of dose absorbed *via* lymphatics within 48 hr	Lymph volume (ml/48 hr)
0.15	1.687±0.026	256± 59
0.60	1.550±0.068	395±116
2.40	1.627±0.117	336± 69

^{3}H-Q-10 was dissolved in 0.75% HCO-60 at the concentration of 0.15 mg/ml. Data are expressed in mean±S.E. (n=3).

Effect of Surface-Active Agents on Lymphatic absorption

The absorption of many drugs has been shown to be affected by surface-active agents.[8] To observe the effect of surface-active agents on lymphatic absorption of ^{3}H-Q-10, HCO-60

Table III. Effect of Surface-active Agents on Lymphatic Absorption of ^{3}H-Q-10

Solvent	Percent of dose absorbed *via* lymphatics in 48 hr	Lymph volume (ml/48 hr)
Sesame oil	1.042±0.313	386± 92
20 mM Sodium taurocholate	b) —0.909±0.017—	278± 59
0.75% HCO-60 a)	—1.550±0.068 d)	395±116
7.5% HCO-60 c)	1.538±0.100—	321± 28

a) presented in Table II *c*) presented in Table I *b*, *d*) significant difference ($p<0.01$)
dose: 0.6 mg/4 ml/Kg of body weight
Data are expressed in mean±S.E. (n=3).

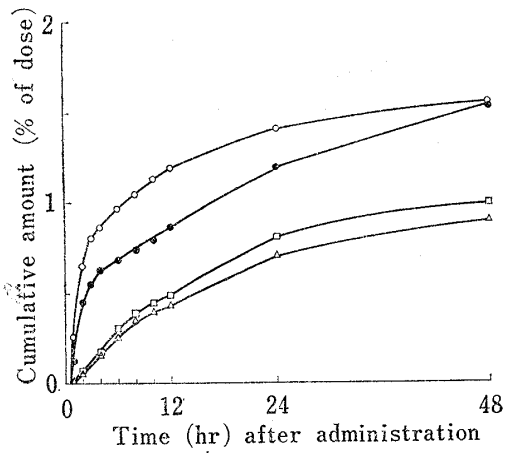

Fig. 1. Recovery of Radioactivity to Lymph after Oral Administration of ^{3}H-Q-10 Dissolved in Sesame Oil and by Surface-active Agents

dose: 0.6 mg/4 ml/kg of body weight
Data are expressed in mean (n=3).
—○—: 0.75% HCO-60
—●—: 7.5% HCO-60
—□—: sesame oil
—△—: 20 mM sodium taurocholate

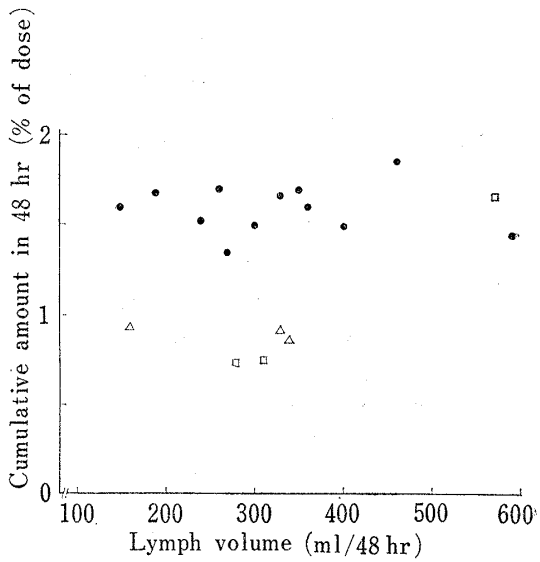

Fig. 2. Correlation between Lymph Volume and Cumulative Amount of Radioactivity in Lymph

●: HCO-60
□: sesame oil
△: sodium taurocholate

8) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **50**, 579 (1970).

which was used in previous papers,[4] was chosen and compared with sodium taurocholate or sesame oil.

Fig. 1 represents the cumulative recovery of radioactivity in lymph after oral administration of ^{3}H-Q-10 dissolved in 0.75% and 7.5% HCO-60, 20 mM sodium taurocholate and sesame oil. The absorption of radioactivity *via* lymphatics after administration of the HCO-60 solution was remarkably high within 2 hr as compared with that of radioactivity *via* lymphatics after administration of the taurocholate solution or the sesame oil solution.

Table III represents the amount of absorbed radioactivity *via* lymphatics during the first 48 hr after administration and the statistical analyses. The significant differences of the percentages of absorbed radioactivity were found only between following administrations of the taurocholate solution and of the HCO-60 solutions.

Effect of Lymph Flow on Lymphatic Absorption of ^{3}H-Q-10

It has been shown that the absorption of salicylamide was affected by blood flow,[9] and the lymph flow rate was quantitatively related to the infusion rate of isotonic saline into intestine.[10] However, the effect of lymph flow rate on lymphatic absorption has not been fully elucidated. The percentages of absorbed radioactivity *via* lymphatics during the first 48 hr were plotted against the lymph volume collected for 48 hr. As shown in Fig. 2, there was no correlation (coefficient of 0.218) between the cumulative amount and the lymph volume.

Identification of ^{3}H-Q-10 in Lymph and Distribution of Radioactivity among Lymph Lypoprotein Fractions

Thin-layer chromatograms of the lymph obtained during the first 48 hr after oral administration of ^{3}H-Q-10 are shown in Fig. 3. The radioactivity in the lymph was observed as a mono-peak at the same area as that of the authentic sample, coenzyme Q_{10}. This fact shows that ^{3}H-Q-10 given orally was absorbed into lymph mainly in the unchanged form.

The distribution of radioactivity among lymph lipoprotein fractions was determined by analytical ultracentrifugation of 4 ml of aliquots of the lymph which was collected for 12 hr after oral administration of ^{3}H-Q-10 dissolved in sesame oil. Table IV shows the amounts of radioactivity in chylomicrons, very low density lipoproteins ($d<1.006$, abbreviated as VLDL) and lipoproteins of density greater than 1.006 (abbreviated as $d>1.006$), respectively. Most of the radioactivity (80% of the total in the lymph) was localized in the chylomicron fraction and remaining radioactivity was almost evenly distributed between VLDL fraction and $d>1.006$ fraction. The radioactivity concentration in VLDL fraction, however, was 2.5—7 fold higher than that in $d>1.006$ fraction.

Table IV. Ultracentrifugal Pattern of Thoracic Duct Lymph

Sample number	Fraction	Volume (ml)	Total activity (dpm)	(%)
1[a]	Chylomicrons	0.38	67,997	81.12
	VLDL[b]	0.52	8,611	10.27
	$d>1.006$	3.10	7,217	8.61
2[c]	Chylomicrons	0.37	27,963	79.24
	VLDL[b]	0.48	1,972	5.59
	$d>1.006$	3.15	5,354	15.17

a), *c*) Lymph in 12 hr after oral administration ^{3}H-Q-10 dissolved in sesame oil
b) very low density lipoprotein ($d<1.006$)

9) W.H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59**, 154 (1970).
10) H.E. Gallo-Torres and O.N. Miller, *Proc. Soc. Exptl. Biol. Med.*, **130**, 552 (1969).

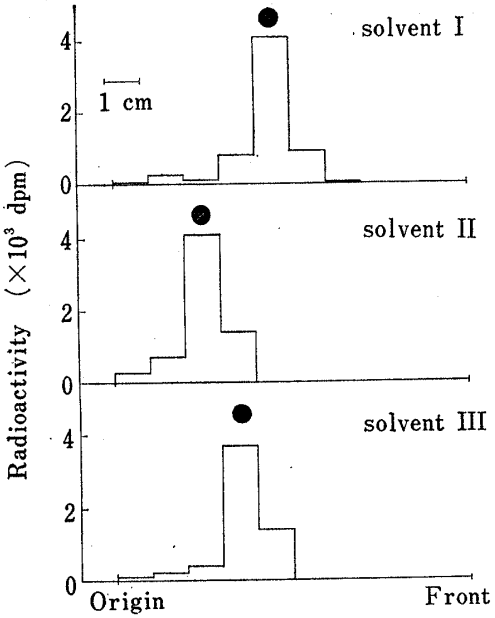

Fig. 3. Thin-Layer Chromatograms of Lymph after Oral Administration of ^{3}H-Q-10 Dissolved in 0.75% HCO-60

solvent I: ether-*n*-hexane (4;6), solvent II: benzene, solvent III: chloroform
authentic sample (black dot): Q-10

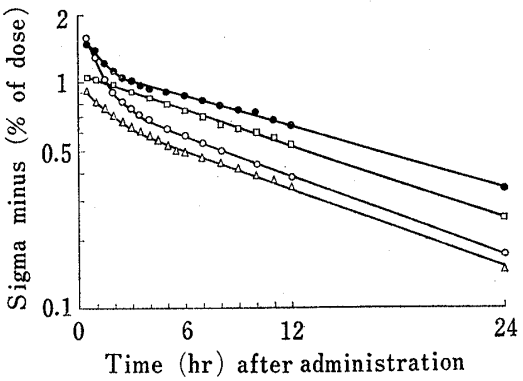

Fig. 4. Semilogarithmic Plots of Sigma Minus Values

dose: 0.6 mg/4 ml/kg of body weight
data are expressed in mean (n=3).
—○—: 0.75% HCO-60
—●—: 7.5% HCO-60
—□—: sesame, oil
—△—: 20 mM sodium taurocholate

Discussion

The data in Table I, IV and Fig. 3 demonstrate that ^{3}H-Q-10 administered orally was absorbed mainly unchanged *via* lymphatics in chylomicrons. These data are comparable with the results by Aso, *et al.*[3] In their report, however, the amount of absorption *via* lymphatics was much higher (30—80% of the dose) than that in the present study. This quantitative discrepancy might come from differences in experimental conditions.

Blomstrand and Gürtler[2c] have shown that the absorption of 1′,2′-(^{3}H)-phytylubiquinone *via* lymphatics in man diminished considerably as the dosage of phytylubiquinone. In contrast, Table II demonstrates that the absorption of ^{3}H-Q-10 *via* rat lymphatics in the dose level of 0.15—2.4 mg/kg was approximately constant. This results suggest the absorption of ^{3}H-Q-10 in rats to be done by passive diffusion. Also, the independence of lymph volume on the lymphatic absorption of ^{3}H-Q-10 (Fig. 2) suggests that the lymphatic transport rate of ^{3}H-Q-10 is not limited by lymph flow rate.

Kinetic studies on the present data were carried out by the sigma minus method[11] to obtain the clearer aspects on the lymphatic absorption in various kinds of dosing forms. Figure 4 shows the semilogarithmic plots of sigma minus values, (L_∞—L), against time (hr), where L_∞ and L represent the toatl amount of radioactivity recovered in lymph during the first 48 hr and the cumulative amount of radioactivity in lymph until a time, t, after the administration of ^{3}H-Q-10, respectively.

As shown in Fig. 4, the absorption patterns were found to be classified into two types. One type, which was observed following administration of ^{3}H-Q-10 dissolved in sesame oil, gave one straight line, and the other type, which was observed after administration of the micellar solution of sodium taurocholate or HCO-60, was composed of two straight lines.

11) *a*) B.K. Martin, *Nature*, **214**, 247 (1967); *b*) A.J. Cumming, B.K. Martin, and G.S. Park, *Brit. J. Pharmac. Chemother.*, **29**, 136 (1967).

Therefore, in the case of one straight line, the sigma minus values were fitted to the mono-exponential equation by the least square method;

$$(L_\infty - L) = B \exp(-\alpha t) \quad (1)$$

and in the case of two straight lines, the values were fitted to the biexponential equation;

$$(L_\infty - L) = A \exp(-\alpha t) + B \exp(-\beta t) \quad (2)$$

Table V shows the averages of the parameters and the results of statistical analyses. The value of β calculated in the sesame oil solution was almost equal to the values of β in the taurocholate micellar solution or the HCO-60, but was lower than the values of α in the micellar solutions.

Table V. Parameters for Lymphatic Absorption of ^{3}H-Q-10
Data are expressed in Mean with (S.E.) ($n=3$)

Parameters	Sesame oil	20 mM sodium taurocholate	HCO-60 0.75%	HCO-60 7.5%
A		0.3617	1.2157[a]	1.1243[a]
		(0.0566)	(0.2960)	(0.1967)
B	1.1200	0.6863	0.9080[a]	1.1187[a,b]
	(0.3424)	(0.0552)	(0.0510)	(0.0436)
α		0.8574	1.0502	1.3883
		(0.3253)	(0.2267)	(0.0500)
β	0.0603	0.0587	0.0798	0.0439
	(0.0105)	(0.0139)	(0.0170)	(0.0098)

a) significant difference from 20 mM sodium taurocholate ($p<0.05$)
b) significant difference from 0.75% HCO-60 ($p<0.05$)

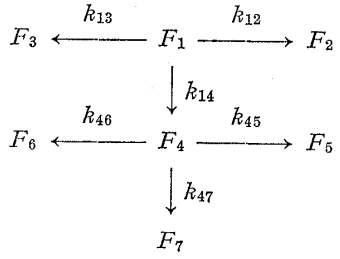

Chart 1. Model for Lymphatic Absorption of ^{3}H-Q-10

F_1: amount of administered radioactivity dissolved by surface-active agents
F_2, F_5: amounts of absorbed radioactivity *via* lymphatics
F_3, F_6: amounts of absorbed radioactivity *via* portal vein
F_4: amount of radioactivity released from the administered micelles
F_7: amount of excreted radioactivity
k_{12}—k_{47}: rate constants

According to present study and previous reports,[4] the lymphatic absorption of radioactivity was almost completed within 48 hr and most of the administered radioactivity was excreted in feces within 48 hr after oral dosing. Also, Table II suggests that the administered radioactivity is apparently absorbed *via* lymphatics at a first order rate process. Therefore, the model in Chart 1 is considered to be the most suitable for analyzing the time course of lymphatic absorption of ^{3}H-Q-10 in rats.

After oral administration of radioactivity (F_1) which was dissolved by surface-active agents, the amount of radioactivity (F_2) is absorbed *via* lymphatics at a rate constant of k_{12} and F_3 *via* portal vein at a rate constant of k_{13}. The radioactivity (F_4) is released from the administered radioactivity in micelles (F_1) at a rate constant of k_{14}. Following solubilization of F_4 by bile salts in intestinal lumen, the amount of radioactivity (F_5) is absorbed *via* lymphatics at a rate constant of k_{45} and F_6 *via* portal vein at a rate constant of k_{46}. From the data in Fig. 4, the assumption is made that the amount of radioactivity (F_5) is absorbed *via* lymphatics at a first order rate process after oral administration of radioactivity (F_4) dissolved in sesame oil, even though the absorption process may be more complicated.

Based on the method of Cummings, *et al.*,[11b] rate constants in Chart 1 are calculated as follows. Sigma minus values according to the model may be expressed;

$$F_{2\infty} - F_2 = F_{2\infty} \exp(-Kt) \tag{3}$$

$$F_{5\infty} - F_5 = F_{5\infty}[K \exp(-K't) - K' \exp(-Kt)]/(K-K') \tag{4}$$

where $F_{2\infty}$ and $F_{5\infty}$ are the sum of the amount of radioactivity absorbed *via* lymphatics within 48 hr, and $F_{2\infty}=F_{10}k_{12}/K$, $F_{5\infty}=F_{10}k_{14}k_{45}/KK'$, F_{10}=dose=100, $K=k_{12}+k_{13}+k_{14}$, $K'=k_{45}+k_{46}+k_{47}$. The sum of the equation (3) and (4) are expressed;

$$(F_{2\infty}+F_{5\infty}) - (F_2+F_5) = [F_{2\infty} - F_{5\infty}K'/(K-K')] \exp(-Kt) + [F_{5\infty}K/(K-K')] \exp(-K't) \tag{5}$$

In equation (2) and (5), $(L_\infty - L)$ is equal to $[(F_{2\infty}+F_{5\infty})-(F_2+F_5)]$ and therefore, $A=F_{2\infty}-F_{5\infty}K'/(K-K')=(F_{10}/K)[k_{12}k_{14}k_{45}/(K-K')]$, $\alpha=K$, $B=F_{5\infty}K/(K-K')=F_{10}k_{14}k_{45}/K'(K-K')$ and $\beta=K'$. Assuming $k_{13}<k_{12}\ll k_{14}$ and $k_{46}<k_{45}\ll k_{47}$ from the data in Table II, K and K' are reduced to the equations; $K=k_{12}+k_{14}$ and $K'=k_{45}+k_{47}$. Accordingly, rate constants are calculated as follows.

$$k_{12} = (A\alpha+B\beta)/100 \tag{6}$$

$$k_{14} = \alpha - k_{12} \tag{7}$$

$$k_{45} = B\beta(\alpha-\beta)/100k_{14} \tag{8}$$

$$k_{47} = \beta - k_{45} \tag{9}$$

Rate constants calculated by the equations (6)—(9) and by the equations; $k_{45}=B\beta/100$, $k_{47}=\beta-k_{45}$ in the case of sesame oil, are shown in Table VI.

Table VI. Rate Constants for Lymphatic Absorption of ^{3}H-Q-10
Data are expressed in Mean with (S.E.) ($n=3$)

Rate constant [hr^{-1}]	Sesame oil	20 mM Sodium taurocholate	HCO-60 0.75%	HCO-60 7.5%
k_{12}		0.003159 (0.000665)	0.014523[a] (0.004871)	0.016240[b] (0.003190)
k_{14}		0.8542 (0.3245)	1.0356 (0.2222)	1.3721 (0.0476)
k_{45}	0.000744 (0.000351)	0.000388 (0.000126)	0.000673 (0.000157)	0.000483 (0.000110)
k_{47}	0.0596 (0.0102)	0.0583 (0.0137)	0.0791 (0.0169)	0.0434 (0.0097)

a) significant difference from 20 mM sodium taurocholate ($p<0.10$)
b) significant difference from 20 mM sodium taurocholate ($p<0.05$)

As for k_{12}, the value of the lymphatic absorption of ^{3}H-Q-10 in the micelles of taurocholate was significantly low as compared with those of HCO-60. This difference might come from an effect of HCO-60 on intestinal permeability. The releasing rate constants of ^{3}H-Q-10 from the micelles of taurocholate or HCO-60, k_{14}, corresponded to half-lives of 30—50 min and were extremely larger than k_{12}. Rate constant of lymphatic absorption, k_{45} following administration of the sesame oil solution, was the value similar to that of lymphatic absorption, k_{45} following administration of ^{3}H-Q-10 dissolved by taurocholate or HCO-60, but was extremely low in comparison with the values of k_{12}. It is, therefore, suggested that the dissolution of ^{3}H-Q-10 by bile salts in intestine is a limiting step in the absorption processes from oil phase and from the post-releasing phase.

The values of fecal excretion calculated using k_{47} were larger than the values shown in Table I but were the values similar to those of previous reports.[4] This quantitative dis-

crepancy might come from the differences in fasting state. Since in these experiment herein, rats were fasted overnight after surgical operation and then for 6 hr after the administration of ^{3}H-Q-10, the excretion to feces might have delayed despite that the lymphatic absorption was almost completed within 48 hr.

The aspect of lymphatic absorption of ^{3}H-Q-10 from micellar solution is shown in the model of Chart 1. But, the process of releasing of ^{3}H-Q-10 from the administered micelles remains unclarified. Further investigations using materials easily absorbable *via* lymphatics will be needed for the evaluation of this model proposed herein.

Acknowledgement The authors are deeply indebted to Mr. K. Hamamura for a supply of 1′,2′-(^{3}H)-coenzyme Q_{10}. We wish to thank Dr. K. Miyao, Director of Department of Pharmacology, Dr. S. Ohtake, Associate Director, for their helpful advices and also to thank Mr. Y. Naito, President of Eisai Co., Ltd., and Dr. K. Satoh, Vice President of Eisai Research Laboratories, for their support of our research.