Conversion of Oxazoles into N-Methylimidazoles——To a chilled solution of the oxazole (0.5 g) in 10—20 ml of dichloromethane was added 0.3—0.4 ml of methyl fluorosulfonate. After 0.5—1 hr, the solvent was evaporated under reduced pressure. The residual oil was dissolved in 10 ml of ammonia-saturated ethanol and the solution was heated at reflux for 2 hr.[14] After evaporation of the solvent, 10% sodium bicarbonate was added and the mixture was extracted with ethyl acetate. The organic layer was dried and evaporated to give a colorless residue. Purification was effected most readily by chromatography on a short silica gel column, using 3% methanol–ethyl acetate for elution. Yields are given in Table I.

3-Benzyl-4-methyl-5-phenyloxazolium Benzenesulfonate (IIe)——A mixture of 4-methyl-5-phenyloxazole (Ie, 100 mg) and benzyl benzenesulfonate (500 mg) was heated at 90° for 5—10 min. The reaction mixture was diluted with dry ether and the resulting precipitate was filtered and recrystallized from ethanol–ether, mp 133—136°.

1-Benzyl-5-methyl-4-phenylimidazole (IIIe)——The N-benzyloxazolium salt (IIe, 0.41 g, 1 mmol) was added to 10 ml of ammonia-saturated ethanol and the solution was refluxed for 2 hr. The product was isolated as described above. Silica gel chromatography provided 0.23 g (97%) of an oil which solidified, mp 76—78°. The picrate was crystallized from ethanol, mp 169—170°.

4-Methyl-5-phenylimidazole (IVe)——Debenzylation of IIIe (248 mg, 1 mmol) was carried out by Na in liq. NH_3[8] to give IVe (109 mg, 69%, mp 184—187°, from benzene, reported[15] mp 192°).

14) Conversion can also be effected at room temperature in a few days.
15) H. Bredereck, R. Gompper, and F. Reich, *Chem. Ber.*, **93**, 733 (1960).

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The Effect of Dosage Form on Absorption of Vitamin A into Lymph

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Some effects of dosage form on absorption of vitamin A into thoracic duct were studied in rats fed vitamin A. Forty-five % of administered vitamin A was recovered in lymph when vitamin A was given in micellar solution form, while 29% of administered vitamin A was recovered when vitamin A was given in triolein solution form. The time courses of appearance of vitamin A in lymph of these two kinds of preparation were very different one another. Vitamin A appearance in lymph showed a sharp peak between 1 and 2 hr after the administration in micellar solution form and indicated the almost complete absorption of vitamin A, while it showed a broad band over 4 hr after the administration in oily solution form. The time courses of vitamin A in serum did not exactly reflect those in lymph.

Most of compounds orally administered are absorbed *via* portal vein system but some fat soluble compounds are absorbed *via* thoracic duct. Some biologically important compounds such as triglyceride, cholesterol, fat soluble vitamins and DDT are known to be absorbed into lymph.[2] The mechanism of lymphatic transport was investigated much about triglyceride and cholesterol, and some about vitamin A (VA). Orally administered VA esters are partially hydrolysed by the pancreatic hydrolase with resultant appearance of VA alcohol and its esters in micellar solution from which the brush border rapidly picks up both ingredients. VA alcohol is directly admitted into the cell, while its esters are hydrolysed by the hydrolase situated on the outer surface of the brush border, and then VA alcohol is

1) Location: *a) Hongo, Bunkyo-ku, Tokyo; b) Kohda-cho, Takada-gun, Hiroshima.*
2) H. Kilian, *Pharmaceutisch Week Blad*, **108**, 1153 (1973).

transfered through the lymph in which VA esters reformed is incorporated with lipoprotein particle.[3] In spite of those investigators, the whole mechanism of lymphatic transport system is not known in detail. In the present study, VA in various dosage forms are investigated in order to clarity the effect of dosage form on release of VA from preparation and the effect of oils and detergents coexisting.

Experimental

Materials——Synthetic VA palmitate (Roche Co., Ltd., 1500 I.U./mg) was used. All other materials used in these experiments were of reagent special grade.

Micellar Solution——Ten mg of VA palmitate was dissolved in 0.05 ml of polysorbate 80 (PS80), then added 1 ml of water.

Oily Solution——Ten mg of VA palmitate was dissolved in 0.5 ml of triglyceride. Three typical triglycerides were chosen, triolein as long chain triglyceride (LCT), tricaproin as medium chain triglyceride (MCT), tributyrin as short chain triglyceride (SCT). A half ml of these oily solutions were administered following 0.5 ml of water.

Emulsified Preparation——Above oily solutions were mixed with 0.5 ml of 10% nonionic detergents mixture (PS80: span 80 was 4: 1) and stirred vigorously in waring blender.

Other Preparation——Ten mg of VA palmitate was dissolved in 0.5 ml of oleic acid or paraffin.

Preparation of Thoracic Duct Cannulated Rats——Male albino Donryu rats weighing 300 g were anesthetized with pentobarbital and a polyethylene catheter (PE 50; I.D., 0.023 inch; O.D., 0.038 inch) was inserted into the thoracic duct by use of the technique described by Bollman.[4] The rats were kept in restraining cages and had access to a solution of 10% glucose in 0.9% saline. Absorption studies were begun 12—24 hr after the surgery. VA was given by gastric intubation under light ether anesthesia followed by the collection of lymph hourly for 8 hr and for all the remainder of the 24 hr period.

Preparation of Femoral Artery Cannulated Rats——Rats were fasted one day before surgery, and under ether anesthesia, polyethylene tube of suitable diameter was inserted into the femoral artery. Blood samples were collected at hourly intervals for 8 hr after dosing of VA in LCT oily solution form, and 30 min intervals for the first 4 hr and at hourly intervals for the next 4 hr after dosing in micellar solution form.

Analytical——Lymph or serum was diluted to proper VA concentration with water. One ml of those samples and 4 ml of ethanol were mixed and 4 ml of cyclohexane was added. The mixture was vortexed for 10 min and centrifuged at 3000 rpm. VA in cyclohexane phase was measured by fluorometrically at 365 nm (excitation) and 485 nm (fluorescence).[5] Identity of VA alcohol and its ester were done by thin layer chromatography on silicagel (Silicagel f spotfilm; Tokyo Kasei Co., Ltd.). The solvent system used was 3% ethanol in cyclohexane.

Results and Discussion

In preliminary experiment, fluorescent intensity ratio of VA alcohol to VA palmitate was found to be 1.05: 1, so that the amount of VA was expressed as total recovery of VA. VA recovered in lymph was analysed by thin-layer chromatography and more than 90% was found to be VA palmitate.

The time course of VA recovered in lymph following the administration of VA in micellar solution form is shown in Fig. 1.

The effect of micellization by detergents on absorption of VA into lymph was remarkable. VA appearance in lymph showed a sharp peak between 1 and 2 hr after VA administration and 22% of VA administered was recovered in this period. Eighty-six % of VA absorbed in lymph in 24 hr was recovered within first 4 hr. Forty-five % of VA administered was recovered in 24 hr and which fact was the highest percentage of recovery among all dosage forms (Table I).

3) J. Ganguly, *Am. J. Clin. Nutr.*, **22**, 923 (1969); H. S. Huang and D.S. Goodman, *J. Biol. Chem.*, **240**, 2839 (1965); N.H. Fidge, T. Shiratori, J. Ganguly, and D.S. Goodman, *J. Lipid Res.*, **9**, 103 (1968); D.L. Yeung and M.J. Veen- Baigent, *Can. J. Physiol. Pharmacol.*, **50**, 753 (1972).

4) J.L. Bollman, J.C. Caine, and J.H. Grindlay, *J. Lab. Clin. Med.*, **33**, 1349 (1948).

5) J. Kahan, *Scand. J. Clin. Lab. Invest.*, **18**, 679 (1966); J. Kahan, *Acta Chem. Scand.*, **21**, 2515 (1967).

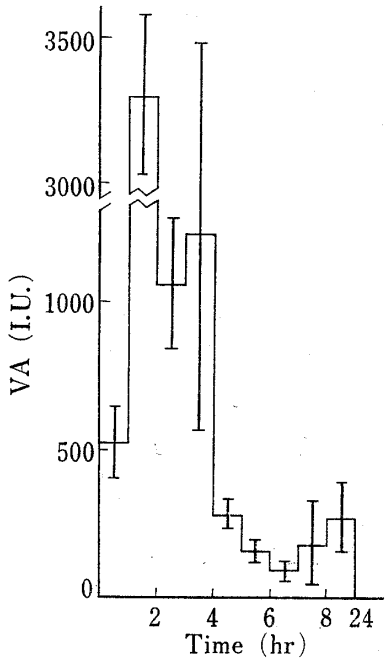

Fig. 1. Appearance of VA in Lymph for 24 hr following the Administration in Micellar Solution Form

Results are mean of three rats.
Vertical bars are S.E. of means.

A possible reason for the good and rapid absorption from micellar solution can be related to the easiness of VA delivery to absorptive cells.

Fig. 2 shows the time course of VA appearance in lymph following the administration in oily solution form. The shapes were very different from that of micellar solution, there was no sharp peak and absorption of VA into lymph continued for over 8 hr after the administration. The percentages of recovery of VA in lymph vary with the carbon chain lengths of triglycerides which were used for vehicle. Twenty-nine % of VA was recovered when LCT was used, and this high percentage was the next to that of micellar solution (Table I). LCT is known to be decomposed to monoglyceride and fatty acid in intestinal lumen by pancreatic lipase, and turns into mixed micelles with the aid of bile salts and phosphatidylcholine to be absorbed by brush border. VA in LCT oily solution was then easily released to be included in micelles with the products of LCT decomposed. On the other hand, percentage of recovery of VA when MCT or SCT was used was relatively low. MCT and SCT are known to be absorbed into blood

Table I. Percentage of Recovery of VA or Sudan Blue in Lymph for 24 hr after Dosing

Dosage form		VA (%)[a]	P[b]	Sudan blue[c] (%)
Micellar solution		45.3±4.0	0.05[d]	—
Oily solution	LCT	29.0±2.3	0.01	5.5
	MCT	12.5±1.5	0.05	1.3
	SCT	6.6±1.2		0.5
Emulsified preparation	LCT	22.1±2.0	N.S.[e]	10.5
	MCT	25.0±2.6	N.S.	3.1
	SCT	20.4±1.2		—
	OAMO[f]	—		12.7
Oleic acid solution		17.1±2.9		2.4
Paraffin solution		1.1±0.4		0.5

a) Values are mean±S.E. from three rats.
b) P values are given for difference of the means.
c) Ten mg of Sudan Blue was orally administered.
d) Micellar solution was significantly different from all other dosage forms.
e) N.S.; not significantly different
f) OAMO; oleic acid and monoolein mixture (2:1 molar ratio)

via portal vein system. As the products of MCT and SCT decomposed do not form micelles so VA is not included in micelles.

Fig. 3 shows VA recovery in lymph following the administration of VA in emulsified preparation. The percentage of recovery of VA showed no significant differences (Table I). The reason for the absence of effect of carbon chain length is suspected that detergents coexisting aided to form micelles including VA.

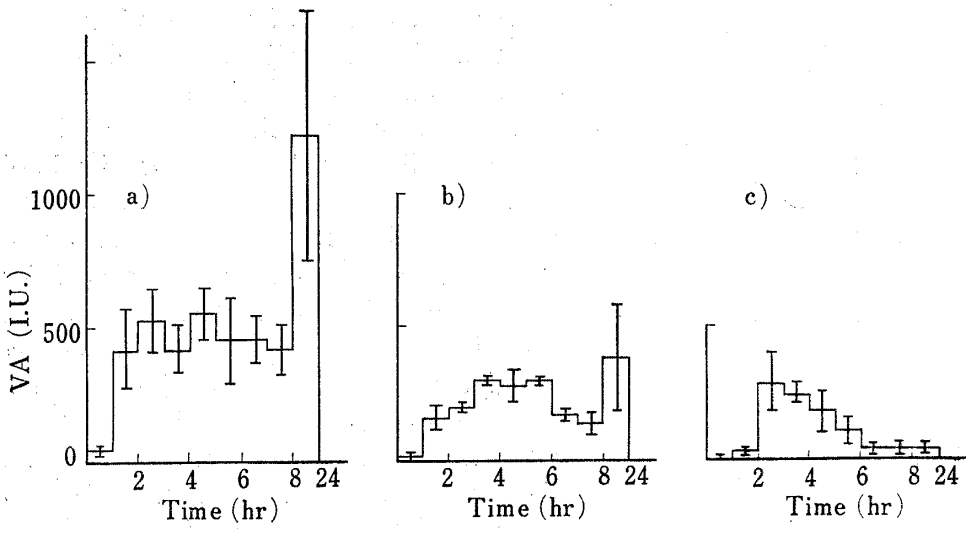

Fig. 2. Appearance of VA in Lymph for 24 hr following the Administration in Oily Solution Form

a) LCT oily solution *b*) MCT oily solution *c*) SCT oily solution
Results are mean of three rats.
Vertical bars are S.E. of means.

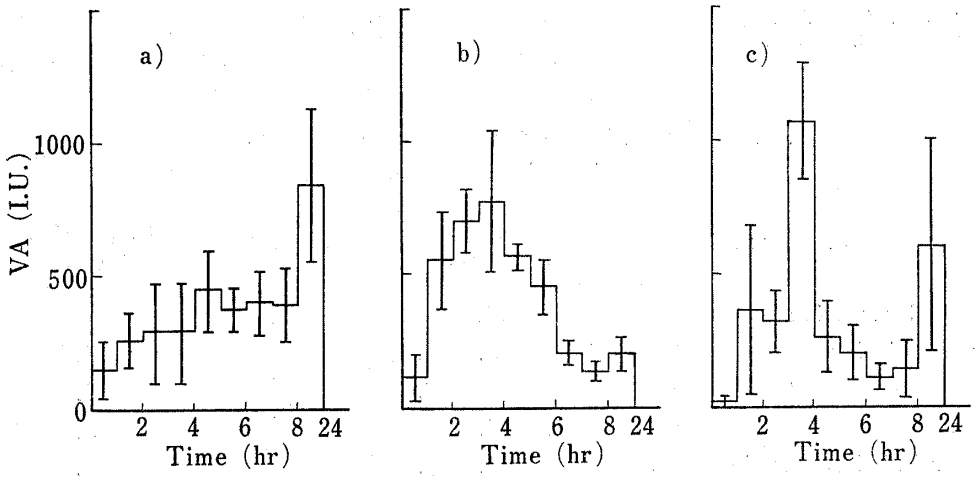

Fig. 3. Appearance of VA in Lymph for 24 hr following the Administration in Emulsified Preparation

a) LCT emulsion *b*) MCT emulsion *c*) SCT emulsion
Results are mean of three rats.
Vertical bars are S.E. of means.

Fig. 4 shows VA recovery in lymph following the administration in oleic acid solution form or paraffin solution form. Seventeen % of VA administered was recovered from oleic acid solution (Table I). Oleic acid is a long chain fatty acid which is readily absorbed into lymph included in micelles of bile salts, but seemed to have little capacity alone to form mixed micelles including VA. Only 1.1% of administered VA was recovered from paraffin solution (Table I). The reason for poor absorption is assumed that VA remains in preparation without releasing since paraffin is not decomposed in intestinal lumen.

One of the factors which affect the time course of appearance of VA in lymph is gastric emptying time. Gastric emptying is known to be delayed when the meal has high viscosity or contains fat. LCT was reported to delay gastric emptying compared to SCT.[6] The slow

6) R.W. Harkins, J.B. Longenecker, and H.P. Sarett, *Federation Proc.*, **22**, 376 (1963).

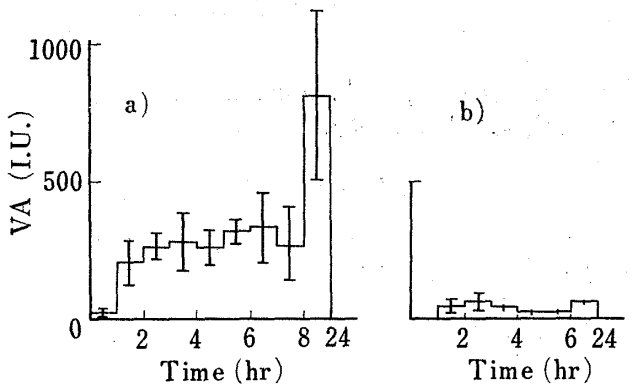

Fig. 4. Appearance of VA in Lymph for 24 hr following the Administration in Oleic Acid Solution Form or Paraffin Solution Form

a) Oleic acid solution *b*) Paraffin solution
Results are mean of three rats.
Vertical bars are S.E. of means.

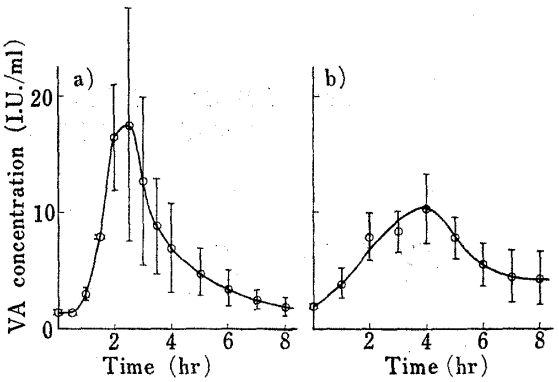

Fig. 5. Appearance of VA in Serum for 8 hr following the Administration in Micellar Solution Form or LCT Oily Solution Form

a) Micellar solution *b*) LCT oily solution
Each point is the mean of three rats.
Vertical bars are S.E. of means.

absorption of VA from preparations except for micellar solution form might be due to the slow gastric emptying.

These data are compared with those by Inoue and others[7] who studied lymphatic transport of Sudan Blue (fat soluble dye) in various dosage forms (Table I). When Sudan Blue was given in oily solution form, the percentage of recovery of Sudan Blue varied with the carbon chain lengths of triglycerides which were used for vehicle. This phenomenon was in agreement with that of VA. In the case of emulsified preparation, 10.5% of Sudan Blue was recovered from LCT emulsion while 12.5% was recovered from oleic acid and monoolein emulsion. These high percentage of recoveries showed that Sudan Blue could be absorbed *via* absorbing route of LCT, namely being carried by the products of LCT decomposed in intestinal lumen. In the case of SCT oily solution, Sudan Blue was scarcely absorbed because of lack of vehicle which carried Sudan Blue. On the other hand, 6.6% of VA was recovered in lymph even in the case of SCT oily solution. This high absorbability might show the existence of absorbing route of VA solely.

Fig. 5 shows the time course of the absorption of VA into blood serum. When VA was given in micellar solution form, there was a sharp peak of absorbed VA at 2 hr after administration. The concentration of VA in this peak was 20 I.U./ml and less than 1% of that in lymph. On the contrary, when VA was given in LCT oily solution form, VA was absorbed more slowly and with lower peak than when VA was given in micellar solution form, although there was no significant difference in VA concentration in each peak between micellar and oily solution. These tendencies in serum coincided with those in lymph, but the very high peak of VA in lymph after dosing in micellar solution form was less remarkable in serum. For the reason, it could be suspected that the peak in lymph was too momentary to detect by these sampling intervals of blood and/or VA in lymph was diluted by blood when lymph fluid flew into blood at vena subclavia and transported to the liver to be stored immediately.

In conclusion, the presented results suggest that VA palmitate was most effectively absorbed into thoracic duct after the administration in micellar solution form, and the time course of VA concentration in serum might not be correct indication of bioavailability.

7) A. Inoue, T. Fuwa, S. Awazu, M. Hanano, and H. Nogami, Abstract of Papers, The 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, May, 1972, Part IV, p. 68.