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Transport of Organic Compounds through Biological Membranes. II.[1] Red Cell Permeability to O-Acyl-S-benzoylthiamines[2]

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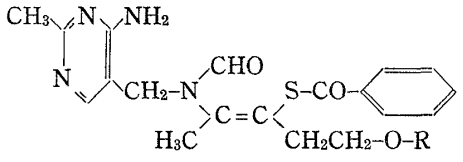
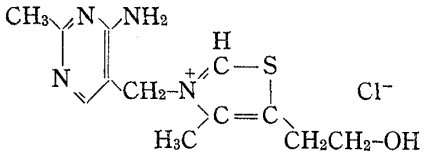
The cell permeability was compared for a series of O-acyl-S-benzoylthiamine in human red cell suspensions. On varying the length of straight chain alkyl group in the acyl part, it was found that i) at the earliest stage of the transport, the red cells showed a rapid and high uptake of the molecules due to the adsorption at the cell membranes, and ii) an equilibrium state was reached within 120 minutes of incubation and the intracellular concentration of total thiamine was in the following order: acetate<propionate<butyrate>valerate>caproate>capryrate, showing a maximum intracellular accumulation (the cell to medium concentration ratio of 2.9) with 4 carbon acyl group. In order to elucidate the mechanism, the followings were compared for the series of compounds: i) the partition coefficient from aqueous to organic phases, ii) the rate of debenzoylation in the cells and at the membrane site, and iii) the rate of conversion of the corresponding O-acylthiamine to thiamine in the cells and at the membrane site. The results indicated that the hydrolytic enzyme systems associated with the cell membrane (acetylcholinesterase) and the cytoplasm (carboxylesterase) play the most important role in determining the intracellular accumulation rather than the lipid solubility of the compound and that the butyrate with the highest rate of conversion to thiamine in the cells results in the highest intracellular accumulation.

In the preceding paper,[1] an accumulative uptake of S-benzoylthiamine (SBT, I) by human red cells was demonstrated and the accumulation was found to be caused from a rapid conversion of SBT to undiffusible thiamine (III) in the cells. It was also found from a comparative study on a series of substituted SBT with a various substituent on the phenyl ring that the extent of accumulation depends mainly on the rate of conversion to thiamine in the cells.

An introduction of O-acyl group in SBT is expected to increase the lipid solubility of the molecule markedly, to a greater extent with increasing the chain length of the acyl group, while not to affect significantly the strength of the S-CO bond electronically. Thus, in order to study how a large increase in the lipophilic character of the molecule can affect the cell permeability and the intracellular accumulation, *in vitro* uptake by human red cells was compared for a series of O-acyl SBT (II) with an acyl group of various length. The results indicated that the hydrolytic enzyme systems associated with the cell membrane and with the



I : R=H
II : R=$-CO(CH_2)_nCH_3$
n=0—6



III

1) Part I: H. Shindo, K. Okamoto and J. Totsu, *Chem. Pharm. Bull.* (Tokyo), **15**, 295 (1967).
2) This work was presented at the 5th Annual Meeting of Biophysical Society of Japan, December 1966 and at the Symposium on the Cell Permeability to Vitamins, Japan Vitamin Society, April 1967. *a*) Abstract: H. Shindo, *Vitamins* (Kyoto), **35**, 409 (1967).
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cytoplasm play the most important role in determining the intracellular accumulation, rather than the lipid solubility of the molecule. In the series of compounds investigated, O-butyryl SBT, the compound with 4-carbon acyl group, showed a maximum accumulation in the cells due to its highest rate of conversion to thiamine in the cells.

Experimental

Human red cells were obtained from blood stored for less than two weeks at 4° in acid–citrate–dextrose solution. Whole blood was centrifuged, the plasma and buffy layer were removed, and the obtained packed red cells were washed three times with isotonic NaCl-phosphate buffer of pH 7.4.

Transport experiments were performed in essentially the same way as described in the preceding paper.[1] The packed red cells were suspended to approximately 40% in the isotonic phosphate buffer containing a given amount of a substrate and the suspension was incubated at 37° under a constant shaking. At an appropriate interval, 5 ml of the suspension was pipetted out, cooled and centrifuged at 4°. Two 1 ml aliquots of the supernatant were pipetted out carefully and the remaining 3 ml of the cellular portion was mixed thoroughly. Two 1 ml aliquots of the cellular portion were pipetted out and the remaining was determined for the Hematocrit value. Each of these 1 ml aliquots was assayed for total thiamine and "free thiamine," respectively.

For determining total thiamine, the sample was heated at 60° for 1 hour with 1 ml of 5% cysteine prior to deproteinization.[4] The mixture was further hemolysed with 2 ml N/5 hydrochloric acid and deproteinized with 3 ml of 15% trichloroacetic acid. The mixture was centrifuged and the precipitates were again extracted with 5 ml of 5% trichloroacetic acid. The combined extract was assayed for total thiamine. For determining "free thiamine," which includes thiamine and O-acylthiamine, 1 ml sample was hemolysed with N/5 hydrochloric acid and deproteinized in the same way as above. The extract was assayed for "free thiamine" and, in some cases, separately for thiamine and O-acylthiamine.

Thiamine was fluorometrically determined by the thiochrome method.[5] Sample was oxidized with bromocyanide at pH 4.5 and the solution was made strongly alkaline with sodium hydroxide to afford thiochrome, which was extracted with *n*-butanol. Thiamine and O-acylthiamine were separately determined by so-called acyl-thiochrome method.[6] The principle is that the oxidized mixture was made weakly alkaline with ammonium hydroxide to form O-acylthiochrome which can be selectively extracted by ethylacetate. Total thiamine was determined by the thiochrome method after an incubation of sample with Takadiastase at 37° at pH 5.6 overnight.

The concentration in the cell (C_c) was obtained from those in the medium (C_m) and in the cellular portion (C_c') and the Hematocrit value of the cellular portion (Ht), according to the following equation.

$$C_c = \frac{C_c' - \left(1 - \frac{Ht}{100}\right) C_m}{\frac{Ht}{100}}$$

The permeability was expressed by the rate constant in the initial rate of penetration, assuming a first order equation for the transport, or in terms of the cell to medium concentration ratio at an equilibrium state or after a constant incubation period.

Hemoglobin–free red cell ghosts were isolated according to the method of Dodge, *et al.*[7] It was confirmed by electron microscopy that the ghosts thus obtained are mostly pure membrane fraction and being retained the unit–membrane structure, after staining with potassium permanganate. The ghosts were suspended in the isotonic NaCl-phosphate buffer to a concentration of 40% with respect to the original packed red cells.

The red cell homogenates were prepared by sonification of the packed cells, applying 25 kc/sec for 10 minutes at 0°. The red cell hemolysates were prepared by hemolysing the packed cells in 5 volumes of distilled water at 4° for 30 minutes under stirring. The mixture was then centrifuged with 28000 rpm for 2 hours at 4° and the supernatant was used.

The organic solvent/water partition coefficients were calculated from the distribution of the compound after shaking a solution in the phosphate buffer of pH 7.4 with ethylacetate at 37°.

4) Because of adsorption of O-acyl SBT molecules at the cell membranes, reduction of S-benzoyl group with cysteine was necessary prior to deproteinization. With this procedure, a recovery of total thiamine from the incubation mixture exceeded over 95%.
5) M. Fujiwara and K. Matsui, *Anal. Chem.*, **25**, 810 (1953).
6) K. Kohno, I. Saito and I. Utsumi, *Vitamins* (Kyoto), **33**, 334 (1966).
7) J.T. Dodge, C. Mitchell and D.J. Hanahan, *Arch. Biochem. Biophys.*, **119**, 100 (1963).

All of O-acyl SBT and O-acylthiamine used in this investigation were synthesized in this laboratories, by a reaction of the corresponding acid chloride with SBT and thiamine, respectively. Other chemicals were of reagent grade and used without further purification.

Results and Discussion

Uptake and Accumulation of O-Acyl-SBT by Human Red Cells

Time courses of the transport of O-acyl-SBT with a various acyl group into human red cells are shown in Table I and the behaviors of those with a straight chain alkyl group are compared in Fig. 1. It was found generally that O-acyl-SBT molecules easily penetrate into the cells, but their behaviors to achieve an equilibrium state are significantly different dep-

Table I. Transport of O-Acyl-SBT into Human Red Cells in the Suspension

Compound No.	Acyl group	Total thiamine, C_{cell}/C_{medium}			
		10 min	30 min	1 hr	2 hr
1	$-CO-CH_3$	1.31	1.30	1.02	1.09
2	$-CO-CH_2CH_3$	1.44	1.26	1.31	1.37
3	$-CO-(CH_2)_2CH_3$	1.76	1.89	2.29	2.90
4	$-CO-(CH_2)_3CH_3$	1.87	1.56	1.89	2.31
5	$-CO-(CH_2)_4CH_3$	2.47	1.74	1.60	2.08
6	$-CO-(CH_2)_6CH_3$	5.90	2.80	1.80	1.36
7	$-CO-(CH_2)_8CH_3$	16.08	5.15	2.95	1.83
8	$-CO-CH_2CH(CH_3)_2$	1.50	1.44	1.24	1.07
9	$-CO-C(CH_3)_3$	1.39	1.08	0.94	0.86
10	$-CO-CH_2OCOCH_3$	1.76	1.75	1.83	2.24
11	$-CO-CH_2N(CH_3)_2$	1.39	1.77	1.85	1.72

The red cell suspensions (*ca.* 40%) containing 0.3 mM/liter substrate were incubated at 37° at pH 7.4. Values represent the cell to medium concentration ratio of total thiamine after a given time of incubation.

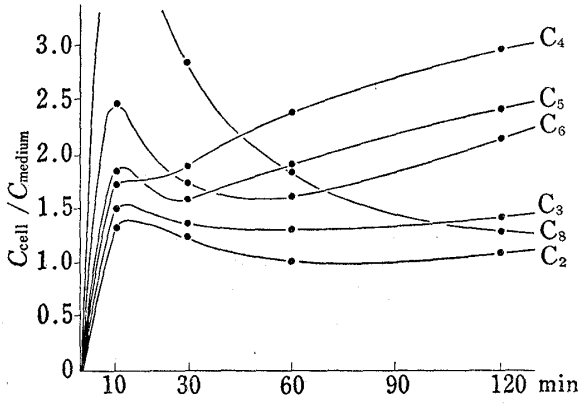

Fig. 1. Time Course of the Transport of O-Acyl-SBT with a Straight Chain Acyl Group into Red Cells

C_2: acetate, C_3: propionate, C_4: butyrate, C_5: valerate, C_6: caproate, C_8: capryrate
ca. 40% human red cell suspensions (pH, 7.4) containing 0.3 mM/liter substrate were incubated at 37°. After a given time, the suspension was cooled, centrifuged and the concentrations of total thiamine in the cells and the medium were determined without washing the cells.

ending upon the length of alkyl group in the acyl part. The following two characteristics are found: i) at the earliest stage of the transport, within the first ten minutes of incubation, the red cells showed a very rapid and high uptake, the extent being increased with increasing the chain length of the acyl group, and ii) after continuing the incubation for about 120 minutes, an equilibrium state was reached and the intracellular concentration of total thiamine was in the following order: acetate<propionate<butyrate>valerate >caproate>capryrate, showing a maximum with 4-carbon acyl group.

A rapid cellular uptake observed at the earliest stage of incubation is considered to be due to an adsorption of O-acyl SBT molecules at the cell membrane, since Hemoglobin-free red cell ghosts showed a similar rapid uptake of the compounds after a short period of incubation. In Table II, the relative rate constants were obtained from the amount of uptake by the red cells and the ghosts in

the first 5 minutes of incubation, assuming a first order reaction for the adsorption process. It is evident from the table that for both the intact cells and the ghosts the rate of adsorption was increased with increasing the chain length of the acyl group involved, and thus with increasing the partition coefficient of the molecule from aqueous to organic phases.

Table II. Partition Coefficient and the Relative Rate of Adsorption of O-Acyl-SBT

R in –O–CO–R	Partition coeff. (EtOAc/buffer)	Rate of adsorption ($k \times 10^2$/min) Red cell	Ghosts
CH_3	28.84	5.156	0.896
CH_2CH_3	55.81	6.128	0.696
$(CH_2)_2CH_3$	137.53	6.552	1.424
$(CH_2)_3CH_3$	205.29	7.056	2.415
$(CH_2)_4CH_3$	216.00	8.309	8.479
$(CH_2)_6CH_3$	228.29	13.970	25.854
$(CH_2)_8CH_3$	274.36	22.470	—

After incubation of 40% or equivalent suspension containing 0.3 mM/liter substrate for 5 minutes at 37°, the suspension was cooled and centrifuged. Apparent k was calculated from a decrease of the concentration in the medium (n_0) according to $k=2.3/5 \log 0.3/0.3-n_0$.

As described in the experimental part, deproteinization of the incubation mixture, in particular of that after a short period of incubation, with trichloroacetic acid gave only a very low recovery of total thiamine. The cellular uptake determined in this way for O-caproyl-SBT is shown as curve 1 in Fig. 2. When the precipitates after extraction with trichloroacetic acid were warmed with an excess of cysteine to reduce the S-benzoyl group some additional thiamine was found to be released (curve 2) and when the incubation mixture was pretreated with cysteine prior to the deproteinization the extract gave a total recovery over 95% (curve 3). Thus, the difference between curve 3 and 1 in Fig. 2 can be regarded as the amount of O-caproyl SBT adsorbed.

After the first ten minutes of incubation, both a release of molecules once adsorbed, probably on the cell membrane, back into the medium and an intracellular accumulation appears to occur simultaneously (Fig. 1) and after 120 minutes of incubation O-butyryl SBT showed a maximum intracellular accumulation, the cell to medium concentration ratio being 2.9.[8] This value is essentially the same to that observed for SBT itself[1] and it can be pointed out here that an increase of the lipid solubility of molecule does not increase the extent of the intracellular accumulation, but all O-acyl SBT except O-butyryl SBT seems to result in a lower intracellular accumulation than SBT.

In Fig. 3, the cell to medium concentration ratio of total thiamine (A) and the con-

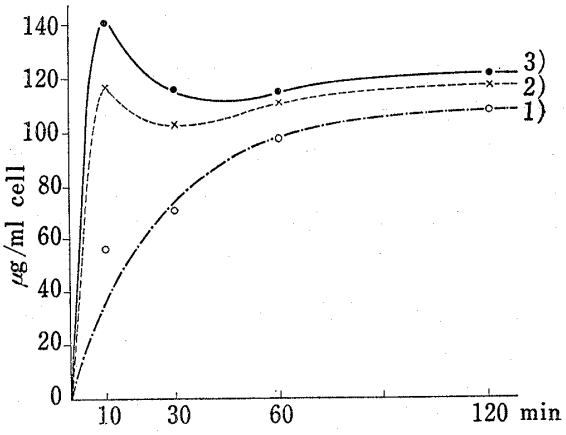

Fig. 2. Adsorption and Transport of O-Caproyl-SBT in Red Cell Suspension

ca. 40% human red cell suspension containing 0.3 mM/liter O-caproyl SBT was incubated at 37°. Total thiamine in the cells was determined after 1) deproteinization with trichloroacetic acid (———O———), 2) 1) and the precipitates were warmed with cysteine (--------×--------) and 3) warming with cysteine prior to the deproteinization (———●———).

8) The ratio is 3.9 if the water content of the red cells is assumed to be 72% (w/v).

centration of "free thiamine" in the cells and the medium (B) after 10 and 120 minutes of incubation are plotted against the carbon number of the acyl group. It was found that the concentration of "free thiamine" in the cells, which includes thiamine and/or O-acylthiamine, showed a sharp maximum with the butyrate and the lowest value with the acetate after 120 minutes. It was also noted that the concentration of "free thiamine" in the medium was highest for the acetate, while lowest for the butyrate. It is therefore suggested that such an intracellular accumulation must be connected in some way to a conversion of O-acyl SBT to a less permeable O-acylthiamine and/or thiamine in the cells.

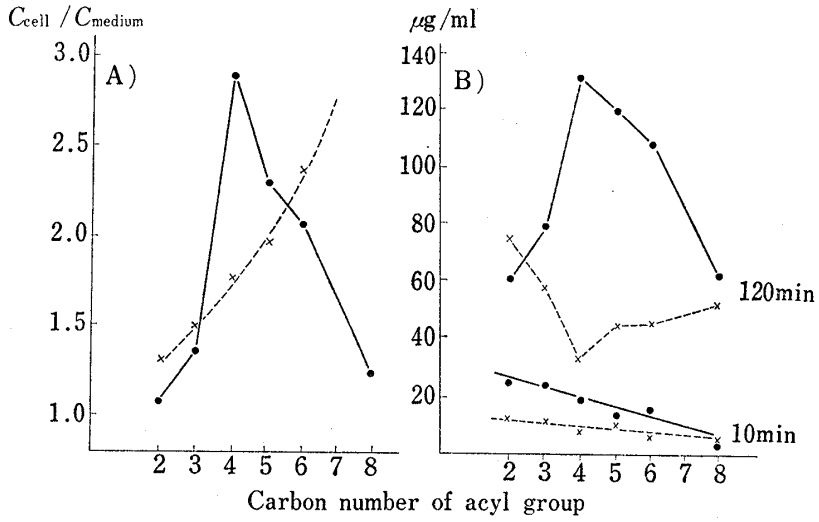

Fig. 3. Relations between Carbon Number of the Straight Chain Acyl Group in O-Acyl-SBT and (A) the Red Cell Uptake and (B) the Conversion to Free Thiamine

ca. 40% red cell suspensions containing 0.3 mM/liter substrate were incubated at 37°. A: The cell to medium concentration ratio of total thiamine after 10 min (------×------) and 120 min (——●——) of incubation was plotted. B: The concentration of "free thiamine" in the cell (——●——) and in the medium (------×------) after 10 and 120 minutes was plotted.

In Table I, some acyl derivatives with other than straight chain alkyl group are involved. Branching of the alkyl chain seems to decrease the extent of accumulation significantly, as revealed from a comparison of the valerate (compound **4**) with compound 8 and 9. The acetoxyacetate (compound **10**) showed a similar intracellular accumulation to that of the valerate. Dimethylaminoacetate (compound **11**), a nitrogen analogue of the iso-valerate (compound **8**), showed a higher accumulation in the cells as compared to the iso-valerate.

Decomposition of S-Benzoyl Group in the Cells

In order to study whether the rate of debenzoylation of O-acyl SBT in the cells can be a determining factor for the intracellular accumulation, the rate of debenzoylation was compared for a series of O-acyl SBT in suspensions of the red cells, the cell homogenates and the cell membranes and in the cell hemolysate, under an equivalent concentration with respect to that of the original packed cells. The formation of "free thiamine" in these systems was found to follow a first order reaction kinetics and the apparent rate constants were determined by the least square method.

The results revealed that, as shown in Table III, i) the rates of debenzoylation in the cell suspension are essentially the same to those in the cell homogenate, indicating that the rate of transport of O-acyl SBT through the membrane into the cells is rapid enough for all the compounds as compared to the rate of debenzoylation in the cells, ii) the rate of debenzoylation with the cell membrane is extremely low, indicating that the debenzoylating activity of

the cell membrane is almost negligible as compared to that in the cells, iii) in consistent with the above findings, the rates of debenzoylation in the cell hemolysate are essentially the same to those in the cell homogenate, and iv) in all systems other than the cell membrane, the rate of debenzoylation decreases with increasing the chain length of the acyl group, which might presumably be ascribed to a steric hindrance of an acyl group.

From these results, it might be concluded that as far as the steps of the membrane transport of O-acyl SBT molecules and of their debenzoylation in the cells are concerned, there is no factor which is responsible for the maximum intracellular accumulation of the compound with 4-carbon acyl group.

Table III. Rate of Decomposition of S-Benzoyl Group in O-Acyl-SBT

R in –O–CO–R	Rate of debenzoylation ($k \times 10^2$/min)			
	Cell susp.	Cell homog.	Cell hemolys.	Cell membr.
CH_3	1.420	1.430	1.216	0.091
CH_2CH_3	1.222	1.298	1.145	0.063
$(CH_2)_2CH_3$	1.134	1.157	1.170	0.063
$(CH_2)_3CH_3$	0.787	0.950	1.119	0.054
$(CH_2)_4CH_3$	0.688	0.775	0.961	0.078
$(CH_2)_6CH_3$	0.279	0.439	—	0.082

The suspensions of 40% or equivalent concentration containing 0.3 mM/liter substrate were incubated at 37° for 1, 3, 5, 7 and 10 min and free thiamine was determined. The amount of "free thiamine" produced per minute (n_m) was obtained by the least square method and the apparent k was calculated accoridng to $k=2.3 \log 0.3/0.3-n_m$.

Transport of O-Acylthiamine into Red Cells

From the foregoing results, it might be deduced that only remaining factor which could contribute for the maximum accumulation of O-butyryl SBT is the step of a conversion of O-acylthiamine to thiamine in the cells. Thus the transport of a series of O-acylthiamine with a straight chain alkyl group in the acyl part was investigated in the red cell suspensions. The results, as shown in Fig. 4, indicated that thiamine acetate and propionate do not penetrate the cell to any appreciable extent, while only the butyrate showed a high accumulation in the cells. In the case of O-acylthiamine, no adsorption phenomenon was observed.

By a separative determination of thiamine from O-acylthiamine, it was found that O-butyrylthiamine was very rapidly converted to thiamine in the cells, while O-acetylthiamine was converted to thiamine very rapidly in the suspending medium, as shown in Fig. 5-A, where percentage of thiamine formed from O-acylthiamine in the cells and the medium after the first 3 minutes of incubation was plotted against the carbon number of acyl group.

The rates of conversion of O-acylthiamine to thiamine were therefore compared in the red cell hemolysate and in the suspension of the ghost membrane. The results, as shown in Fig. 5-B, indicated that the rate of decomposition in the cell hemolysate was in the following order: butyrate>valerate>caproate≫acetate∼propionate, while that with the cell membrane the order was the following: acetate>propionate≫butyrate∼valerate.

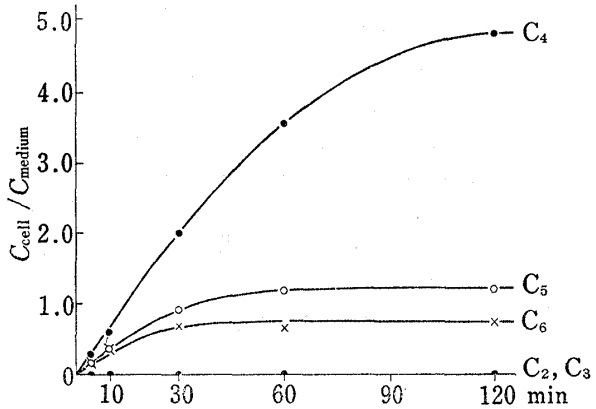

Fig. 4. Transport of O-Acylthiamine into Red Cells

C_2: acetate, C_3: propionate, C_4: butyrate, C_5: valerate, C_6: caproate

ca. 40% human red cell suspensions (pH, 7.4) containing 0.3 mM/liter substrate were incubated at 37°. Concentrations of total thiamine in the cell and the medium were determined.

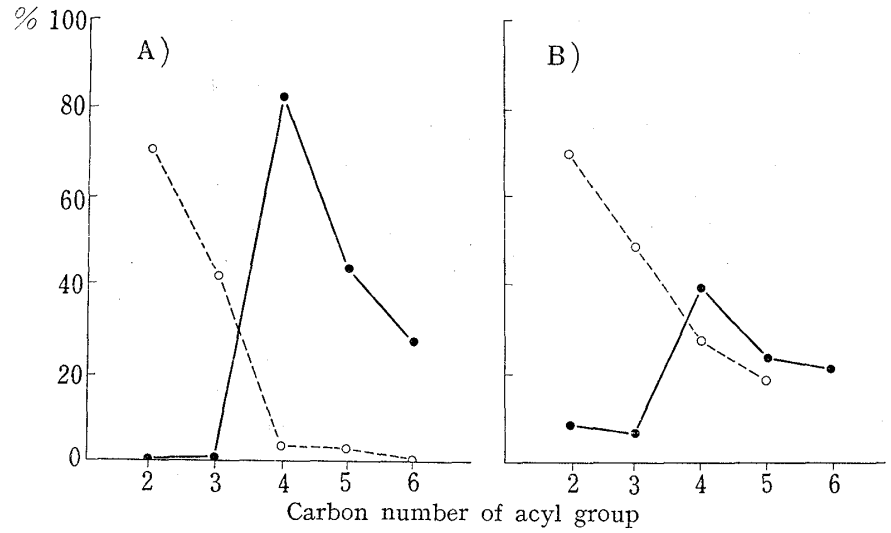

Fig. 5. Conversion of O-Acylthiamine to Thiamine in (A) the Red Cell Suspension and (B) the Ghost Suspension and the Cell Hemolysate

A): *ca.* 40% human red cell suspensions containing 0.3 mM/liter substrate were incubated at 37° for 3 minutes. Concentration of thiamine was determined in the cell (——●——) and the medium (------O------). B): Suspensions of the ghosts membranes, *ca.* 40% equivalent to the packed cell, (------O------) and solutions of the cell hemolysate, *ca.* 10% equivalent to the packed cell, (——●——), both containing 0.3 mM/liter substrate, were incubated at 37° for 60 and 10 minutes, respectively, and the formation of thiamine was determined.

From these results, the observed differences among the red cell uptake of O-acylthiamine can be explained in the following way.[9] O-Acetyl and O-propionylthiamines are deacylated very rapidly on the surface of, or in, the cell membrane before entering into the cells and thus afforded thiamine cannot penetrate the cells appreciably, while O-butyrylthiamine is not easily deacylated at the cell membrane and the molecules penetrated in the cells are rapidly deacylated and thus afforded thiamine is accumulated in the cells in a high concentration. For O-valeryl and O-caproylthiamines, although the rate of transport through the membrane is expected to be larger than that of the butyrate because of their higher lipid solubility, the rate of conversion to thiamine in the cells is smaller than that of the butyrate and no significant accumulation might occur in the cells. In the case of O-caproylthiamine, in fact, the cell to medium concentration ratio of total thiamine approached to 1.0 (Fig. 4).

The membrane acetylcholinesterase in red cells has been known to hydrolyse also noncholin esters and to have a sharp optimum with O-acetate and the activity falls to almost zero with O-butyrate.[10] Since it has been shown by electron microscopy[11] that acetylcholinesterase locates at the outer layer of the red cell membrane, it is plausible that O-acetyl and O-propionylthiamines are deacylated rapidly in the medium in contact with the cell surface. On the other hand, the conversion to thiamine in the cells is considered to be due to the action of carboxylesterase[12] (so-called aliesterase) which has been known to locate in the endoplasm of red cells.[13] The enzyme is known, generally, to have an optimum with 4 to 6 carbon chain of the acyl group depending upon the length of the alkyl part[12] and it is quite possible that the erythrocyte carboxylesterase has a sharp optimum with O-butyrylthiamine.[9]

9) The same considerations have been presented by Utsumi, *et al.*, independently. I. Utsumi, K. Kohno, I. Saito and M. Mizobe, *Vitamins* (Kyoto), 37, 250 (1968).
10) D.H. Adams and V.P. Whittaker, *Biochim. Biophys. Acta*, 3, 358 (1949).
11) Y. Shinagawa and M. Ogura, *Kagaku* (Tokyo), 31, 554 (1961).
12) M. Dixon and E.C. Webb, "Enzymes," 2nd Ed., Longmans, London, 1964, p. 218.
13) C. Huggins and J. Lapides, *J. Biol. Chem.*, **170**, 467 (1947); K. Irino, *Seikagaku* (Tokyo), **30**, 603 (1958).

Under a presence of 10^{-3}M difluoro-diisopropyl phosphate, which inhibits both acetylcholinesterase and carboxylesterase, it was found that O-acetyl and O-propionylthiamines penetrate into red cells appreciably and that O-butyrylthiamine does not show any intracellular accumulation. The cell to medium concentration ratio after the first ten minutes of incubation was in the following order: acetate, 0.10<propionate, 0.19<butyrate, 0.36<valerate, 0.47, indicating that the initial rate of the transport of O-acylthiamine molecules is increased with increasing the chain length of the acyl group.

Mechanism of the Highest Intracellular Accumulation of O-Butyryl-SBT

From the foregoing results, it became clear that i) for all O-acyl SBT investigated, the rate of transport into red cells is rapid enough as compared to the rate of decomposition of S-benzoyl group in the cells, ii) the rate of debenzoylation of O-acyl SBT in the cells is decreased slightly with increasing the chain length of the acyl group, iii) thiamine does not penetrate the cell membrane appreciably,[1)] while O-acylthiamine does rather easily, iv) O-acetyl and, to a less extent, O-propionylthiamine are rapidly deacylated at the outer layer of the cell membrane. Therefore, it can be considered that the main reason which is responsible to the highest accumulation of O-butyryl SBT in the cells must be the highest rate of conversion of O-butyrylthiamine to thiamine in the cells.

Since the debenzoylation reaction of O-acyl SBT in the cells might be ascribed mainly to a non-enzymatic reduction with intracellular SH group, most probably of glutathione,[1,14)] the transport experiment was carried out under an inhibition of esterase with 10^{-3}M difluoro-diisopropyl phosphate. The results (Fig. 6) revealed that after 120 minutes of incubation all O-acyl SBT with 2, 4 and 5 carbon acyl group showed no intracellular accumulation and approached the cell to medium concentration ratio of about 1.0. This can be interpreted as indicating that when the conversion of O-acylthiamine to thiamine is inhibited all O-acyl SBT reaches a final equilibrium state with the cell to medium concentration ratio of 1.0 with respect to O-acylthiamine molecules. At the first stage of incubation, a high uptake which can be ascribed to the adsorption of O-acyl SBT at the cell membrane was clearly observed, the amount being increased with increasing the chain length.

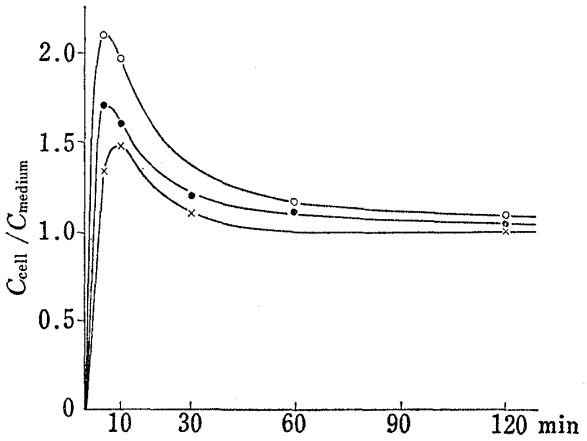

Fig. 6. Transport of O-Acyl-SBT into Red Cells under DFP Inhibition

—×—: acetate, —●—: butyrate, —○—: caproate
ca. 40% human red cell suspensions containing 0.3 mM/liter substrate and 10^{-3}M/liter difluoro-diisopropyl phosphate were incubated at 37°.

It was confirmed by a separate experiment that O-acyl SBT cannot be deacylated by the membrane acetylcholinesterase to any significant extent, presumably because of the steric effect of S-benzoyl group. Therefore, in summarizing, the mechanism of the highest intracellular accumulation of O-butyryl SBT can be explained in the following way. Both O-acetyl and O-butyryl SBT may enter the red cells very easily and may be reduced to the corresponding O-acylthiamine rapidly. However, O-acetylthiamine is not easily deacylated in the cells, while the outflux of the O-acetylthiamine molecules may be accompanied with a very rapid conversion to thiamine at the outer layer of the cell membrane. This conversion may be assumed to accelerate the outflux of O-acetylthiamine, resulting in a high concentration of thiamine in the suspending medium. On the other hand, O-butyrylthiamine is rapidly deacylated in the cells, while

14) H. Shindo, K. Okamoto, J. Totsu and I. Takahashi, *Vitamins*, (Kyoto), **38**, 21 (1968).

the conversion to thiamine at the outer layer of the membrane is very low, resulting in an intracellular high accumulation of thiamine. For the compounds with an acyl group longer than 5 carbon chain, the extent of the intracellular accumulation may depend on the easiness of conversion of the corresponding O-acylthiamine to thiamine in the cells.

The present investigation provides an example which indicates that generally the cell uptake of an organic molecule can be affected not only by the physico-chemical nature of the molecule such as partition coefficient, but more significantly by enzymatic degradations of the side chain in the cells and at the membrane site. It seems interesting, furthermore, to note that the molecular modification can affect the cell permeability of the molecule very significantly in such a way that one derivative does not penetrate the cell at all, the other results in a high intracellular accumulation.

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