FIGURE LEGEND

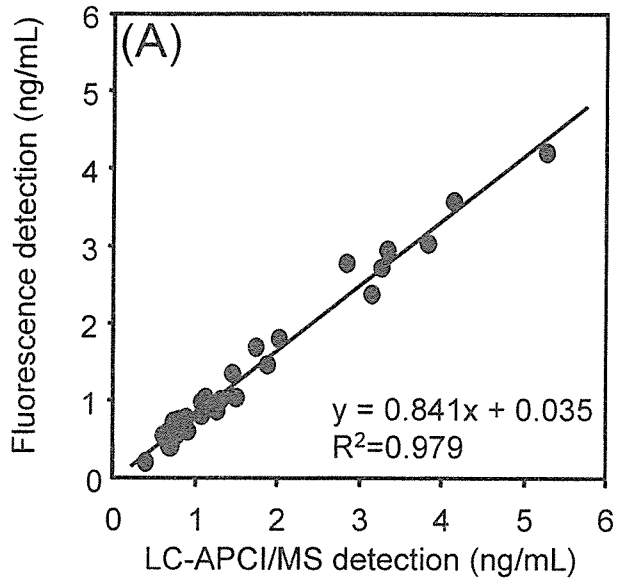
Figure 1. Correlation between the values obtained by HPLC with fluorescence detection using internal standards and those obtained by LC-APCI/MS. (A) PK; (B) MK-4; (C) MK-7.

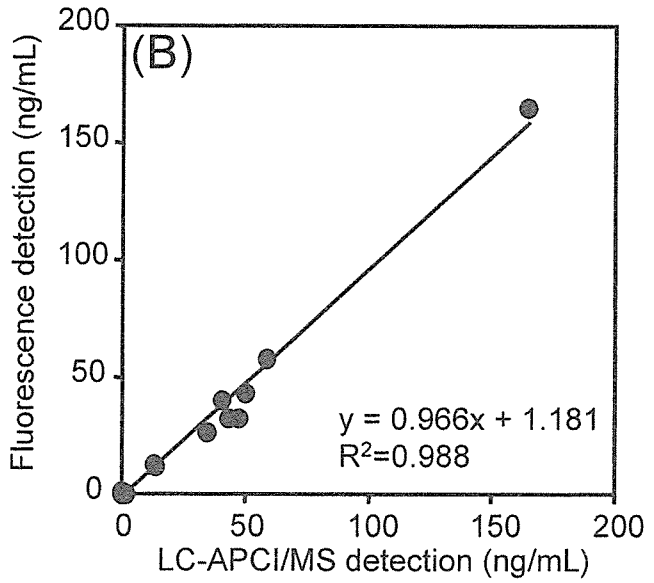
Table 1. Concentrations of fat-soluble vitamins in human milk and estimated infant's intake

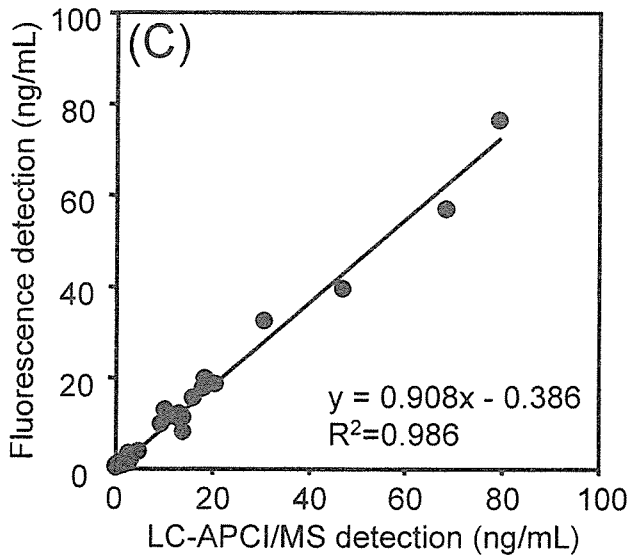
Vitamin	Compound	Concentration in human milk[1]	Estimated infant's intake[2]
A	all-*trans*-retinol	0.39 ± 0.14 (μg/mL)	335 μg RE/day[3]
	β-carotene	0.05 ± 0.04 (μg/mL)	
D	vitamin D_3	0.10 ± 0.15 (ng/mL)	0.47 μg/day[4]
	vitamin D_2	0.09 ± 0.19 (ng/mL)	
	25(OH)D_3	0.08 ± 0.04 (ng/mL)	
	25(OH)D_2	0.003 ± 0.002 (ng/mL)	
E	α-tocopherol	3.96 ± 1.84 (μg/mL)	3.09 mg/day
K	PK	3.56 ± 2.19 (ng/mL)	4.79 μg/day[5]
	MK-4	1.77 ± 0.68 (ng/mL)	
	MK-7	1.19 ± 1.54 (ng/mL)	

[1] Values are the means ± S.D., n=51.
[2] The product of the concentrations of fat-soluble vitamins in human milk and infant's consumption of human milk (780 mL/day).
[3] The sum of all-*trans*-retinol and β-carotene expressed as retinol equivalent (RE) value.
[4] The sum of vitamin D and vitamin D equivalent 25(OH)D [25(OH)D x 5, vitamin D conversion factor of 25(OH)D=5].
[5] The sum of PK, MK-4 and MK-4 equivalent MK-7 (MK-7 content x 444.7/649).

Fig. 1


(A)
Fluorescence detection (ng/mL)
LC-APCI/MS detection (ng/mL)
y = 0.841x + 0.035
R²=0.979
0
1
2
3
4
5
6


(B)
Fluorescence detection (ng/mL)
LC-APCI/MS detection (ng/mL)
y = 0.966x + 1.181
R²=0.988
0
50
100
150
200


(C)
Fluorescence detection (ng/mL)
LC-APCI/MS detection (ng/mL)
y = 0.908x - 0.386
R²=0.986
0
20
40
60
80
100

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Note

Increase in *S*-Adenosylhomocysteine Content and Its Effect on the *S*-Adenosylhomocysteine Hydrolase Activity under Transient High Plasma Homocysteine Levels in Rats

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Summary The objective of this study was to examine how transient high plasma homocysteine (Hcy) levels affect the metabolism of Hcy, the activity and expression of *S*-adenosylhomocysteine (SAH) hydrolase which catalyzes both SAH hydrolysis and SAH synthesis. Wistar ST rats (males) were cannulated in the right jugular vein for intravenous infusion of physiological saline or DL-Hcy solutions (15 and 30 mg/mL) for 1 h at 1.1 mL/h/rat. The content of *S*-adenosylmethionine (SAM), SAH-synthetic activity of SAH hydrolase and the expression of SAH hydrolase mRNA in liver extracts showed no significant difference in the Hcy infused groups as compared to the Control group. On the other hand, the contents of hepatic SAH in the Hcy infused groups were dose-dependent and significantly higher than that of the Control group. Thus, this study showed that hepatic SAH increased without any increase in the SAH-synthetic activity and the expression of SAH hydrolase mRNA under transient high plasma Hcy levels after intravenous infusion of Hcy.

Key Words plasma homocysteine, *S*-adenosylhomocysteine, *S*-adenosylhomocysteine hydrolase, methionine metabolism

Homocysteine (Hcy) is an intermediate of the methionine metabolic pathway, and it occupies a branch point of two metabolic pathways, the remethylation pathway to methionine and the transsulfuration one to cysteine. These two pathways are coordinated by *S*-adenosylmethionine (SAM), which acts as an allosteric inhibitor of the methylentetrahydrofolate reductase (EC 1.1.1.68) and as an activator of cystathionine β-synthase (EC 4.2.1.22; CBS) (*1*). SAM is converted to *S*-adenosylhomocysteine (SAH) by some transmethylation reactions, and then SAH is hydrolyzed by SAH hydrolase (EC 3.3.1.1), a reversible enzyme which catalyzes SAH-hydrolytic reaction to Hcy and adenosine, and SAH-synthetic reaction from Hcy and adenosine. In general, equilibrium of the SAH hydrolase reaction favors the direction of SAH synthesis, while the SAH-hydrolytic reaction predominates under physiological conditions. Predominant SAH hydrolysis is maintained by rapid removal of Hcy. However, if it is difficult for sufficient Hcy to be removed from this cycle due to CBS deficiency or inhibition of methionine synthase, it means that the methionine cycle can not maintain its balance. The balance of the methionine cycle is maintained intricately by methionine metabolites. Therefore disturbed methionine metabolism induces several diseases, e.g. hyperhomocysteinemia.

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An increase of plasma Hcy is caused by nutritional deficiencies or genetic mutations. Vitamin B_6 (B_6) deficiency (*2*, *3*) and folic acid deficiency (*4*) were reported as the factors affecting Hcy accumulation. Furthermore, the relationship between elevated plasma Hcy and accumulated SAH was observed in several studies using rats (*5*, *6*). Elevated plasma Hcy and accumulated SAH are likely to be caused by disturbed methionine metabolism. We reported in our previous study that the elevation of plasma Hcy and accumulation of hepatic SAH were observed in B_6-deficient rats (*7*). In B_6 deficiency, the transsulfuration pathway is suppressed due to the fact that B_6 is a coenzyme of CBS and γ-cystathionase. Therefore, abnormal methionine metabolism is caused by B_6 deficiency. Moreover, the increase in SAH-synthetic activity of SAH hydrolase was also observed in B_6 deficiency (*7*), the cause of which was not clarified. It was surmised that the elevation of Hcy, a substrate of SAH-synthetic reaction of SAH hydrolase, might induce the increase of SAH-synthetic activity of SAH hydrolase.

In this experiment, we examined the SAH content and the SAH-synthetic activity of SAH hydrolase in the liver of rats with transiently elevated plasma Hcy levels after intravenous infusion of Hcy.

Materials and Methods

Reagents. Somnopentyl® was purchased from

Schering-Plough Co. (Kenilworth, USA). SAH, SAM, adenosine and L-Hcy thiolactone were purchased from Sigma Chemicals Co. (St.Louis, USA). DL-Hcy was purchased from Nacalai Tesque Inc. (Kyoto, Japan). SBD-F (7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate) and 1-heptanesulfonic acid sodium salt were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methanol (HPLC grade) was purchased from Kanto Kagaku Co. (Tokyo, Japan).

Animal and diets. Male Wistar ST rats, from 8 to 10 wk old, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The rats had free access to MF chow diet and water during the experimental period. The rats were housed in a temperature-controlled room (23±1°C) with alternating 12 h cycle of light (light: 6:00 a.m. to 6:00 p.m.). The experiments were performed in accordance with the governmentally legislated guidelines in Japan on the proper use of laboratory animals (1980), and our experiments were approved by the ethical committee of the Faculty of Applied Biological Sciences in Gifu University.

Surgical operation. The right jugular vein was cannulated for intravenous infusion of physiological saline or Hcy solution, and the surgical method followed was of Kraegen et al. (*8*). After a 3 d recovery term from surgery, the rats were used for the following infusion study.

Homocysteine infusion. DL-Hcy was dissolved in physiological saline at the concentration of 15 (15-Hcy group) or 30 (30-Hcy group) mg/mL, and infused at a rate of 4 mL/h/kg body weight using a syringe pump (Model IVAC P3000, IMI Co., Ltd., Saitama, Japan) for 1 h. Transient high plasma Hcy levels were achieved by the same amount of Hcy infusion (*9*). The rats in the Control group were given physiological saline at the same infusion rate as the Hcy groups. Rats were randomly divided into three groups ($n=7$) before intravenous infusion of Hcy. The body weights (g) were 284±7,284±5, and 283±4 (means±SE) in the Control, 15-Hcy, and 30-Hcy groups, respectively.

Sample preparation. Under Somnopentyl® anesthesia, blood samples were drawn from the abdominal aorta with a heparinized syringe, and then rat livers were immediately excised and weighed. Plasma was obtained by centrifugation at 2,000×*g* at 4°C for 20 min. Plasma and liver were stored at −20°C until analyses.

Assay of plasma Hcy and cysteine. Hcy in plasma was measured by the method of Yamaguchi et al. (*10*).

Assay of SAM and SAH. SAM and SAH were measured by the method of She et al. (*11*).

Assay of hepatic SAH hydrolase. The SAH-synthetic activity of SAH hydrolase was measured using a modified method of She et al. (*11*). Liver was homogenized with 5 volumes of 0.25 M sucrose/3.3 mM $MgCl_2$/2 mM glutathione (reduced form)/50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 100,000×*g* (4°C) for 1 h. The supernatant was used for assay of SAH hydrolase.

For the assay of the SAH-synthetic activity of SAH hydrolase, the incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.3), 2 mM dithiothreitol, 5 mM L-Hcy thiolactone, 100 μM adenosine and 50 μM liver extract (1 mg protein/mL) in a total volume of 0.3 mL. After incubation at 37°C for 10 min, the reaction was stopped by adding 50 μL of 3 N perchloric acid. The content was mixed and then centrifuged at 2,000×*g* for 10 min. The supernatant was filtered through a Millipore membrane (0.45 μM) and applied to HPLC for analysis of SAH. SAH-synthetic activity of SAH hydrolase was estimated by formed SAH.

Extraction of total RNA. Total RNA in each homogenate was isolated by the acid guanidium-phenol-chloroform method, using TRIzol (Invitrogen Corporation, Carlsbad, CA, USA). RNA concentration was determined by absorption at 260 nm using HITACHI U-2001 spectrophotometer (Tokyo, Japan).

Assay for expression of SAH hydrolase mRNA. Expression of SAH hydrolase mRNA was determined by RT-PCR using a TaKaRa One Step RNA PCR Kit (Takara Bio Inc., Otsu, Japan). The RT-PCR partly followed the method of Ohmori et al. (*12*). The primer of SAH hydrolase used was as follows: the upstream primer sequence was 5′-AAGCTGCCATGGAGGGCTACGA-3′ and the downstream primer sequence was 5′-GATGGCAGCTGGAAGGTGAAGG-3′. For the primer of β-actin (used as an invariant control), β-actin RT-PCR Primer set (Toyobo Co., Ltd., Osaka, Japan) was used.

The samples were amplified by 30 PCR cycles, where each consisted of denaturation at 94°C for 60 s, annealing at 58°C for 60 s and extension at 72°C for 75 s. Each PCR product was resolved by electrophoresis on 1% agarose gel stained with ethidium bromide, and photographed under UV light. Band intensity was evaluated by the NIH image program, which was developed by U.S. National Institutes of Health.

Statistical analysis. The statistical difference among mean was estimated at $p<0.05$ according to ANOVA and Scheffe's test (Excel Statistics 2006 for Windows, Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results and Discussion

Hcy is an important intermediate of methionine metabolism because it occupies a branch point in the metabolism. Therefore its metabolites and activity of relevant enzyme would change under disturbed Hcy metabolism. Elevated plasma Hcy and the accumulation of SAH were observed in rats with disturbed methionine metabolism (*5*, *6*). This suggests that metabolic situations in which plasma Hcy is increased by intravenous infusion of Hcy is more likely to induce the accumulation of SAH.

In this study, intravenous infusion of Hcy was performed to make transient high plasma Hcy levels in rats. Plasma Hcy concentration after intravenous infusion of physiological saline or Hcy solution is shown in Fig. 1. The 15-Hcy and 30-Hcy groups showed significant increase in plasma Hcy concentrations. Plasma Hcy concentrations in the 15-Hcy and 30-Hcy groups were approximately 46 times and 73 times higher as

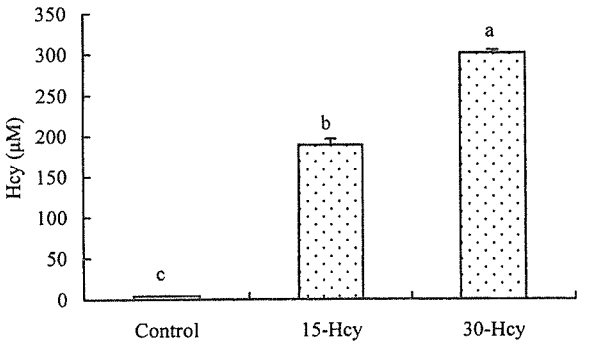

Fig. 1. Concentration of homocysteine in plasma. After 1 h infusion of physiological saline or each concentration of Hcy solution, plasma Hcy concentration was measured by HPLC following the method described by Yamaguchi et al. (*10*). Values without a common superscript letter are significantly different at $p<0.05$. Values are mean±SE for seven rats.

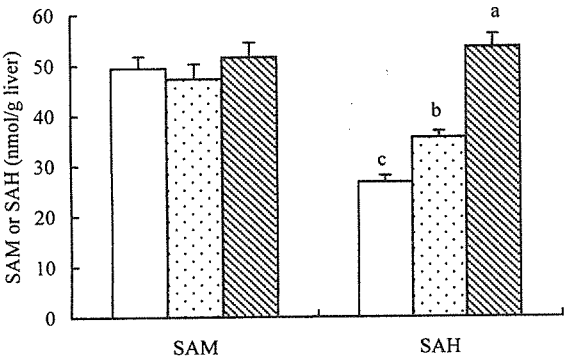

Fig. 2. Contents of SAM and SAH in liver. After 1 h infusion of physiological saline or each concentration of Hcy solution, rats were dissected and livers were excised immediately. SAM and SAH in livers were measured by HPLC following the method of She et al. (*11*). White bars: Control group, dotted bars: 15-Hcy group (15 mg/mL Hcy), hatched bars: 30-Hcy group (30 mg/mL Hcy). Values without a common superscript letter are significantly different at $p<0.05$. Values are mean±SE for seven rats.

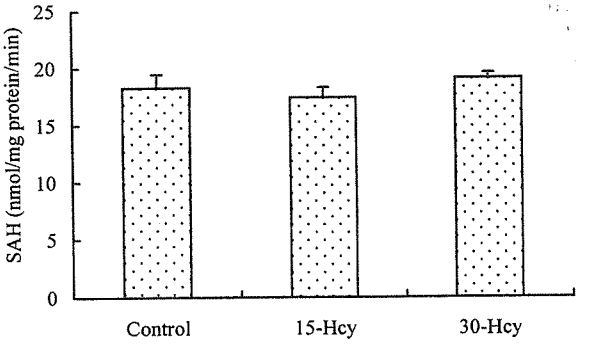

Fig. 3. Activity of SAH hydrolase in liver. SAH hydrolase activity was measured by HPLC following the modified method described by She et al. (*11*). SAH-synthetic activity of SAH hydrolase was estimated by increased SAH. Values are means±SE for seven rats.

compared to that in the Control group, respectively. This result showed that the elevated plasma Hcy concentration was achieved by intravenous infusion of Hcy.

As shown in Fig. 2, the content of hepatic SAH in the 15-Hcy and 30-Hcy groups were significantly higher than that in the Control group. There was a positive correlation ($r=0.877$, $p<0.001$) between the amount of infused Hcy and SAH content in liver. On the other hand, the content of SAM in liver did not increase by Hcy infusion (Fig. 2). Fu et al. (*9*) and Kloor et al. (*13*) reported significantly increased SAH in examined tissues of the rats received Hcy infusion, while SAM was not significantly different as compared to the Control group. The results of our study were similar to their reports. The content of SAM in liver might not be affected by the elevated plasma Hcy unlike the case of SAH. Moreover, a decrease in the SAM/SAH ratio, which is used as an indicator of methylation capacity, was significant under transient high plasma Hcy levels (1.85±0.09, 1.33±0.08, and 0.96±0.02 in the Control, 15-Hcy, and 30-Hcy groups, respectively). The decrease of the ratio indicated a block in methylation reactions (*14*). Therefore, the accumulation of SAH might be caused by the SAH-synthetic reaction.

Under physiological conditions, SAH hydrolysis works predominantly, in which Hcy and adenosine are removed efficiently via the remethylation and transsulfuration pathways. In contrast, when nutritional deficiencies (*2–4*) and genetic mutations (*5*, *6*) induced plasma Hcy elevation, the SAH-synthetic reaction was surmised to be favored. However, under these conditions, the SAH-synthetic activity of SAH hydrolase was not examined. In this study, the SAH-synthetic activity of SAH hydrolase in liver extracts was assayed under transient high plasma Hcy levels (Fig. 3). SAH-synthetic activity of SAH hydrolase in liver extracts showed no significant difference among the three groups. In addition, the ratio of SAH hydrolase mRNA versus β-actin mRNA in liver extracts (0.81±0.06, 0.76±0.02, and 0.89±0.04 in the Control, 15-Hcy, and 30-Hcy groups, respectively) showed no significant difference among the three groups. Thus SAH hydrolase mRNA in liver extracts could not be upregulated by transiently elevated plasma Hcy concentration. These results indicate that the accumulation of hepatic SAH is likely to be caused by a SAH-synthetic reaction although its activity was unchanged in liver.

In our previous study when B_6-deficient rats were used, an increase in the SAH-synthetic activity of SAH hydrolase was observed under elevated plasma Hcy and accumulated hepatic SAH (*7*). However, in this study using normal rats, transient high plasma Hcy induced the accumulation of SAH without any change in SAH-synthetic activity of SAH hydrolase. In this study, Hcy rapidly decreased from plasma after discontinuance of intravenous Hcy infusion (data not shown), which showed that further metabolism of Hcy was working

properly. SAH-synthetic activity of SAH hydrolase may change under conditions of abnormal methionine metabolism in which remethylation and transsulfuration pathways are disturbed. Our next concern is the effects of long-term infusion of Hcy on methionine metabolism including the SAH-synthetic activity of SAH hydrolase.

In conclusion, this study showed that there was an increase in hepatic SAH without any increase in SAH-synthetic activity of SAH hydrolase in liver extracts under transient high plasma Hcy levels.

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Effect of Vitamin B_6 Deficiency on *S*-Adenosylhomocysteine Hydrolase Activity as a Target Point for Methionine Metabolic Regulation

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Summary The objective of this study was to clarify the relationship between the accumulation of *S*-adenosylhomocysteine (SAH) and the change in the SAH hydrolase activity in vitamin B_6 (B_6). Male Wistar rats were fed a control diet (control and pair-fed groups) or B_6-free diet (B_6-deficient group) for 5 wk. Although the SAH-synthetic activity of SAH hydrolase significantly increased in the B_6-deficient group, SAH-hydrolytic activity of SAH hydrolase showed no significant difference in the liver among the three groups. On the other hand, SAH hydrolase mRNA in the liver did not show any significant change. Thus, the accumulation of SAH would be due to the increased SAH-synthetic activity of SAH hydrolase. The disturbed methionine metabolism by B_6-deficiency, such as a significant increase of plasma homocysteine, might induce the activation of SAH hydrolase in the direction of SAH synthesis.

Key Words Vitamin B_6, *S*-adenosylhomocysteine hydrolase, *S*-adenosylhomocysteine, homocysteine, methionine metabolism

Vitamin B_6 (B_6) is known as a coenzyme related to amino acid metabolism, including methionine. Methionine is metabolized to *S*-adenosylmethionine (SAM) by L-methionine *S*-adenosyltransferase (EC 2.5.1.6) (*1*, *2*). SAM is a methyl donor for transmethylation in vivo, involving protein, histones, DNA and RNA as cellular methyl acceptors (*3*). SAM is converted to *S*-adenosylhomocysteine (SAH) by some transmethylation reactions, and then SAH is hydrolyzed to homocysteine (Hcy) by SAH hydrolase (EC 3.3.1.1). Hcy was reported to be an independent risk factor for cardiovascular diseases and arterial sclerosis (*4*), so it is important to remove Hcy from the metabolic pathway. Hcy is catabolized by cystathionine β-synthase (EC 4.2.1.22; CBS) and γ-cystathionase (EC 4.4.1.1), both of which require PLP as a coenzyme. This route is called the transsulfuration pathway. On the other hand, Hcy is remethylated to methionine and this route is called the remethylation pathway. This pathway requires betain, folic acid and vitamin B_{12}. Under normal physiological conditions, the remethylation pathway predominates rather than the transsulfuration pathway (*5*). Additionally, it was reported that appropriate intake of vitamin B_{12}, folic acid and B_6 is important to avoid Hcy accumulation (*6*, *7*).

SAH hydrolase plays an important role in the metabolism of SAH. SAH is reversibly hydrolyzed to adenosine and Hcy by SAH hydrolase. Thermodynamically, an equilibrium of SAH hydrolase reaction favors the direction of SAH synthesis. However, under physiological conditions, SAH hydrolase directs toward the hydrolysis of SAH because adenosine and Hcy are rapidly catabolized.

Our previous report showed the accumulation of SAH and an increase of the SAH-synthetic activity of SAH hydrolase in the liver and thymus of B_6-deficient rats (*8*). This indicated the possibility that SAH accumulated in the liver and thymus due to a dominant increase in the SAH-synthetic activity of SAH hydrolase. Since SAH hydrolase is a reversible enzyme, it is needed to examine whether the SAH-hydrolytic activity of SAH hydrolase was also activated or SAH hydrolase, per se, increased during B_6 deficiency. Therefore, we measured the activity of SAH hydrolase in both directions and examined the expression of SAH hydrolase mRNA to clarify the mechanism of SAH accumulation during B_6 deficiency.

MATERIALS AND METHODS

Reagents. Somnopentyl® was purchased from Schering-Plough Co. (NJ, USA). SAH, SAM, adenosine and L-homocysteine thiolactone were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). SBD-F (7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate) and 1-heptanesulfonic acid sodium salt were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methanol and acetonitrile were purchased from Kanto Kagaku Co. (Tokyo, Japan). Other reagents were purchased from Nacalai Tesque Inc. (Kyoto, Japan) unless otherwise indicated.

Animal and diets. Male Wistar rats, 4-wk old and weighing 80–100 g, obtained from Japan SLC, Inc. (Hamamatsu, Japan) were acclimatized on AIN-76 diet

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Table 1. Composition of the experimental diets (g/kg).

Ingredients	Group	
	Control and P.F.[1]	B_6-def.[2]
Casein (vitamin-free)	700	700
Sucrose	173	173
Soybean oil	50	50
Cellulose powder	20	20
Vitamin mix. (AIN-76)	10	—
Vitamin B_6-free vitamin mix.[3]	—	10
Mineral mix. (AIN-76)	35	35
DL-Methionine	10	10
Choline bitartrate	2	2

[1] Pair-fed group: Control diet was pair-fed to the B_6-deficient group.
[2] Vitamin B_6-deficient group: Vitamin B_6-deficient diet was fed ad libitum.
[3] Composition of vitamins except vitamin B_6 basically followed the AIN-76 vitamin mixture.

for 3 d. The animals were housed individually in a stainless-steel cage in a room maintained at constant temperature (23±1°C) with alternating 12-h cycles of light (light: 6:00 a.m. to 6:00 p.m.) and dark. They were randomly divided into three groups of six rats each. The B_6-deficient (B_6-def.) group and the control group were given free access to their respective diets (Table 1) for 5 wk, and the pair-fed control (P.F.) group was pair-fed to the B_6-def. group. The food intake and body weight were measured daily.

At weekly intervals, the 24 h-urine of each rat was collected for measurement of xanthurenic acid excretion. It was determined colorimetrically in the presence of Fe^{2+} at 610 nm (*9*).

The experiments were performed in accordance with the guidelines of governmental legislation in Japan on the proper use of laboratory animals (1980), and our experiments were approved by the ethical committee of the Faculty of Applied Biological Sciences in Gifu University.

Sample preparation. Under Somnopentyl® anesthesia, blood samples were drawn from the abdominal aorta with a heparinized syringe, and the liver was then immediately excised and weighed. Plasma was obtained by centrifugation at 2,000×*g* (4°C) for 20 min. Plasma and liver were stored at −20°C until analyzed.

Assay of plasma PLP. The contents of PLP in the plasma were determined using the method described by Tsuge (*10*).

Assay of plasma Hcy. Total Hcy in the plasma was measured by the method of Yamaguchi et al. (*11*).

Assay of SAM and SAH. SAM and SAH were measured by the method of She et al. (*12*).

Assay of hepatic SAH hydrolase. The SAH-synthetic activity of SAH hydrolase was measured using a modified method of She et al. (*12*), and the SAH-hydrolytic activity of SAH hydrolase was determined using the method of Aksamit et al. (*13*). Livers were homogenized with five volumes of 0.25 M sucrose/3.3 mM $MgCl_2$/2 mM glutathione (reduced form)/50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 100,000×*g* (4°C) for 1 h. The supernatant was used for assaying SAH hydrolase.

For the assay of the SAH-synthetic activity of SAH hydrolase, the incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.3), 2 mM dithiothreitol (DTT), 5 mM L-homocysteine thiolactone, 100 μM adenosine and 50 μL homogenate (1 mg protein/mL) in a total volume of 0.3 mL. For the assay of the SAH-hydrolytic activity of SAH hydrolase, the incubation mixture consisted of 50 mM potassium phosphate buffer (pH 6.9), 0.2 mM SAH, 2 mM DTT, 1.7 units of adenosine deaminase (purified from calf intestine following the method of Coddington (*14*)) and 50 μL homogenate (1 mg protein/mL) in a total volume of 0.3 mL. After incubation at 37°C for 10 min, the reaction was stopped by adding 50 μL of 3 N perchloric acid (PCA). The content was mixed and then centrifuged at 2,000×*g* for 10 min. The supernatant was filtered through a Millipore membrane (0.45 μm) and applied to HPLC for the analysis of SAH. SAH-synthetic activity was estimated by the increase in SAH, and SAH-hydrolytic activity was estimated by the decrease in SAH.

Extraction of total RNA. Total RNA in each homogenate was isolated by the acid guanidium-phenol-chloroform method, using TRIzol (Invitrogen Corporation, Carlsbad, CA, USA). RNA concentration was determined by absorption at 260 nm using a Hitachi U-2001 spectrophotometer (Tokyo, Japan).

Assay for expression of SAH hydrolase mRNA. Expression of SAH hydrolase mRNA was determined by RT-PCR using a TaKaRa One Step RNA PCR Kit (TaKaRa Bio Inc., Otsu, Japan). The RT-PCR process partly followed the method of Ohmori et al. (*15*). The primer for SAH hydrolase was as follows: the upstream primer sequence was 5′-AAGCTGCCATGGAGGGCTACGA-3′, and the downstream primer sequence was 5′-GATGGCAGCTGGAAGGTGAAGG-3′. For the primer of β-actin (used as an invariant control), the β-actin RT-PCR Primer set (Toyobo., Ltd. Osaka, Japan) was used.

The samples were amplified by 30 PCR cycles, where each consisted of denaturation at 94°C for 60 s, annealing at 58°C for 60 s and extension at 72°C for 75 s. Each PCR product was resolved by electrophoresis on 1% agarose gel stained with ethidium bromide, and photographed under UV light. Band intensity was evaluated by a NIH image program, which was developed by the U.S. National Institute of Health.

Statistical analysis. The statistical difference among means was estimated at $p<0.05$ according to ANOVA and Duncan's multiple-range test (*16*).

RESULTS

Effect of B_6 deficiency on growth parameters and liver weight

The final body weight, body weight gain and total food intake are shown in Table 2. The parameters of the B_6-def. group were the lowest of the three groups. Liver

Table 2. Growth parameters and tissue weights of the experimental rats.

Group	Control	P.F.[1]	B_6-def.[2]
Final body weight (g)	303±11[a]	152±2[b]	146±9[b]
Body weight gain (g)	194±10[a]	43±4[b]	37±10[b]
Total food intake (g)	515±17[a]	268±3[b]	254±15[b]
Liver weight (g/100 g B.W.)	2.82±0.41[a]	2.12±0.04[b]	2.92±0.18[a]

Values are means±SE for six rats.
[1,2] See the legend for Table 1.
Values without a common superscript letter are significantly different at $p<0.05$.

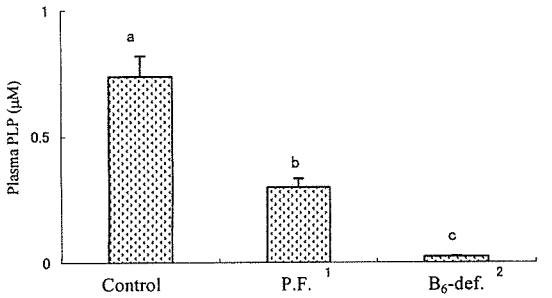

Fig. 1. Concentration of plasma PLP. Values are means±SE for six rats. [1,2] See the legend for Table 1. Values without a common superscript letter are significantly different at $p< 0.05$. Each concentration was measured by HPLC following the method described by Tsuge (*10*).

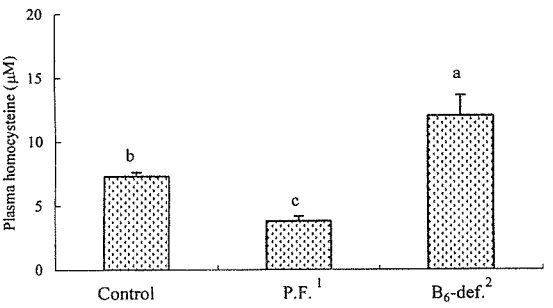

Fig. 2. Concentration of homocysteine in plasma. Values are means±SE for six rats. [1,2] See the legend for Table 1. Values without a common superscript letter are significantly different at $p< 0.05$. Homocysteine was measured by HPLC following the methods described by Yamaguchi et al. (*11*).

weights (g/100 g body weight) in the B_6-def. group were higher than those of the P.F. group (Table 2). Urinary xanthurenic acid excretion as an index of B_6 deficiency (*17*) was significantly high in the B_6-def. group, indicating B_6 deficiency in this group (data not shown).

Concentrations of plasma PLP and Hcy

In general, it is known that the plasma PLP concentration is the most suitable index for internal B_6 nutrition (*18*). Concentrations of plasma PLP after the feeding period are shown in Fig. 1. The plasma concentrations of PLP in the B_6-def. group were lower than those of the other groups. This indicates that the B_6-def. group did not hold enough B_6 in the body.

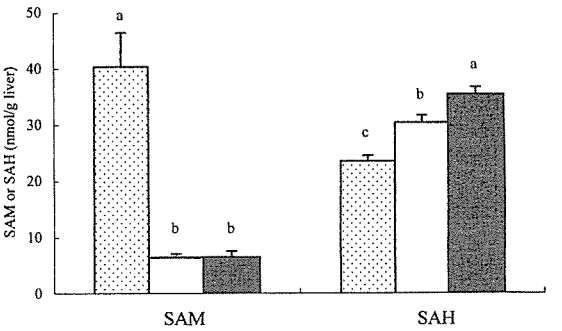

Fig. 3. Contents of SAM and SAH in livers. Values are means±SE for six rats. [1,2] See the legend for Table 1. Values without a common superscript letter are significantly different at $p< 0.05$. SAM and SAH were measured by HPLC following the method of She et al. (*12*). Dotted bars: Control group, White bars: P.F. group. Dark bars: B_6-def. group.

Concentrations of plasma Hcy are shown in Fig. 2. It was significantly higher in the B_6-def. group than in the other groups. Hcy concentration was about two times higher in the B_6-def. group as compared to those in the other groups. This would be caused by impaired CBS and γ-cystathionase activities in the liver due to B_6 deficiency, as reported previously (*19*).

Contents of SAM and SAH in liver

The content of SAM in the livers of the B_6-def. group was significantly lower than that of the control group; however, it was almost the same as in the P.F. group (Fig. 3). On the other hand, the content of hepatic SAH was significantly higher in the B_6-def. group than in that of the other groups (Fig. 3).

Change in the SAH hydrolase activity under B_6-deficiency

The activity of SAH hydrolase was measured in both the direction of SAH-synthesis and SAH-hydrolysis, as shown in Table 3. The SAH-hydrolytic activity was not significantly different among the three groups. On the other hand, SAH-synthetic activity was significantly higher in the B_6-def. group than in that of the other groups.

Expression of SAH hydrolase mRNA

The expression of SAH hydrolase mRNA was evaluated by RT-PCR. There were no significant differences in mRNA expression in the livers among the three groups (date not shown).

Table 3. Activity of SAH hydrolase in the livers of the experimental rats.

Activity (nmol/mg protein/min)	Control	P.F.[1]	B_6-def.[2]
SAH hydrolysis	6.1±1.3	6.2±1.4	7.3±1.0
SAH synthesis	22.3±2.6[b]	26.8±0.8[b]	37.7±1.3[a]

Values are means±SE for six rats.
[1,2] See the legend for Table 1.
Values without a common superscript letter are significantly different at $p<0.05$.
SAH-synthetic activity was estimated by inceased SAH, and SAH-hydrolytic activity was estimated by decreased SAH. For the details about the assay, see "Materials and Methods."

DISCUSSION

Nguyen et al. reported abnormal methionine metabolism using B_6-deficient rats (*8*), in which the accumulation of SAH and increase of SAH-synthetic activity in both the liver and thymus were observed. There were two explanations for the mechanism of SAH accumulation. One explanation is the inhibition of SAH hydrolysis by adenosine, because SAH hydrolase is an adenosine binding protein and bound adenosine was reported to inhibit the activity (*20*). Another explanation is due to enhancement of the SAH-synthetic rate from adenosine and Hcy. According to previous results (*8*), it was presumed that SAH accumulation was caused by the increased SAH-synthetic activity of SAH hydrolase. However, we could not ascribe SAH accumulation to the increased activity of SAH synthesis, because this enzyme is a reversible one. Namely, we could not explain the accumulation of SAH until we measured both directions of SAH hydrolase activity.

In this study, we observed a significant decrease of PLP in the plasma (Fig. 1) and accumulation of SAH in the liver (Fig. 3) of the B_6-def. group, and these results were similar to a previous report (*8*). On the other hand, the content of SAM in the liver was significantly lower in the B_6-def. and P.F. groups than in the control group, which would reflect the amount of methionine supplied from the diet.

As noted above, SAH hydrolase is a reversible enzyme in the methionine metabolism and catalyzes the conversion between SAH and Hcy. Although the equilibrium dynamics of the SAH hydrolase reaction strongly favor SAH formation rather than SAH hydrolysis, the reaction under the physiological conditions was supposed to proceed in the hydrolytic direction of SAH because adenosine was removed rapidly through adenosine deaminase or adenosine kinase (*21–23*), and Hcy was consumed via transsulfuration and remethylation so as to be removed from this system (*5*). In this experiment, we measured the activity of SAH hydrolase in both directions. SAH-synthetic activity of SAH hydrolase was significantly higher in the livers of the B_6-def. group; however, the SAH-hydrolytic activity of SAH hydrolase in this group did not show a significant increase (Table 3). Although the same enzyme catalyzes the hydrolysis and synthesis of SAH, the changes in both activities were not parallel. These results indicate that SAH-synthetic activity increased, in particular. Furthermore, we examined hepatic SAH hydrolase mRNA expression. There was no significant difference in SAH hydrolase mRNA expression in the livers among the three groups. It was confirmed that B_6 deficiency did not affect SAH hydrolase at the mRNA level. Therefore the accumulation of SAH in B_6 deficiency would be caused by an increase in the SAH-synthetic activity of SAH hydrolase because the SAH-hydrolytic activity of SAH hydrolase was not increased and there was no significant difference in the expression of SAH hydrolase mRNA among the three groups.

On the other hand, we observed an increase of plasma Hcy in the B_6-def. group (Fig. 2). Smolin and Benevenga (*24*) reported the accumulation of plasma Hcy under B_6 deficiency using the model for CBS-deficient rats. She et al. (*19*) also reported that CBS and γ-cystathionase were greatly diminished in the livers of the B_6-def. group, and suggested that the accumulation of Hcy was derived from B_6 deficiency. It was reported that excess methionine impaired the Hcy metabolism in B_6-deficient rats (*25*). In our study, we added methionine at 1% into the diet and excess methionine might be metabolized through an unusual fashion because the enzyme activities involved in the transsulfuration pathway were significantly decreased by B_6 deficiency (*19*). Under such conditions, there was an increase in the Hcy concentration, and the resulting increase needed to be metabolilzed by either remethylation or a SAH-synthetic reaction. The remethylation pathway was reported to be suppressed by excess methionine intake (*25*). Therefore, under B_6 deficiency, accumulated Hcy might be re-converted to SAH again due to limitations of the remethylation pathway together with a diminished transsulfuration pathway. This would be the circumstances under which the SAH-synthetic activity of SAH hydrolase needs to increase.

In in virto experiments, some factors were reported to be involved in the increase of SAH hydrolase in SAH-synthetic direction. Rat liver SAH hydrolase is a homotetrametric enzyme which contains NAD^+ as a co-factor, and its activity is regulated by the oxidation-reduction cycle of the enzyme-bound NAD^+ (*26*). Kloor et al. (*27*) reported that the SAH-synthetic activity of SAH hydrolase increased in the presence of phosphate at physiological concentrations in bovine kidney. They suggested that the phosphate would facilitate adenosine-mediated conversion of enzyme-bound NAD^+ to NADH. Furthermore, Finkelstein and Harris (*28*)

reported that hormone treatment caused a marked change in the SAH-synthetic activity of SAH hydrolase. It is also reported that B_6 binds to steroid hormone receptors and controls steroid hormone functions (*29*). These factors might be involved in the specific increase of SAH-synthetic activity of SAH hydrolase observed in this study.

In summary, we measured methionine metabolites and SAH hydrolase activity to determine the mechanism of SAH accumulation in B_6-deficient rats. We observed SAH accumulation and a significant increase of SAH-synthetic activity of SAH hydrolase in the liver although SAH-hydrolytic activity showed no significant increase. Furthermore, there was no significant difference in the hepatic mRNA level of SAH hydrolase. Thus, SAH hydrolase mRNA was not involved in the changes in SAH hydrolase. We assume that the observed non-parallel increase in the SAH-synthetic activity of SAH hydrolase might be a cause of SAH accumulation. Further research is needed to clarify the mechanism why the SAH concentration and SAH-synthetic activity of SAH hydrolase alone were significantly enhanced in the liver under our experimental conditions.

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B 免疫疾患 《免疫不全、HIV感染症》

まとめ

1. 免疫不全の多くは後天性免疫不全症である。
2. 食事からの感染防止に配慮する。
3. 的確な栄養アセスメントを実施する。
4. 栄養アセスメントに基づく栄養補給により、栄養不良に陥ることを避ける。
5. 体重減少が治療の良悪の指標となる。
6. 消化管機能の低下が著しい場合には吸収のよい遊離アミノ酸を用いる。
7. 免疫賦活作用を有する栄養補助剤投与が有効である。
8. 自己免疫疾患も続発的に免疫不全を発症し、n-3系脂肪酸摂取が有効とするエビデンスがある。

1 病態と栄養

免疫系に異常をきたし、生体防御機構が正常に働かないために生じる病態を免疫不全症と呼んでいる。免疫不全症はその原因が遺伝的要因による先天性(原発性)免疫不全症と産まれたときはまったく免疫系には問題はなかったが、成長・発達に伴いなんらかの原因で免疫系が著しい低下を示す後天性(獲得性あるいは続発性)免疫不全症に大別される。いずれも体液性免疫あるいは細胞性免疫の片方かあるいは両方の破綻を示し、細菌やウイルスなどの易感染性と腫瘍などの発生をきたす疾患である。先天性免疫不全症では、多くの場合、生存して産まれてくることは少なく、産まれたとしても長期の生存は難しい。そのため、先天性免疫不全症の頻度は低く、臨床の現場において散見されることは少ない疾患である。

一方、後天性免疫不全症はその頻度も高く、臨床の現場においても頻繁に散見される。その代表的なものがヒト免疫不全ウイルス(HIV)の感染によって発症するエイズ(AIDS)である。本稿では免疫不全を中心とする疾患の栄養管理だけでなく免疫能が異常に亢進した場合の自己免疫疾患の栄養管理についても併せて叙述する。一般に、免疫不全の際の栄養管理としては、①食品からの感染防止、②食欲低下への対応、および③免疫賦活を目的とした栄養補給、などが中心となる。また、自己免疫疾患では異常に亢進した免疫能とそれに伴う炎症を抑制するためにしばしばステロイド(プレドニゾロン)が使用され、それが原因で続発性免疫不全症を発症し、易感染性を示すことが知られていることから、それに対する栄養補給が必要となる。

2 栄養管理

1. 先天性免疫不全症

先天性免疫不全症は造血系や免疫系の発生・分化のいずれかの段階で遺伝的に欠陥がみられる疾患である。その頻度は10万人あたり数人と非常に低いが、中には選択的免疫グロブリンA(IgA)欠損症のように頻度の高いものもある。そのほかに代表的な先天性免疫不全症として伴性無γグロブリン血症や重症複合型免疫不全症(SCID)などがあるが、正常に産まれることは少なく、たとえ産まれたとしても長期生存は難しい。治療としては欠損している免疫系を修復するためにγグロブリンによる置換療法ならびに骨髄、胸腺や胎児細胞などの移植が実施される。栄養療法としては特にはないが、食事からの感染を防止するために無菌食や加熱食などが適用とされている。また、栄養不良状態は易感染性を高めることから、患者の栄養アセスメントを行い、栄養不良をきたさないように十分なエネルギーとたんぱく質補給を行うことが基本となる。

表1　骨髄移植時における栄養管理

1．抗癌薬大量投与による食欲低下への対応
　①患者の嫌いな食品や調理法は避け、なるべく嗜好に合ったものを出す。
　②通常の食事時間にこだわることなく、食べたくなったときに与える。
　③消化のよい食品や調理法を用いる。
　④味の濃いもの、刺激の強いものは避ける。

2．抗癌薬、放射線照射に伴う免疫低下への対応
　食品からの感染を防止するために無菌食や加熱食が実施される。
　①無菌食
　　ガスオーブン法(乾熱滅菌法)
　　オートクレーブ法(高圧蒸気滅菌法)
　　電子レンジ法(高周波滅菌法)[*1]
　②加熱食
　　調理済み食品を耐熱容器に入れ、100℃、13分間、蒸気加熱する。[*2]

[*1]水分の少ない食品は不適。
[*2]リンゴ、ミカンなどの果物やキュウリ、トマトなどの生野菜は加熱処理せず、適当な消毒液にて処理して、喫食する。
(森口　覚，高杉美佳子：免疫・アレルギー疾患(アレルギー，免疫不全症)．看護のための最新医学講座，第29巻，武田英二(編)，pp367-380，中山書店，東京，2002による)

表2　エイズにおける栄養アセスメントと栄養管理

1．栄養アセスメント
　①体重、皮下脂肪、上腕の測定
　②血清蛋白質、血清アルブミン、レチノール結合蛋白質(RBP)の測定
　③血清カリウム：体細胞量や貯蔵蛋白質量の指標
　④その他の血清電解質：下痢に伴う水分、電解質補給の指標
　⑤問診により食物摂取状況、悪心、嘔吐、下痢、便秘などの胃腸機能のチェック
　⑥CD4(ヘルパー)T細胞数の変化：病態の進行度の指標

2．栄養管理
　①バランスのとれた食事：高たんぱく質、下痢がある場合は低脂肪
　②ビタミンとミネラルの十分な摂取
　③エネルギーの十分な摂取：体重減少を防止するために頻回食、高脂肪食(吸収機能が正常な場合)、高糖質食
　④食品からの感染防止：生の肉、魚、卵は避ける。十分な加熱調理。手指や食品の消毒

(森口　覚，高杉美佳子：免疫・アレルギー疾患(アレルギー，免疫不全症)．看護のための最新医学講座，第29巻，武田英二(編)，pp367-380，中山書店，東京，2002による)

2．後天性免疫不全症

後天性免疫不全症は腫瘍、自己免疫疾患、HIV感染、薬剤、放射線、臓器移植、栄養障害、内分泌異常ならびに加齢などさまざまな要因によって続発的に発症する免疫不全症である。本稿では骨髄移植やウイルス感染によって発症する免疫不全症の代表例として白血病とエイズを取りあげた。

㋐白血病 (357頁)

白血病は遺伝、放射線、化学物質、ウイルス感染、癌遺伝子の活性化ならびに癌抑制遺伝子の不活性化などさまざまな要因によって発症する。癌化した白血球細胞の成熟度により急性骨髄性白血病(AML)と慢性骨髄性白血病(CML)に分類され、前者は進行するにつれて、出血、貧血ならびに感染などの症状を呈するが、適切な治療により高率に症状の改善を認める。しかし一方、CMLは慢性期、移行期を経て急性期へと進展し、予後は一般に悪い。わが国ではヒトT細胞白血病ウイルス1型(HTLV-1)感染(母子感染、夫婦感染、輸血による感染)による成人T細胞白血病(ATL)が注目され、西日本を中心にキャリアが多く分布することが知られている。AMLに対してはビタミンA(レチノイン酸)の大量投与が実施され、高い寛解率が得られている[1]。また、CMLでは複数の抗癌薬などを併用した化学療法やインターフェロンαなどのサイトカイン投与が実施されるが、最終的には骨髄移植を行う。骨髄移植にはまずドナーの確保が第一で、次いで大量の抗癌薬と放射線照射によって自己の免疫細胞を根絶しておく必要がある。この際、患者は食欲低下と免疫低下を示すことから、これらに対する栄養管理が必要となる(表1)。

㋑エイズ(AIDS)

HIV感染後、エイズ関連症候群(ARC)を発症し、最終的にAIDSとなり死に至る疾患である。この疾患の根幹はHIVのヘルパーT(CD4陽性)細胞への感染・増殖に伴う細胞性免疫能の著しい低下であることから、ヘルパーT細胞数のモニタリングは疾患の進展を知るうえで重要な指標となる。一般にヘルパーT細胞数により＞500/μl(免疫不全に関係した危険性は低い)、200～500/μl(中等度の免疫不全)、＜200/μl(重度の免疫不全)のように分類されている。

栄養管理としてはまず、患者の栄養アセスメント(表2)を実施し、それに見合う食事計画を立てる。HIV感染初期(無症候期)は健常者と同様の食事摂取でよいが、感染細胞内ではHIVが多量のフリーラジカルを産生し、それが免疫低下やHIVの増殖を刺激することから、この時期には十分な抗酸

化ビタミン(β-カロテン、C、E)の摂取が必要である[2]。また、良質のたんぱく質摂取や食品衛生にも配慮が必要である。ARCからAIDSへと進展するに伴い消化管機能の低下がみられるので、刺激性の少ない軟食(流動食、三分粥、五分粥、全粥食)を用いる。さらに、経口摂取が困難な場合は経腸栄養剤などの非経口栄養法を実施する。

3．自己免疫疾患

㊐関節リウマチ(RA)

RAは原因不明の多発性、破壊性の非化膿性慢性関節炎を主徴とする疾患である。マクロファージ、好中球などの免疫細胞から分泌される種々のサイトカイン、蛋白分解酵素や血管新生因子などにより、軟骨や骨が次第に破壊されていくため、治療には非ステロイド抗炎症薬、抗リウマチ薬、免疫抑制薬などが使用される。栄養療法としては除去療法および補充療法がある。除去療法ではRAの増悪因子である食事由来の抗原の除去や細胞膜を構成する脂質を変化させることによってロイコトリエンやリゾチーム産生の抑制を図るもので、200～300kcalのエネルギー制限が実施されている[3]。補充療法とは体内で催炎性物質の基質となるアラキドン酸と競合するeicosapentaenoic acid(EPA)やdihomogammalinolenic acid(DGLA)を多く含む食品摂取により、拮抗的にRA症状の改善を目指すものである。EPA供給源として魚油、DGLA供給源としてはその前駆物質であるgamma linolenic acid(GLA)を多く含む月見草種子油が用いられ、これまで副作用もなく、疼痛減弱、抗炎症作用が見い出されている[4]。

㊐全身性エリテマトーデス(SLE)

SLEはTおよびB細胞の機能的異常、免疫複合体により多臓器病変を示し、血中に抗DNA抗体をはじめとする自己抗体を認める全身性の自己免疫疾患である。栄養管理としては低エネルギー、動物性たんぱく質制限によりSLE患者血中の抗DNA抗体、抗核抗体などの自己抗体量の減少が認められており、その機序として低栄養による免疫抑制効果や細胞膜構成脂質の変化などが考えられている。そのほかに低脂肪食や魚油摂取が効果があったとする報告もあるが、今後さらに検討が必要である。

3 日常生活管理

免疫不全を示す疾患のいずれにおいても多かれ少なかれみられるのが体重減少である。特に、HIV感染に伴い発症するAIDSではその感染初期から徐々に体重の減少がみられる。疾患の進展に伴い1ヵ月に5kgを超える急激な体重減少がみられるが、その主たる原因は摂食量の低下による。その後、慢性的に体重減少が続くが、それは下痢などの胃腸障害が主な原因となる。これら、病態の進展に伴う体重減少が何によるものかをしっかりと把握し、そのうえで適切な栄養補給を行うことが大切である。また、臨床的にはAIDS患者の生存日数が栄養状態や体重減少および血清アルブミン値の低下と密接に関連することが知られていることから、患者の栄養状態の保持、体重維持、血清アルブミン値の改善を図ることが重要である。また、最近では癌患者や外傷患者を対象として免疫賦活を図り、治療効果の改善と入院日数の短縮を目指した栄養補助製剤が開発され、臨床において使用されている。①Impact(免疫賦活作用を有する栄養素としてアルギニン、核酸、n-3系脂肪酸を含む)：T細胞や食細胞機能の亢進、感染性合併症の防止、②Immun-Aid(免疫賦活作用を有する栄養素としてグルタミン、アルギニン、核酸、n-3系脂肪酸、分岐鎖アミノ酸を含む)：T細胞数の増加、感染性合併症の防止、③Alitra-Q(免疫賦活作用を有する栄養素としてグルタミンを含む)：敗血症の防止、肺炎発生率の抑制、などがあり、これらはAIDSをはじめとする免疫不全を有する患者に臨床応用されている。また、たんぱく質栄養では分子量500未満の遊離アミノ酸が消化吸収機能の低下時においても比較的高率に吸収されることから、遊離アミノ酸を基本としたたんぱく質補給が推奨される。

以上、免疫不全時においては体重や血液生化学的パラメーターを指標として栄養アセスメントをしっかりと行い、それに基づいて適切な栄養補給を実施し、栄養状態の改善・保持を図ることが肝要である。

(森口　覚)

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第2章　加齢と免疫力

2.1　高齢者の疾患と健康保持

21世紀には高齢者人口の増加に少子化の加速が相まって，4人に1人が65歳以上の高齢者という超高齢化社会の到来することが予測されており，医療の分野のみならず，社会，経済的にも大きな問題となっている．また，人口構成の高齢化は疾病構造に対しても大きな影響をもたらしており，心疾患や脳血管疾患などの高齢者特有の慢性疾患に加え，ガンおよび肺炎，結核などの感染性疾患による死亡者の増加が高齢者において際立っている[1]（図2.1）．昭和10年頃は高齢者だけでなく，乳・幼児においても肺炎は致死率の高い疾患であったが，抗生物質の発見，普及により肺炎による死亡者は激減した．しかし，65歳を越える高齢者では現在でもなお肺炎などの感染症によ

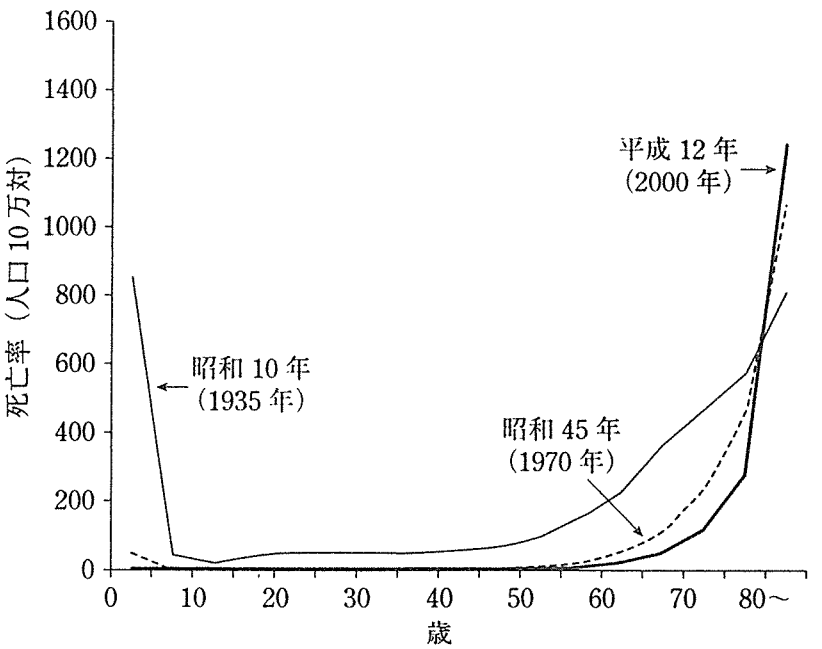

図2.1　肺炎による年齢階級別死亡率（人口10万対）の年次比較[1]

る致死率が高い．また，昨今では老人保健施設などに入所している高齢者において，インフルエンザやノロウイルス感染による死亡者が相次いでみられている．さらに，近年の高齢者におけるインフルエンザなどの感染症による死亡者の増加は，日本人の男女における平均寿命の伸びをはじめてマイナスに転じる一因となっていることから，高齢者における感染症対策は高齢者の健康を保持するうえで最重要課題であると考えられる．この高齢者における易感染性や疾病の重症化の背景として，体内に侵入した細菌やウイルスなど外来性の微生物を排除する働きをしている免疫系の破綻との関連が指摘されている．本章では加齢に伴う免疫系の変化について説明するとともに，栄養がこの加齢に伴う免疫能の変化とどのように関連するかについて，これまでの研究成果をもとに解説する．

2.2　加齢に伴う免疫能の変化

加齢により免疫能が低下することが知られているが，すべての免疫系が一様に低下するのではなく，低下する機能もあれば，ほとんど変化しないか，あるいは逆に加齢に伴い亢進する機能さえある．この加齢に伴う免疫能の変化は免疫老化と呼ばれ，昔は免疫不全状態と解釈されていたが，現在は免疫能の調節不良状態であると考えられている．一般に，T細胞を中心とする細胞性免疫能が加齢に伴い顕著に低下するが，抗体，補体などの体液性免疫能はほとんど変化しないか，あるいは上昇することが知られている[2]（図2.2）．まず，本節では加齢に伴う免疫組織および免疫細胞機能の変化について解説する．

2.2.1　胸腺の変化

胸腺は骨髄で産生された未熟T細胞を成熟T細胞へと分化＋成熟する組織，換言すると未熟なT細胞を教育しておとなの（成熟）T細胞へと転換する場である．実際には胸腺皮質においてネガティブセレクションが，そして皮質・髄質境界域においてマクロファージや樹状細胞によりポジティブセレクションが行われ，自己反応性T細胞やMHC（主要組織適合遺伝子複合体）

非拘束性T細胞は排除され，末梢血中に現れることはない．胸腺へ入った未熟T細胞が無事，末梢血中に現れる確率は5%程度であると言われており，これら選択の厳しさが想像できる．

胸腺は思春期の頃に最大となるが，実際には生後すぐから徐々に実質組織が消失し，それに代わり結合組織，脂肪組織が増加し，60歳になると生後まもなくの胸腺と比べると数%程度の実質組織しか残存しておらず，高齢者では上述の胸腺機能が極端に低下していることがわかる[3]（図2.3）．その結果，胸腺における厳しい選択がくずれ，自己反応性T細胞や非拘束性T細胞が末梢血中に多数出現することになり，後述の自己免疫疾患発症の契機となる．高齢者では胸腺機能の低下した

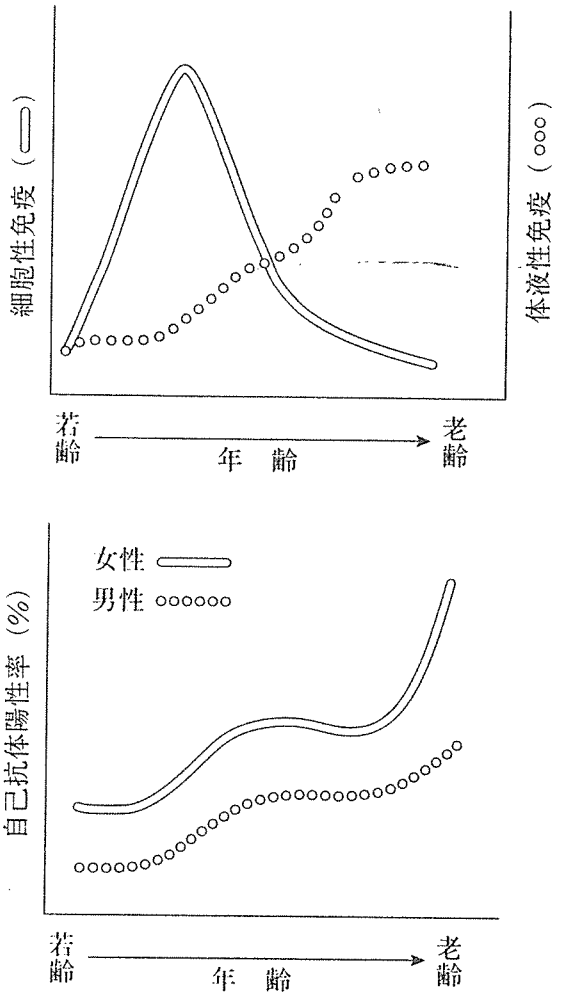

図 2.2 加齢に伴う細胞性および体液性免疫能の変化[2]

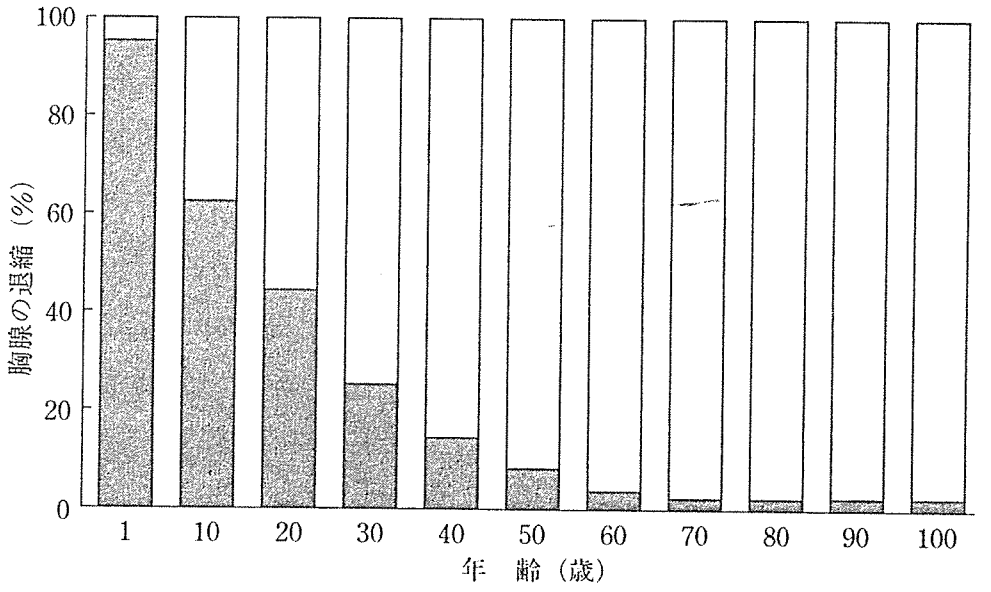

図 2.3 加齢に伴う胸腺の生理的萎縮（退縮）[3]

分，骨髄，脾臓（ひぞう），リンパ節，パイエル板および肝臓などのリンパ系器官においてT細胞の分化，選択が行われる．これをTリンパ球の胸腺外分化と呼んでいる．その他の免疫組織，例えば脾臓やリンパ節も加齢により変化を示すが，胸腺に比べ，その変化が出るのは遅く，50歳以降である．

2.2.2　末梢血中の免疫細胞割合の変化

加齢に伴う血液中の免疫細胞割合の変化としては，抗原に暴露されたことのないナイーブT細胞の割合が減少し，メモリーT細胞の割合が増加する[4]．T細胞の中でもCD4抗原陽性（$CD4^+$）T細胞やCD8抗原陽性（$CD8^+$）T細胞の割合も加齢に伴い低下し，特に抗体産生反応に対して抑制的に働く$CD8^+$ T細胞割合の低下が顕著である．その結果，$CD4^+/CD8^+$が高齢者では上昇する．しかし，若年者，中年者および高齢者を対象とした最近の研究では，高齢者においてもメモリーT細胞中の$CD4^+$および$CD8^+$ T細胞割合は若年者および中年者と比べても有意な低下はしないことが見出されている．

2.2.3　B細胞機能の変化

B細胞は抗体を産生する形質細胞の前駆細胞であるが，B細胞が形質細胞へと分化するためには活性化T細胞によって産生されるサイトカインなどの補助因子が必要である．つまり，加齢に伴いT細胞機能が低下することから，B細胞機能も加齢に伴い低下することが考えられる．実際，大腸菌やサルモネラ菌由来のリポポリサッカライド（LPS），抗Ig抗体あるいは免疫グロブリン（Ig）のFcフラグメントに対するB細胞増殖能は加齢に伴い低下することが知られている．しかし，高齢者の血中IgGやIgM濃度は若年者に比べ高値である．この相違の説明として，高齢者における自己の組織や細胞に対する自己抗体濃度の上昇と関連することが示唆されている．しかし，細菌やウイルスなどの外来性抗原に対する抗体価は高齢者では減少しており，体格指数（BMI）の改善や血清タンパク質，アルブミン濃度の改善により抗体価が上昇することが認められていることから，細菌，ウイルスなどの感染症から自分自身の免疫系を介して身を守るためには良好な栄養状態を維持することが高齢者では重要である．