BIOMEDICAL AND ENVIRONMENTAL SCIENCES **17**, 266-272 (2004)

May Chronic Childhood Constipation Cause Oxidative Stress and Potential Free Radical Damage to Children?

JI-YUE WANG[*,1], YE-LING WANG[**], SHENG-LI ZHOU[**], AND JUN-FU ZHOU[**]

[*]*Affiliated Children's Hospital, College of Medicine, Zhejiang University, 57 Zhugan Lane, Hangzhou 310003, Zhejiang, China;* [**]*Second Affiliated Hospital, College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, Zhejiang, China*

Objective To investigate whether chronic childhood constipation (CCC) may cause oxidative stress and potential free radical damage to children, and to explore the mechanisms by which CCC may cause oxidative stress and potential free radical damage to chronic constipation patients (CCPs). **Methods** Sixty CCPs and sixty healthy child volunteers (HCVs) whose ages, gender and others were matched for the CCPs were enrolled in a randomized controlled study, in which levels of vitamin C (VC) and vitamin E (VE) in plasma as well as activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometric analytical methods. **Results** Compared with average values of the above biochemical parameters in the HCVs group, the average values of VC and VE in plasma as well as those of SOD and CAT in erythrocytes in the CCPs group were significantly decreased ($P<0.0001$). Linear regression and bivariate correlation analysis showed that with prolonged course of the CCPs, the levels of VC and VE in plasma as well as the activities of SOD and CAT in erythrocytes in the CCPs were decreased gradually ($P<0.0001$). **Conclusion** The findings in the present study suggest that chronic childhood constipation causes oxidative stress and potential free radical damage to children with chronic constipation.

Key words: Chronic childhood constipation; Oxidative stress; Free radicals; Free radical damage; Antioxidant; Antioxidase

INTRODUCTION

Constipation is a symptom rather than a specific disease. It is generally defined by patients as defecation frequency of twice weekly or less, and the defecation frequency of patients with chronic constipation is much less still. However, the patients may differ in their perception about constipation depending on psychological and social backgrounds. Other descriptive features of constipation that may be independent of bowel habits include stool consistency, sense of incomplete evacuation, and difficulty during defecation[1-8]. Up to now, there have been neither reports on the abnormal free radical chain reactions in chronic childhood constipation patients (CCPs), nor reports about the relationship between oxidative stress, oxidative damage and chronic childhood constipation (CCC). In order to investigate whether CCC could cause oxidative stress and potential free radical damage in CCPs, and to

[1]Correspondence should be addressed to Dr. Ji-Yue WANG, Tel: 86-571-87061007. Fax: 86-571-86554032. E-mail: wjiyue@mail.hz.zj.cn

Biographical note of the first author: Ji-Yue WANG, male, born in 1959, M. D., associate professor, majoring in child health medicine.

0895-3988/2004
CN 11-2816

explore the mechanisms by which CCC could cause oxidative stress and potential free radical damage, 60 CCPs and 60 healthy child volunteers (HCVs) were enrolled in a randomized controlled study design, in which the levels of vitamin C (VC) and vitamin E (VE) in plasma as well as the activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometric analytic methods. At the same time, the differences between the average values of the biochemical parameters in the CCPs group and the HCVs group were compared, the linear regression and bivariate correlation of the course of CCPs and the biochemical parameters were analyzed.

MATERIALS AND METHODS

Study Design

A randomized controlled study design was used in this study. In order to obtain an objective research conclusion, the principles of random, control, replication and equilibrium, and the management factor, experimental effect and subjects, and the inclusion and exclusion criteria of subjects, etc. were taken into full consideration, and were strictly executed in the study[9-12].

Subjects

Chronic childhood constipation patients (CCPs) Sixty chronic childhood constipation patients (CCPs) were randomly selected from 103 CCPs who were confirmed by the diagnostic criteria[1-8] and their samples were collected according to the inclusion and exclusion criteria[1-8], with "Select Cases-Random Sample" of "SPSS 11.0 for Windows". Their course of disease ranged from 1 to 5 (2.2 ± 1.4) years, and they were agreed by their parents and all were volunteers in this study.

Healthy child volunteers (HCVs) Sixty healthy child volunteers (HCVs) were randomly sampled from 100 HCVs confirmed by comprehensive physical examination at the Affiliated Children's Hospital, College of Medicine, Zhejiang University, with "Select Cases-Random Sample" of "SPSS 11.0 for Windows". They had not any medical history of acute or chronic constipation, they were agreed by their parents and all were volunteers in this study.

There was no significant difference between average age, systolic and diastolic pressures, hemoglobin, albumin and body mass index, and gender in the CCPs group and the HCVs group.

In the above subjects, the common diseases associated with constipation were all excluded, such as colon cancer, colonic neoplasms, benign strictures of the colon, colonic ischemia, diverticular disease, inflammatory bowel disease，anorectal diseases, inflamed hemorrhoids, anal fissure, rectal inflammation (e.g., proctitis) or rectal trauma, diabetes mellitus, hypothyroidism, etc.[1-8].

The demographic data and some other data of 60 CCPs and 60 HCVs are presented in Table 1.

In the medical history of the above CCPs and HCVs, the disorders associated with brain, heart, lung, liver, kidney and other organs as well as blood system, circulatory system, respiratory system and other systems were all excluded by their routine blood, urine and feces examinations as well as radiographs, cardiogram and other necessary examinations. Patients having a history of inflammation, hypertension, hyperlipidemia, acute or chronic bronchitis, asthma, autoimmune disease, diabetes, atherosclerosis, tumors and other diseases,

and subnutrition, malnutrition and other nutritional diseases were also all excluded.

TABLE 1

Demographic Data and Some Other Data in the CCPs Group and HCVs Group

Items	CCPs (*n*=60)	HCVs (*n*=60)	*P*
Age (year)	8-14 (10.8±1.9)	8-14 (10.9±1.8)	0.7299[a]
Gender	M=34 F=26	M=30 F=30	0.5832[b]
Systolic Pressure (mm Hg)	59-107 (84.4±10.7)	58-108 (84.2±10.6)	0.9115[a]
Diastolic Pressure (mm Hg)	41-65 (55.7±6.2)	43-65 (55.2±6.1)	0.6316[a]
Hemoglobin (g/L)	112-139 (133.7±6.0)	111-139 (134.0±5.3)	0.8181[a]
Albumin (g/L)	30.84-42.75 (38.24±2.48)	31.02-42.34 (38.18±2.34)	0.9013[a]
Body Mass Index	20.81-24.98 (23.28±1.07)	21.14-24.83 (23.22±1.14)	0.7691[a]

Note. [a]Independent samples *t* test; [b]Pearson Chi-square test (Exact Sig.).

Within the prior month in which the above subjects volunteered the experimentation in this study, none of the subjects had taken any antioxidant supplements such as VC, VE, ginkgo biloba, tea polyphenols or other similar substances.

Methods

Collection and pretreatment of blood samples Fasting venous blood samples from elbow vein in left arm were collected in the morning from all the subjects and heparin sodium was added as anticoagulant, and the plasma and erythrocytes separated promptly were stored at -50℃ immediately[9,10]. The blood samples collected did not undergo any hemolysis.

Determination of biomedical substances and enzymes plasma VC level Trichloroacetic acid solution was used to sedimentate proteins in plasma and to extract VC from plasma. The VC in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product that was detected at 563 nm, and the VC level was expressed as μmol/L[9,10].

Plasma VE level Absolute ethanol was used to sedimentate proteins in plasma and to extract VE from plasma. The VE in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product that was detected at 563 nm, and the VE level was expressed as μmol/L[9,10].

Erythrocyte SOD activity Spectrophotometry of inhibiting pyrogallol auto-oxidation was used to determine erythrocyte SOD activity expressed as U/g•Hb[9,10].

Erythrocyte CAT activity Spectrophotometry of coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocyte CAT activity expressed as K/g•Hb[9,10].

The main analytical reagents in determining the above biochemical substances and enzymes, such as vitamin C, vitamin E, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), Cu/Zn-superoxide dismutase, catalase, 1,2,3-trihydroxybenzene (pyrogallol), were purchased from SIGMA® Chemical Company, USA. The other analytical reagents were produced in China. Fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. In the determination of the above biochemical substances and enzymes, the main analytical instruments included Hewlett Packard 8453-Spectrophotometer, USA, and others.

In determination of the above biochemical substances and enzymes, the standardization of experiment, e.g. the same batch number of each reagent, the same quality control, the same laboratory assistant, and the identical analytical apparatus were strictly used for each experiment in order to decrease errors, and to ensure the analytical quality of determinations[9-12].

Medical Statistical Analysis

All data were statistically analyzed by SPSS 11.0 for Windows statistic software using a Compaq Pentium Ⅳ/2.4 GHz computer. The biochemical parameters in this study presented normal distributions by Kolmogorov-Smirnov *Z* test, and were expressed as $\bar{x} \pm s$ and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples *t* test, Pearson Chi-square test (χ^2 test), linear regression and bivariate correlation analysis. In the statistical analysis in this study, the level of hypothesis testing (α) was $\leqslant 0.05$ in order to avoid false positives (Type Ⅰ error), and the power of hypothesis testing (*power*) was $\geqslant 0.80$ to avoid false negatives (Type Ⅱ error)[9-12].

RESULTS

Comparison Between Average Values ($\bar{x} \pm s$) of Above Biochemical Parameters in the CCPs Group and the HCVs Group, and 95% CI

Compared with the average values of the biochemical parameters in the HCVs group, the average values of VC and VE in plasma as well as those of SOD and CAT in erythrocytes in the CCPs group were significantly decreased (Table 2). The upper limits of 95% CI of the average values of VC and VE in plasma as well as those of SOD and CAT in erythrocytes in the CCPs group were less than the lower limits of 95% CI of those in the HCVs group (Table 2).

TABLE 2

Comparison Between Average Values ($\bar{x} \pm s$) of Biochemical Parameters in the CCPs Group and the HCVs Group

Group	*n*	Plasma		Erythrocytes	
		VC (μmol/L)	VE (μmol/L)	SOD (U/g · Hb)	CAT (K/g · Hb)
CCPs	60	45.38±12.03 (42.27-48.49)	20.18±5.04 (18.88-21.48)	1927±152 (1887-1966)	261.0±68.5 (243.3-278.7)
HCVs	60	56.84±15.07 (52.95-60.73)	26.49±6.87 (24.72-28.26)	2187±179 (2141-2234)	318.7±86.0 (296.5-340.9)
t^a		4.6045	5.7385	8.5925	4.0646
P		<0.0001	<0.0001	<0.0001	<0.0001

Note. [a]Independent-samples *t* test. The figures in parentheses are 95% confidence interval (95% CI).

Linear Regression and Bivariate Correlation Analysis Between Course of Disease and Each Biochemical Parameter for 60 CCPs

The findings in linear regression and bivariate correlation analysis between the course of disease and each biochemical parameter for 60 CCPs showed that when the course of CCPs was prolonged, the levels of VC and VE in plasma as well as the activities of SOD and CAT in erythrocytes were decreased gradually (Table 3).

TABLE 3

Linear Regression and Bivariate Correlation Analysis Between Course of Disease and Each Parameter in CCPs

Items	*n*	Linear Regression	Bivariate Correlation		
			r	*t*	*P*
Course and VC	60	Y = 54.9622 - 4.3556 X	- 0.4934	4.3205	<0.0001
Course and VE	60	Y = 24.5166 -1.9712 X	- 0.5332	4.8000	<0.0001
Course and SOD	60	Y = 2065.87 - 63.319 X	- 0.5656	5.2234	<0.0001
Course and CAT	60	Y = 318.456 - 26.098 X	- 0.5190	4.6244	<0.0001

DISCUSSION

It is well known that VC and VE are important antioxidants, and SOD and CAT are important antioxidases in the human. They play important roles in scavenging superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (·OH) and other free radicals (FRs) as well as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) which are excessively generated in the human, and in preventing physiological and pathological aggravation of a series of FRs chain reactions induced by excessive $O_2^{\cdot-}$, thereby protecting the biological membranes of cells against oxidative stress and free radical damage[9-23]. Significantly decreased antioxidant levels and antioxidase activities in the human might cause metabolic disorders and pathological aggravation of a series of FRs chain reactions, thus inducing a variety of diseases associated with abnormal reactions of FRs[9-23].

The findings in this study suggested that chronic constipation might induce oxidative stress and potential oxidative damage in the bodies of children. There might be several interpretations.

In fact, constipation is a common complaint in clinical practice, and its pathophysiologic mechanisms most often involve poor colonic propulsive activity, colonic dysfunction or colonic motor disorders[1-8]. The cause of the majority of constipation patients could not be obviously identified, nor could the childhood constipation[1-8]. However, besides psychological and physiologic factors, disordered colonic transit (e.g. reduced smooth muscle contractility, lowered and disturbed bowel motility, and prolonged gut transit time) and disordered anorectal function (e.g. inhibition of the desire to evacuate and impaired defecation) might play, in all likelihood, an important role in this disorder[1-8]. Such phenomena could result gradually in increased water absorption as well as solid and hard consistency of stools, and at the same time, toxicants in stools such as ammonia, hydrogen sulfide, indole, etc. were absorbed largely by intestinal tracts, and entered into blood circulation[1-8]. In addition, such phenomena might cause intestinal flora imbalance, thereby drying up feces and aggravating constipation[1-8,24-27].

A large amount of FRs and ROS could be generated both by excessive ammonia in intestinal tracts and blood and by intestinal flora imbalance[24-34]. The excessive FRs and ROS could interact directly with DNA, thus causing DNA damage, inhibiting or depressing

DNA replication, and could attack strongly the active sites and active groups in the molecular structures of VC, VE, SOD and CAT, thereby deactivating them[9-23]. The excessive FRs and ROS could also cause oxidative decomposition and peroxidative modification of many organic compounds in the body of CCPs, thereby further producing a large amount of FRs and ROS, damaging DNA, deactivating antioxidants and antioxidases. As a consequence, the levels of VC and VE as well as the activities of SOD and CAT in CCPs were decreased markedly[9-23].

According to the data that we collected in this study, most children in the CCPs (41/60, 68.3%) often liked fried foods such as fried chicken, fried meat, fried pie, fried pork chop, fried roll, etc. from KFC® and/or McDonald's®, and did not like foods rich in vitamins and celluloses. Such phenomena not only increased the solid and hard consistency of stools, disordered the colonic transit and anorectal function, thus forming constipation[1-8,35,36], but also decreased the intake of VC, VE and other antioxidant vitamins, enhanced the oxidative decomposition and peroxidative modification of lipids and other organic compounds, thereby generating and releasing excessive FRs and ROS.

The findings in linear regression and bivariate correlation analysis in this study showed that the values of the above biochemical parameters were closely related to the course of disease in the CCPs, in other words, the longer the course of child constipation, the severer the oxidative stress and the potential free radical damage in his or her body. In addition, such a correlation further suggested that chronic constipation was a risk factor affecting children's physical and mental health.

In conclusion, the findings in the present study suggest that chronic childhood constipation causes, in all likelihood, oxidative stress, thereby resulting in potential free radical damage in the bodies of chronic childhood constipation patients.

REFERENCES

1. Hammer, H. F. (1999). Constipation. In *Gastroenterology and Hepatology* (Porro, G. B., Cremer, M., Krejs, G., Ramadori, G., Rask-Madsen, J., and Isselbacher, K. J. Eds., 1st ed.), pp. 77-82. London, McGraw-Hill International (UK) Ltd.
2. Snape, W. J. Jr. (2000). Disorders of gastrointestinal motility. In *Cecil Textbook of Medicine* (Goldman, L. and Bennett, J. C. Eds., 6th ed.), pp. 694-702. London, W.B. Saunders Company.
3. Barnett, J. L. (2000). Approach to the patients with constipation, fecal incontinence, and gas. In *Kelley's Textbook of Internal Medicine* (Humes, H. D. Ed., 4th ed.), pp. 755-763. New York, Lippincott Williams & Wilkins Inc.
4. Harty, R. F. and Ojeas, H. S. (1996). Colon. In *Essentials of Pathophysiology* (Kaufman, C. E. and Mckee, P. A. Eds., 1st ed.), pp. 199-210. Philadelphia, Lippincott Williams & Wilkins.
5. Friedman, L. S. and Isselbacher, K. J. (1998). Diarrhea and constipation. In *Harrison's principles of internal medicine* (Fauci, A. S., Braunwald, E., Isselbacher, K. J., Wilson, J. D., Martin, J. B., Kasper, D. L., Hauser, S. L., and Longo, D. L., Eds., 14th ed.), pp. 236-244. London, McGraw-Hill Health Professions Division.
6. Lingappa, V. R. and Farey, K. (2000). *Physiological Medicine: A Clinical Approach to Basic Medical Physiology* (1st ed.), pp. 914-917. New York, McGraw-Hill Press.
7. Lingappa, V. R. (2000). Gastrointestinal disease. In *Pathophysiology of disease. An introduction to clinical medicine* (McPhee, S. J., Lingappa, V. R., Ganong, W. F. and Lange, J. D., Eds., 3rd ed.), pp. 293-326. New York, McGraw-Hill Companies, Inc.
8. Lennard-Jones, J. E. (1998). Constipation. In *Sleisenger & Fordtran's Gastrointestinal and Liver Disease Pathophysiology/Diagnosis/Management* (Feldman, M., Scharschmidt, B. F., and Sleisenger, M. H. Eds., 6th ed.), pp. 174-197. New York, Harcount Publishers Limited.
9. Zhou, J. F., Cai, D., and Tong, G. Z. (2003). Oxidative stress and potential free radical damage associated with photocopying. A role for ozone? *Free Radic. Res.* **37**, 137-143.
10. Zhou, J. F., Chen, P., Zhou, Y. H., Zhang, L., and Chen, H. H. (2003). 3,4-Methylenedioxymeth-amphetamine (MDMA) abuse may cause oxidative stress and potential free radical damage. *Free Radic. Res.* **37**, 491-497.
11. Zhou, J. F., Zhou, Y. H., Zhang, L., Chen, H. H., and Cai, D. (2003). 3,4-Methylenedioxymeth-amphetamine (MDMA) abuse markedly inhibits acetylcholinesterase activity and induces severe oxidative damage and

lipoperoxidative damage. *Biomed. Environ. Sci.* **16**, 53-61.
12. Zhou, J. F., Chen, W. W., and Tong, G. Z. (2003). Ozone emitted during copying process - a potential cause of pathological oxidative stress and potential oxidative damage in the bodies of operators. *Biomed. Environ. Sci.* **16**, 95-104.
13. Zhou, J. F., Yan, X. F., Guo, F. Z., Sun, N. Y., Qian, Z. J., and Ding, D. Y. (2000). Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. *Biomed. Environ. Sci*. **13**, 44-55.
14. Zhou, J. F., Cai, D., Zhu, Y. G., Yang, J. L., Peng, C. H., and Yu, Y. H. (2000). A study on relationship of nitric oxide, oxidation, peroxidation, lipoperoxidation with chronic cholecystitis. *World J. Gastroenterology* **6**, 501-507.
15. Zhou, J. F., Xu, G. B., and Fang, W. J. (2002). Relationship between acute organophosphorus pesticide poisoning and oxidative damages induced by free radicals. *Biomed. Environ. Sci*. **15**, 177-186.
16. Xie, B., Zhou, J. F., Lu, Q., Li, C. J., and Chen, P. (2002). Oxidative stress in patients with acute coxsackie virus myocarditis. *Biomed. Environ. Sci*. **15**, 48-57.
17. Chen, P. and Zhou, J. F. (2001). Abnormal metabolism of nitric oxide, oxidative stress and lipoperoxidative stress in patients with acute viral myocarditis. *Chinese Medical Journal* **114**, 1132-1135.
18. Zhou, J. F., Yan, X. F., Ruan, Z. R., Peng, F. Y., Cai, D., Yuan, H., Sun, L., Ding, D. Y., and Xu, S. S. (2000). Heroin abuse and nitric oxide, oxidation, peroxidation, lipoperoxidation. *Biomed. Environ. Sci.* **13**, 131-139.
19. Zhou, J. F. and Chen, P. (2001). Studies on the oxidative stress in alcohol abusers in China. *Biomed. Environ. Sci.* **14**, 180-188.
20. Chen, H. H. and Zhou, J. F. (2001). Low cholesterol in erythrocyte membranes and high lipoperoxides in erythrocytes are the potential risk factors for cerebral hemorrhagic stroke in human. *Biomed. Environ. Sci.* **14**, 189-198.
21. Zhou, J. F., Chen, J. X., Shen, H. C., and Cai, D. (2002). Abnormal reactions of free radicals and oxidative damages in the bodies of patients with chronic glomerulonephritis. *Biomed. Environ. Sci.* **15**, 233-244.
22. Zhou, J. F., Si, P. L., Ruan, Z. R., Ma, S. H., Yuan, H., Peng, F. Y., Sun, L., Ding, D. Y., and Xu, S. S. (2001). Primary studies on heroin abuse and injury induced by oxidation and lipoperoxidation. *Chinese Medical Journal* **114**, 297-302.
23. Zhou, J. F., Chen, P., Yang, J. L., Zhu, Y. G., Peng, C. H., and Wu, Y. L. (2000). Oxidative stress before and after operation in patients with chronic cholecystitis containing gallstone. *Biomed. Environ. Sci.* **13**, 254-262.
24. Johnson, S. (2001). Clostridial constipation's broad pathology. *Med. Hypotheses* **56**, 532-536.
25. Peshenko, I. V., Singh, A. K., and Shichi, H. (2001). Bovine eye 1-Cys peroxiredoxin: expression in *E. coli* and antioxidant properties. *J. Ocul. Pharmacol. Ther.* **17**, 93-99.
26. De-Koning-Ward, T. F. and Robins-Browne, R. M. (1997). A novel mechanism of urease regulation in Yersinia enterocolitica. *FEMS. Microbiol. Lett.* **147**, 221-226.
27. Martin, G., Haehnel, W., and Boger, P. (1997). Oxidative inactivation of glutamine synthetase from the cyanobacterium Anabaena variabilis. *J. Bacteriol.* **179**, 730-734.
28. Dakshayani, K. B., Velvizhi, S., and Subramanian, P. (2002). Effects of ornithine alpha-ketoglutarate on circulatory antioxidants and lipid peroxidation products in ammonium acetate treated rats. *Ann. Nutr. Metab.* **46**, 93-96.
29. Rouach, H., Fataccioli, V., Gentil, M., French, S. W., Morimoto, M., and Nordmann, R. (1997). Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. *Hepatology* **25**, 351-355.
30. Kosenko, E., Kaminsky, Y., Kaminsky, A., Valencia, M., Lee, L., Hermenegildo, C., and Felipo, V. (1997). Superoxide production and antioxidant enzymes in ammonia intoxication in rats. *Free Radic. Res.* **27**, 637-644.
31. Kosenko, E., Kaminski, Y., Lopata, O., Muravyov, N., and Felipo, V. (1999). Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication. *Free Radic. Biol. Med.* **26**, 1369-1374.
32. Iino, T., Tashima, K., Umeda, M., Ogawa, Y., Takeeda, M., Takata, K., and Takeuchi, K. (2002). Effect of ellagic acid on gastric damage induced in ischemic rat stomachs following ammonia or reperfusion. *Life Sci.* **70**, 1139-1150.
33. Kosenko, E., Kaminsky, Y., Lopata, O., Muravyov, N., Kaminsky, A., Hermenegildo, C., and Felipo, V. (1998). Nitroarginine, an inhibitor of nitric oxide synthase, prevents changes in superoxide radical and antioxidant enzymes induced by ammonia intoxication. *Metab. Brain. Dis.* **13**, 29-41.
34. Murthy, C. R., Rama-Rao, K. V., Bai, G., and Norenberg, M. D. (2001). Ammonia-induced production of free radicals in primary cultures of rat astrocytes. *J. Neurosci. Res.* **66**, 282-288.
35. Clark, J. H., Russell, G. J., Fitzgerald, J. F., and Nagamori, K. E. (1987). Serum beta-carotene, retinol, and alpha-tocopherol levels during mineral oil therapy for constipation. *Am. J. Dis. Child.* **141**, 1210-1212.
36. Laurin, D., Brodeur, J. M., Bourdages, J., Vallee, R., and Lachapelle, D. (1994). Fibre intake in elderly individuals with poor masticatory performance. *J. Can. Dent. Assoc.* **60**, 443-449.

(*Received September* 4, 2003 *Accepted December* 30, 2003)