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# EFFECT OF DIETARY ADDITION OF myo-INOSITOL ON THE METABOLIC CHANGES IN RATS EXPOSED TO 1,1,1-TRICHLORO-2,2-BIS(P-CHLOROPHENYL)ETHANE

Tetsuyuki Katayama Ph.D.
Laboratory of Nutritional Science,
Faculty of Education, Hiroshima University,
Higashi-Hiroshima 724, Japan

## ABSTRACT

Feeding of 0.1% 1,1,1-trichloro-2,2-bis(P-chlorophenyl)ethane (DDT) -containing diet for 13-14 days to rats caused a reduction in growth rate, which was significantly ameliorated by dietary addition of myo-inositol. Dietary DDT increased liver weight, liver lipids, serum cholesterol, serum phospholipid, serum copper or ceruloplasmin activity, liver and urinary ascorbic acid and activities of serum glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) as well as liver and serum thiobarbituric acid reactive substances (TBA-RS). The increases in liver weight, liver cholesterol, liver triglyceride, liver total lipids, serum cholesterol and serum GOT activity due to DDT were significantly suppressed by dietary addition of myo-inositol. In addition, dietary myo-inositol caused a decreasing trend in serum phospholipid and serum GPT activity in DDT-fed animals. On the other hand, the increase in urinary ascorbic acid due to DDT was potentiated with dietary myo-inositol. In the animals without receiving DDT, dietary myo-inositol caused no significant effect on these metabolic parameters except serum ceruloplasmin activity, which was decreased by myo-inositol intake. These results suggest that rats fed DDT containing diet may require an exogenous source of myo-inositol.

KEY WORDS: 1,1,1-trichloro-2,2-bis(P-chlorophenyl)ethane, Dietary myo-inositol, Glutamate-oxaloacetate transaminase, fatty liver, Ascorbic acid, Copper

## INTRODUCTION

Many reports have showed that myo-inositol functions as a lipotropic factor (1-8). Burton and Wells demonstrated that under stress of lactation, myo-inositol deprived diet leads to a formation of fatty liver provided phthalylsulfathiazole is added to inhibit the growth of intestinal bacteria which are known to synthesize myo-inositol (4). A similar effect had been previously noted in laying hens (6). From these facts, Kuksis and Mookerjea speculated that the physiological stress of lactation or laying might greatly increase the requirement of myo-inositol indispensable for the normal function of a tissue (7).

On the other hand, some inducers of drug-metabolizing enzymes, or xenobiotics including DDT, polychlorinated biphenyls (PCB) and phenobarbital, induce fatty liver and liver lesion in laboratory animals (9-12). In addition, these chemicals result in an increase in serum cholesterol and phospholipid (13-14). Recently, Nagaoka et al. have reported that PCB intake causes an increase in the urinary excretion of histamine and catecholamines, which is generally increased with stressful situations (15). These facts mentioned above seems to suggest that animals

fed xenobiotics may require an exogenous source of myo-inositol unless they possess the enzymes of the biosynthetic pathway or biosynthesis by intestinal flora.

Furthermore, previous studies demonstrated that administration of some xenobiotics to rats also increased tissue and urinary ascorbic acid and serum copper or ceruloplasmin activity (16-17). In addition, it has been reported that the metabolic changes in serum and liver lipids due to xenobiotics intake are influenced by nutritional status of ascorbic acid and copper (18-19). Therefore, an attempt was made to investigate the effect of dietary addition of myo-inositol on the changes in concentrations of liver and serum lipids, liver and urinary ascorbic acid and serum copper and the activity of serum ceruloplasmin due to DDT intake in rats.

## MATERIAL AND METHOD

After feeding the commercial stock diet (MF, Oriental Yeast Co. Ltd., Tokyo) for 4 days, male rats of the Wistar strain weighing 83 to 115g were divided into four groups of six rats each. The basal diet was according to the AIN formula, which did not contain myo-inositol (20). Half of the groups were fed diets containing 0.1% DDT (Tokyo Kasei Ind. Ltd., Tokyo). In the myo-inositol supplemented groups, the diets contained 0.2% myo-inositol (Nakarai Chemicals Ltd., Kyoto). The dietary addition of DDT and myo-inositol was made at the expense of sucrose. All rats were individually housed and were provided feed and deionized water ad libitum. The animals room was maintained at 24 ± 1 ℃ and illuminated for 12 h from 8:00 a.m. On day 9-11, urine was collected into 30ml of 5% metaphosphoric acid solution, filtered and used for the determination of ascorbic acid. After feeding the test diets for 13 to 14 days, the diets were removed from cages at 8:00 a.m. and the rats were lightly anesthetized with ether and killed between 1:00 p.m. and 3:00 p.m. Aliquots of blood were allowed to clot and serum samples were isolated by centrifugation at 4℃. Liver was immediately excised and weighed. Serum and liver were stored in a freezer maintained at -20℃ until analysis.

Activities of GOT and GPT in serum were determinated with a commercially available kit (Transaminase CⅡ-Test, Wako Pure Chemical Industries Ltd., Osaka). Oxidase activity of serum ceruloplasmin was determined by the method of Schosinsky et al., in which o-dianisidine dihydrochloride was used as substrate (21). Serum TBA-RS were fluorometorically measured by the method of Yagi (22). Serum copper was determined With a commercial available kit (Cu-Neotest, Sinotest Ltd., Tokyo). Serum total cholesterol, free cholesterol, high density lipoprotein (HDL) cholesterol, phospholipid and triglyceride were determined as described previously (18). Serum free fatty acid was determined by the Duncombe procedure (23). Serum protein was measured according to the method of Gornall et al.(24). Liver lipids were extracted by the method of Folch et al.(25). Liver total lipids and triglyceride were determined as described previously (18). Liver phospholipid was measured with the method of Stewart (26). Liver free cholesterol was isolated by the method of digitonin precipitation (27). Liver total and free cholesterol were determined by the method of Zlatkis et al.(28). Esterified cholesterol in liver and serum was calculated from total and free cholesterol. Liver TBA-RS were measured according to the method of Masugi and Nakamura (29). Ascorbic acid in urine and liver was determined by the 2,4-dinitrophenyl hydrazine method (30). Liver protein was measured by using a Bio-Rad Protein Assay, which is a dye-binding assay.

The statistical significance of difference between values of the data

was analyzed by Duncan's multiple-range test (31).

## RESULTS

Table 1 describes the influence of dietary 0.2% myo-inositol on gains in body weight, liver weight, liver concentrations of protein, lipids, TBA-RS and ascorbic acid and urinary excretion of ascorbic acid in rats fed DDT. Rats were maintained on the test diets for 13-14 days, since some xenobiotics intake and a special myo-inositol deficient diet cause fatty liver and metabolic changes in rats in a short period time (4-14 days). Growth was significantly depressed by dietary 0.1% DDT. The growth depression due to DDT was ameliorated by dietary addition of 0.2% myo-inositol to the diet. In the animals without receiving DDT, myo-inositol caused no significant effect on growth.

As shown in table 1, DDT intake caused an increase in liver weight (% of body wt.), liver total lipids, cholesterol and triglyceride. These effects were clearly depressed by dietary myo-inositol.

Table 1

Effect of dietary myo-inositol on growth, liver weight, protein, lipids, thiobarbituric acid reactive substances(TBA-RS) and liver and urinary ascorbic acid in rats fed 0.1% DDT-containing diet[1].

| Groups | Normal | Normal+ myo-inositol | DDT | DDT+ myo-inositol |
|---|---|---|---|---|
| Gains in body wt. g/13 days | 117 ± 4[a 2] | 110 ± 4[a b] | 93 ± 3[c] | 105 ± 4[b] |
| Liver % of body wt. | 5.43 ± 0.10[a] | 5.25 ± 0.13[a] | 7.56 ± 0.20[c] | 6.59 ± 0.13[b] |
| Protein mg/g tissue | 176 ± 5[a] | 166 ± 5[a] | 169 ± 5[a] | 175 ± 6[a] |
| Total lipids mg/g tissue | 52.8 ± 5.5[a] | 41.6 ± 1.0[a] | 91.8 ± 8.1[b] | 56.6 ± 2.5[a] |
| Cholesterol mg/g tissue | 4.21 ± 0.29[a] | 3.80 ± 0.14[a] | 6.91 ± 0.47[b] | 4.56 ± 0.14[a] |
| Phospholipid mg/g tissue | 25.1 ± 0.62[a] | 24.2 ± 0.81[a] | 31.3 ± 0.26[b] | 33.8 ± 1.1[c] |
| Triglyceride mg/g tissue | 23.5 ± 5.8[a] | 13.6 ± 1.2[a] | 53.4 ± 7.9[b] | 18.2 ± 1.4[a] |
| TBA-RS nmol/g tissue | 103 ± 4[a] | 99 ± 7[a] | 139 ± 7[b] | 153 ± 10[b] |
| Ascorbic acid µg/g tissue | 221 ± 17[a] | 253 ± 12[a] | 434 ± 11[b] | 482 ± 12[c] |
| Urine Ascorbic acid mg/100g body wt/day | 0.87 ± 0.16[a] | 0.85 ± 0.11[a] | 6.62 ± 0.52[b] | 8.56 ± 1.04[c] |

1. Initial body weight, average 101g(83-115g) and feeding period 13 to 14 days.
2. Mean ± SE (N=6). Means not followed by the same letter are significantly different($p<0.05$).

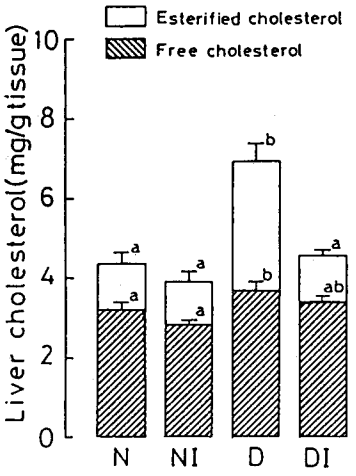


Figure 1. Effect of dietary 0.1% DDT and myo-inositol on liver free and esterified cholesterol. White bars indicate esterified cholesterol. Hatched bars indicate free cholesterol. Each bar indicates mean ± SE (N = 6). Means not followed by the same letter are significantly different ($p<0.05$). N; Normal, NI; Normal-inositol, D; DDT, DI; DDT-inositol.

Table 2

Effect of dietary addition of myo-inositol on protein, lipids, TBA-RS, copper and ceruloplasmin activity in serum of rats fed 0.1% DDT.

| Groups | Normal | Normal+ myo-inositol | DDT | DDT+ myo-inositol |
|---|---|---|---|---|
| Serum | | | | |
| Protein (mg/100 ml) | 5.46 ± 0.09[a][1] | 5.32 ± 0.08[a] | 5.57 ± 0.06[a] | 5.53 ± 0.09[a] |
| Cholesterol | | | | |
| Total(mg/100 ml) | 109 ± 7[a] | 115 ± 4[a] | 158 ± 6[c] | 141 ± 3[b] |
| Free(mg/100 ml) | 32 ± 3[a] | 32 ± 2[a] | 46 ± 3[b] | 41 ± 1[b] |
| Ester(%) | 71.0 ± 0.6[a] | 72.2 ± 0.7[a] | 70.8 ± 0.5[a] | 71.0 ± 0.7[a] |
| HDL-cholesterol (mg/100 ml) | 67 ± 5[a] | 65 ± 3[a] | 88 ± 4[b] | 76 ± 2[a] |
| Phospholipid (mg/100 ml) | 233 ± 10[a] | 233 ± 8[a] | 298 ± 6[b] | 277 ± 5[b] |
| Triglyceride (mg/100 ml) | 164 ± 18[a] | 207 ± 28[a] | 161 ± 20[a] | 217 ± 26[a] |
| Free fatty acid (mEq/100 ml) | 0.329 ± 0.035[a] | 0.436 ± 0.054[a] | 0.445 ± 0.033[a] | 0.434 ± 0.060[a] |
| TBA-RS (nmol/100 ml) | 294 ± 36[a] | 298 ± 22[a] | 598 ± 126[b] | 621 ± 73[b] |
| Copper (μg/100 ml) | 95 ± 3[a] | 80 ± 8[a] | 128 ± 5[b] | 115 ± 4[b] |
| Ceruloplasmin (IU/100 ml) | 10.7 ± 0.5[b] | 8.4 ± 1.0[a] | 16.4 ± 0.6[c] | 15.6 ± 0.5[c] |

1. Mean ± SE (N=6). Means not followed by the same letter are significantly different ($p<0.05$).

Figure 1 indicates that the change in liver concentration of cholesterol due to these dietary treatment is mainly attributable to the change in liver concentration of esterified cholesterol, but not to that of free cholesterol. In addition, liver phospholipid was significantly increased by DDT feeding, which was enhanced by dietary myo-inositol when the data were expressed per g tissue (Table 1). However, the increasing effect of dietary myo-inositol on liver phospholipid in rats fed DDT was not observed when expressed per total liver per 100g body weight (data not shown). Although liver TBA-RS was clearly increased by dietary DDT, myo-inositol had no effect. Liver concentration of protein was not appreciably affected by these dietary manipulation (Table 1).

Dietary DDT drastically increased urinary excretion of ascorbic acid, which was potentiated with a myo-inositol containing diet (Table 1). Similarly, liver ascorbic acid was remarkably increased by DDT feeding, which was also potentiated with a myo-inositol containing diet when the data were expressed per g tissue (Table 1), although dietary myo-inositol had no significant effect on liver ascorbic acid when expressed per total liver per 100g body weight (data not shown). In the rats not receiving DDT, dietary myo-inositol had no significant influence on liver weight, lipids and ascorbic acid and urinary excretion of ascorbic acid (Table 1).

Table 2 presents the effects of DDT intake and dietary myo-inositol on serum protein, lipids, copper or ceruloplasmin activity and TBA-RS levels. There were no significant differences in serum protein found among the four groups. Serum total and HDL-cholesterol levels were elevated in rats fed DDT. These effects were depressed with a myo-inositol containing diet. A similar trend of change in free cholesterol was observed in these dietary manipulation. As a result, the proportion of the ester to total cholesterol remained unchanged (Table 2). Dietary DDT also increased serum phospholipid. Depressing effect of myo-inositol on the high level of serum phospholipid due to DDT feeding was also observed, although this effect was not significant (Table 2). In the rats without receiving DDT, dietary myo-inositol had no effect on serum cholestrol and phospholipid.

There was a trend of increment in serum triglyceride with dietary addition of myo-inositol regardless of DDT intake. Serum free fatty acid was not influenced by these dietary manipulation. TBA-RS was remarkably increased in the DDT-treated groups, which was not influenced by dietary myo-inositol (Table 2).

As shown in table 3, serum GOT activity, an indication of liver injury, was elevated by DDT intake, which was significantly depressed by myo-inositol intake. A similar trend of change in serum GPT activity was observed in these dietary manipulation (Table 3).

Table 3

Effect of dietary addition of myo-inositol on the activities of serum glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) in rats fed DDT-containing diets.

| Groups | Normal | Normal+ myo-inositol | DDT | DDT+ myo-inositol |
|---|---|---|---|---|
| GOT activity (IU/1) | 31.0 ± 3.3[a] [1] | 32.2 ± 1.9[a] | 46.2 ± 7.8[b] | 31.9 ± 2.8[a] |
| GPT activity (IU/1) | 5.5 ± 0.2[a] | 5.8 ± 0.2[a] | 12.2 ± 2.9[b] | 8.7 ± 0.8[a b] |

1. Mean ± SE (N=6). Means not followed by the same letter are significantly different ($p<0.05$).

## DISCUSSION

The present study showed that dietary addition of myo-inositol at least in part alleviated DDT toxicity in rats. This effect of dietary myo-inositol was defined by improvement of growth retardation and prevention of the increase in liver weight and serum GOT activity, an indication of liver injury, due to DDT intake (Table 1, 3). In the rats receiving DDT, there was also a decreasing trend of serum GPT activity in rats fed myo-inositol (Table 3).

In consistency with previous reports (11-13), dietary DDT increased total lipids, triglyceride, cholesterol (mainly esterified cholesterol) and phospholipids in liver (Table 2, Figure 1). On the other hand, a number of authors (1-8) have reported that myo-inositol deficiency induced fatty liver in animals. Burton and Wells (4) and Hayashi et al.(5) demonstrated that the accumulation of liver lipids produced by myo-inositol deficiency was due to the accumulation of liver triglyceride and cholesterol (mainly esterified cholesterol). The rise in liver total lipids, triglyceride and cholesterol due to DDT was clearly reduced to the normal level by myo-inositol feeding (Table 1, Figure 1). These facts imply that rats fed DDT may require an exogenous source of myo-inositol because of an increased requirement of myo-inositol in rats exposed to the chemical.

In general, triglyceride and esterified cholesterol are present mainly as lipid droplets in cytosol, while phospholipid and free cholesterol are components of membrane structure (32-33). Dietary xenobiotics such as DDT cause an increase in lipid droplets of liver cytosol and in endoplasmic reticulum (34-35). The present results suggest that dietary addition of myo-inositol depresses the increment of lipid droplets in liver cytosol caused by DDT intake, but not of endoplasmic reticulum. Because, the depressing effect of dietary myo-inositol on fatty liver caused by DDT intake was mainly due to the reduction of triglyceride and esterified cholesterol, while phospholipid and free cholesterol were not apparently affected by myo-inositol in rats fed DDT (Table 1, Figure 1).

Although there have been numerous studies on the production of fatty liver in rats brought about by myo-inositol deficiency (1-8), its mechanism is not completely understood. Several investigators (3-4) have suggested the inhibition of lipoprotein formation or secretion as the major cause. Hayashi et al. (5) considered excessive mobilization of free fatty acids from adipose depots to be the more direct cause of fatty liver syndrome in myo-inositol deficiency. Recently, Beach and Flick (36) have reported that enhanced accumulation of liver triglyceride observed during myo-inositol deficiency in rats might be due in part to elevated levels of lipogenic enzymes. On the other hand, the increment of liver cholesterol caused by xenobiotics intake such as DDT is attributable to the stimulation of cholesterogenesis (14,37), although the mechanism of triglyceride accumulation due to these chemicals is largely unknown. In the present study, dietary myo-inositol not only depressed the accumulation of liver cholesterol and triglyceride in liver due to DDT intake, but also decreased serum cholesterol in rats fed DDT (Table 2). A similar decreasing effect of myo-inositol was observed in serum phospholipid of rats fed DDT (Table 2). Serum triglyceride and free fatty acid were not apparently affected by these dietary manipulation (Table 2). The present results suggest that curative effect of myo-inositol on fatty liver in rats fed DDT containing diet may result from the depression of de novo synthesis of triglyceride and cholesterol and/or the enhanced degradation of these lipids. Further studies are in progress to examine the mechanism of curative effect of myo-inositol on fatty liver in rats fed DDT.

The enhanced lipid peroxidation in liver by $CCl_4$ administration to rats is known to repress the release of very low density lipoprotein (VLDL) from liver into the blood, which in turn results in the accumulation of liver triglyceride (38). Some inducers of drug-metabolizing enzymes including DDT, PCB and phenobarbital are known to induce a lipid peroxidation in the liver (9-10). However, Kato et al. (12) and Oda et al.(39) considered that lipid peroxidation is not responsible for the fatty liver by these inducers; since high dietary levels of vitamin E and dietary antioxidant, N,N'-diphenyl-p-phenylenediamine, effectively depressed the enhancement of lipid peroxidation due to PCB, but not fatty liver due to the chemical. This idea is further supported by the present data that dietary myo-inositol depressed an increase in liver lipids due to DDT intake, but the nutrient caused no influence on the increase in liver and serum TBA-RS (Table 1, 2).

The administration of DDT causes a marked increases in urinary excretion of ascorbic acid and tissue level of ascorbic acid in rats (16). There is substantial evidence that this phenomenon is caused by a stimulation of ascorbic acid biosynthesis in the liver (16). In accordance with previous reports (13,16), DDT intake remarkably increased liver ascorbic acid and urinary excretion of ascorbic acid. It is interesting that the increase in urinary ascorbic acid due to DDT intake was significantly potentiated with dietary myo-inositol. Myo-inositol is oxidized to D-glucuronic acid in animals (40). The D-glucuronic acid undergoes reduction to L-gulonic acid, which serves as the precursor of L-ascorbic acid (41). Thus, increasing effect of dietary myo-inositol on urinary ascorbic acid in rats fed DDT might relate to this pathway.

The type of dietary carbohydrate has been shown to influence copper metabolism (42). In the present study, supplementation with myo-inositol produced a slight but significant decrease in the activity of serum ceruloplasmin in rats fed DDT uncontaining diet (Table 2). A similar trend was observed in serum copper in rats fed DDT uncontaining diet (Table 2). Thus, myo-inositol also may affect the metabolism of copper.

The present results seems to suggest that the exposure to certain xenobiotics including DDT might be useful to elucidate the physiological and metabolic functions of myo-inositol. In this study, the experimental diets were fed to young rats for 13-14 days, but a longer period of experiment will be necessary to investigate the effect of dietary addition of myo-inositol on the metabolic changes in rats exposed to DDT to obtain further information on the physiological and metabolic functions of myo-inositol.

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## REFERENCES


1. Gavin G, McHenry EW. Inositol: A lipotropic factor. J Biol Chem 1941; 139:485.

2. Handler P. Dietary factors in the regulation of liver lipid concentration. J Biol Chem 1946; 162:77-85.

3. Kotaki A, Sakurai T, Kobayashi M, Yagi K. Studies on myo-inositol: Ⅳ. Effect of myo-inositol on the cholesterol metabolism of rats suffering from experimental fatty liver. J Vitaminol 1968; 14:87-94.

4. Burton LE, Wells WW. Characterization of the lactation-dependent fatty liver in myo-inositol deficient rats. J Nutr 1977; 107:1871-1883.

5. Hayashi E, Maeda T, Tomita T. The effect of myo-inositol deficiency on lipid metabolism in rats: Ⅱ. The mechanism of triacylglycerol accumulation in the liver of myo-inositol-deficient rats. Biochim Biophys Acta 1974; 360:146-155.

6. Reed JR, Deacon LE, Far F, Couch JR. Inositol and the fatty liver syndrome. 23rd Annual Texas Nutrition Conference 1968; pp40-54.

7. Kuksis A, Mookerjea S. Inositol. Nutrition Reviews 1978; 36:233-238.

8. Andersen DB, Holub BJ. Myo-inositol responsive liver lipid accumulation in the rat. J Nutr 1980; 110:488-495.

9. Innami S, Ono S, Nakamura A. Effect of polychlorinated biphenyls (PCB) on lipid peroxide formation in rats. Fukuoka Igakuzzashi 1979; 70:102-108.

10. Hahn HKJ, Tima DJ, Bark AJ, Sorrell MF. Effect of phenobarbital on lipid peroxidation in the liver. Biochem Pharmacol 1976; 25:769-772.

11. Itokawa Y. Effect of simultaneous administration of polychlorinated biphenyls and alkylbenzene sulfonic acid salt in rats. Arch Environ Contam 1975; 3:115-131.

12. Kato N, Kawai K, Yoshida A. Effects of dietary polychlorinated biphenyls and protein level on liver and serum lipid metabolism of rats. Agric Biol Chem 1982; 46:703-707.

13. Katayama T, Momota Y, Watanabe Y, Kato N. Elevated concentrations of α-tocopherol, ascorbic acid, and serum lipids in rats fed polychlorinated biphenyls, chlorobutanol, or phenobarbital. J Nutr Biochem 1991; 2:92-96.

14. Nagaoka S, Masaki H, Aoyama Y, Yoshida A. Effects of excess dietary tyrosine or certain xenobiotics on the cholesterogenesis in rats. J Nutr 1986; 116:726-732.

15. Nagaoka S, Kato M, Aoyama Y, Yoshida A. Comparative studies on the hypercholesterolaemia induced by excess dietary tyrosine or polychlorinated biphenyls in rats. Brit J Nutr 1986; 56:509-517.

16. Horio F, Yoshida A. Effects of some xenobiotics on ascorbic acid metabolism in rats. J Nutr 1982; 112:416-425.

17. Ohchi H, Kusuhara T, Katayama T, Ohara K, Kato N. Effects of dietary

xenobiotics on the metabolism of copper, α-tocopherol and cholesterol in rats. J Nutr Sci Vitaminol 1987; 33:281-288.

18. Kato N, Kawai K, Yoshida A. Effect of dietary level of ascorbic acid on the growth, hepatic lipid peroxidation, and serum lipids in guinea pigs fed polychlorinated biphenyls. J Nutr 1981; 111:1727-1733.

19. Katayama T, Ohara K, Kusuhara T, Momota Y, Kato N. Influence of copper deficient diet on the metabolic changes in rats exposed to PCB. Nutr Rep Int 1989; 39:963-971.

20. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 1977; 107:1340-1348.

21. Schosinsky KH, Lehmann HP, Beeler MF. Measurement of ceruloplasmin from its oxidase activity in serum by use o-dianisidine dihydrochloride. Clin Chem 1974; 20: 1556-1563.

22. Yagi K. A simple fluorometric assay lipoperoxide in blood plasma. Biochemical Medicine 1976; 15:212-216.

23. Duncombe WG. The colorimetric micro-determination of long-chain fatty acids. Biochem J 1963; 88:7-10.

24. Gornall AG, Bardawill CJ, David MM. Determination of serum protein by means of the biuret reaction. J Biol Chem 1949; 177:751-766.

25. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; 226:497-507.

26. Stewart JCM. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. Anal Biochem 1980; 104:10-14.

27. Sperry WM, Webb M. A revision of the Schoenheimer-Sperry method for cholesterol determination. J Biol Chem 1950; 187:97-112.

28. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med 1953; 41:486-492.

29. Masugi F, Nakamura T. Measurement of thiobarbituric acid value in liver homogenate solubilized with sodium dodecylsulphate and variation of the values affected by vitamin E and drugs. Vitamins (Kyoto) 1977; 51:21-29.

30. Ohara T. Shokuhinbunseki Handbook pp301-305, Kenpaku Co., Tokyo (1973).

31. Duncan DB. Multiple range and multiple F test. Biometrics 1955; 11:1-6.

32. Sugano M, Imaizumi K. Cholesterol (in Japanese) pp122-145, Sankyo pub. Inc., Tokyo (1986).

33. Spiro MJ, Mckibbin JM. The lipides of rat liver cell fractions. J Biol Chem 1956; 219:643-653.

34. Kimbrough RD, Linder RE, Chamblee TBG. Morphological changes in livers of rats fed polychlorinated biphenyls. Arch Environ Health 1972; 25:354-364.

35. Jonsson HT, Walker EM, Hughson MD, HEnnigar GR. Effects of prolonged

exposure to dietary DDT and PCB on rat liver morphology. Arch Environm Contam Toxicol 1981; 10:171-183.

36. Beach DC, Flick PK. Early effect of myo-inositol deficiency on fatty acid synthetic enzymes of rat liver. Biochim Biophys Acta 1982; 711:452-459.

37. Jenke HS. Polychlorinated biphenyls interfere with the regulation of hydroxymethylglutaryl-coenzyme A reductase activity in rat liver via enzyme-lipid interaction and at the transcriptional level. Biochim Biophys Acta 1985; 837:85-93.

38. Luzio NR. Antioxidants, lipid peroxidation and chemical-induced liver injury. Fed Proc 1973; 32:1875-1881.

39. Oda H, Yamashita K, Sasaki S, Horio F, Yoshida A. Long-term effects of dietary polychlorinated biphenyls and high level of vitamin E on ascorbic acid and lipid metabolism in rats. J Nutr 1987; 117:1217-1223.

40. Charalampous F. Biochemical studies on inositol: Ⅵ. Mechanism of cleavage of inositol to D-glucuronic acid. J Biol Chem 1960; 235:1286-1293.

41. Conny AH, Bray GA, Evans C, Burns JJ. Metabolic interactions between L-ascorbic acid and drugs. Ann. NY Acad Sci 1961; 92:115-127.

42. Fields M, Ferretti RJ, Smith JC, Reiser S. The interaction of type of dietary carbohydrate with copper deficiency. Am J Clin Nutr 1984; 39:239-245.

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