Cadmium Induced Serum Biochemicals Changes in Subcronically Exposed Rats

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The increasing enviromental and occupational exposure to cadmium reveals the need for biochemical indicators of cadimum exposure and toxicity. Since the most important effects of cadmium poisoning were nephrotoxicity and hepatoxicity, the indices of renal and hepatic function and lipid peroxide product in serum were determinated. Serum concentration of calcium and phosphate were also monitored during our experiment. Wistar rats were given Cd doses, 0, 0.5, 1, 2 mg/kg, dailly gavage, for up to 8 weeks. Serum activities of aspartate and alanine aminotransferase were elevated as early as 6 weeks of Cd exposure and exhibited a dose dependence of Cd treatment. Renal dyfunction were observed at the 8th week in the maximum dose of Cd treated groups shown by th elevation of blood urea nitrogen and creatinine concentration. Lipid peroxide product reveals significant elevation at the 8th week. Calcium and inorganic phosphate exhibited a earlier reduction to that observed at renal dynfunction. These data suggested that the early indicators of Cd induced toxicity were those biochemicals altered by liver damage.

Key Words: Cadmium, Renal Dynfunction, Lipid Peroxide

Cadmium, one of the most toxic metal in our enviroment, is known to induce various human diseases for decades. Chronic human expouse to cadmiun results in different maladies including osteomalacia[1], hypertension[2], proteinuria[3] and emphysema[4]. The previous reports indicate that the kidney and liver are the target organ of cadmiun poisioning and distribution.[5, 6]

Friberg et al[7] first discoverd the ratio of renal dynfunction in workers occupationally exposed to cadmium were higher than the non-exposed workers. For this metal there seems to be abudent evidence from several laboratories that cadmium bond to metallothionien enter the renal proximal tubular cells after filtration of the protien-metal complex [8, 9] Once inside the cell, the complex decomposed to release cadmiun, this accumulation disturbs the activity of metal binding enzyme[10], which reduce the renal damage

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including proximal tubule atrophy, and fiberosis [11]. Jonhan et al[12] postulated that once absorbed the first distributed organ of cadmium was liver. There is abudent evidences that cadmium induce toxic hepatic damage both in acute or chronic exposure [13, 14], ranging from minor hepatic dynfunction to necrosis or cirrhosis.[15, 16]

Klimegak, J et al[17] propose that by the inhibition of reductase and catalase activity, lipid peroxidation were stimulated by cadmium exposure and may play an important role both in liver and kidney injury. Nephrotoxicity also reduce renal hydroxylation of vitamin D to its active metabolities 1,25 -dihydroxy-cholecaciferol [18, 19]. These factor results in decreasing calcuim absorption and reducing bone mineralization. Koji Nogawa et al[20] have demonstrated that serum calcium and phosphate concentration were clearly reduced in the Cd-exposed people while the serum parathyroid hormone was elevated to mediate the calcium metabolism. Though only a small portion of dietary cadmium (<0.6%) is absorbed, the ingestion were the main path of human cadmium intake.[21]

The purpose of our study were to evaluate the alteration of some represantive biochemicals in rat's serum following gastional exposed to cadmium and the prograssing interaction of renal dynfunction and hypocalcium were monitored. The results obtained may serve as a basis for future clinical diagnosis in human beings poisoned by cadmium.

MATERIALS AND METHODS

Animals and treatments

Wistar rats weighing 200-250g were obtained from the animal center of the National Tainwan University and were kept at six per cage in a standard-controlled room. Animals were allowed appropriate rodent chow and water *ad libitum*. Animals were given cadmium ($CdCl_2 \cdot H_2O$; Merk Co.) at dose 0, 0.5, 1.0, 2.0 Cdmg/kg in 2 ml normal saline by gavaging with 4-inch feeding needle.

Animals were anesthetized with ether and blood (2 c.c.) was obtained by cardiac puncure two weeks a time. The blood was allowed to clot, centrifuged and the serum aspirated. In that time inorganic phosphate and calcium value in serum were monitored. After 8 weeks of administration rats were killed and the major organ were excised, rinsed with normal saline, and weighted. Whole blood were collected and portion of liver tissure were frozen for later determination of biochemical value.

Hepatic damage

The damage of liver were evaluated by the elevation of enzyme activity as asparate aminotransferase (AST), alanine aminotranaferase (ALT), which were determinated by the kinetic method postulated by Karmen [22].

Renal damage

The elevated level of creatinine and blood urea nitrogen in serum were used as a means to evaluate renal damage. Creatinine was measured by Jaff's method and blood urea nitrogen were detected by urease-indophenol method. Calcium and inorganic phosphate were also evaluated in serum by o-cresolphthalein complexone and molybdenum blue method to show the intention of hypocalcium. [22] The former parameter in serum were measured by Beckman Co. Synchron clinical system AS^{TM}8.

Serum lipid peroxide level

20 μl of rat serum were used for the assay of lipoperoxide according to the method demostrated by Burnheim and modi-

fied by Kunio Yagi[23], 1, 1, 3, 3-tetraethoxy propane (TEP) was used as a standard for the thiobarbituric acid (TBA) reaction. The TBA reactive substances were measured by Perkin-Elmer fluorescence spectrometer LS–3.

RESULT

Body and specific organ weight

Figure 1 illustrated the weight grains of variously treated rats for the duration of this experiment. For the data reported here, there were no significant differences in the final weight among the four groups of animals. The specific weight of kidney, liver, pencreas, testes (g/100g body weight) were shown in table 1, All of the Cd-treated groups showed significant ($P < 0.05$) inereased in specific liver weight after 8 weeks of treatment when compared to the rats receiving the normal diet. Kidney weight also increase significantly ($P < 0.05$) in groups 2.0, 1.0 mgCd/kg. There were no considerable difference of other organ weight among the Cd-treated and normal groups.

Liver damage

Biochemical evidence of cd-induced hepatic damage was observed 6 weeks at the beginning of Cd treatment (Figure 2). At this time, serum AST activity in Cd treated has all increased more than 85% above normal group, and rose gradually at 8th week of treatment. Similar inceasing pattern was observed with respect to ALT activity.

Renal damage

Figure 3 illustrated the concentration (μg/dl) of blood urea nitrogen and creatinine in serum. After 8 weeks of Cd administration, 2.0mg Cd/kg treated group showed a higher creatinine value (56%) than normal group, simular but less results were observed in 1.0 and 0.5 treated groups. Urea nitrogen also shows a medial increasing, but was not so obvious as creatinine in this study.

Lipid peroxide product in serum

Figure 4, showed a significent ($P < 0.05$) increase in TBA-reactive substance (A523 values x 10/20mg serum) was obsreved in rats treated with different doses of Cd relative to the rats without Cd treatment. A dose dependence also reveals in our data.

Calcium and Inorganic phosphate in serum concentration

The serum concentration of calcium and inorganic phosphate (ug/dl) during our experiment were illustrated in figure 5. Only 2.0mg treated rats showed a decreasing of calcium value from 6th to 8th week.

Inorganic phosphate were also observed reductions in 2.0mg and 1.0mg treated rats after 6 weeks of Cd treatment but simular results were not observed in 0.5 mg treated group.

DISSCUSSION

There is no significantly reduced weigh gains in the Cd treated groups during our experiment. Leskey et al[24] reported that reduced weight grains in rats were observed after 2 week injection with 3.66 mg/kg $CdCl_2$ but similar effect was not shown at the lower dose 2.8mg/kg. Baranski et al[25] indicated that there were no significant difference on body weight after gavaging rat with 4mg/kg Cd for 14 weeks. The ambigurous effect of low-dose cadmium to rat weight gain may be supposed to the compensation of cd-induced elevation of serum corticosterone level.[26]

Our study indicated that the occurence of Cd-indued heptic dyfunction is prior to renal dynfuction. As early as 6 weeks of Cd

treatment, serum enzyme activites indices of hepatic injury were elevated. In contrast, execpt the hightest Cd dosing group, comparable changs in indices of renal dynfunction were not demostrated until 8th week of Cd treatment. Our results were supported by the previous reports that once absorbed by ingestion the early ratio of ^{109}Cd in liver is ten times greater than in kidney[27]. Numerous reports[28, 29] indicated that the accumulation of Cd-Metallothionine may play an important part in nephrotoxicity induced by chronic Cd exposure. Duley et al[14] also demonstrated that minor Cd-induced hapatic damage resulted in a substantial increase in circulating Cd-MT. Which was freely filtered at the glomerulus and reasorbed by a proximal system which appears to specific for anionic proteins.[30]

The serum TBA reactive substance seen in Cd treated rats was higher than the normal group after 8 weeks of treatment. This result indicated that lipid peroxidation might involve in the toxicity of Cd exposure in our experiment.

It is interest to note that calcium and inorganic phosphate were show reduction at the 6th week in the group 3. Which is prior to the elevation seen in serum creatinine and urea nitrogen. This result may be supposed that there must be a less severe renal leision occure before either blood urea nitrogen and creatinine level are significantly raised[31]. The calcium metabolism may be disturbed by the renal damage not revealed in those indices of serum. In summary, our result proposes that the early biocamical indicators of cadmium poisoning may be the indices of liver dynfuntion, which could be served as the assistance of clinical diagonisis of cadmium induced damage.

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Table 1. Effect of oral cadmium intake on spacific organ weight of male wistar rat after eight week of treatment

Dose Group $CdCl_2$/kg	Specific organ weight (g/100g body weight)[a]				
	Liver	Kidney	Pancreas	Lung	Testes
Normal[b]	2.10 ± 0.03	0.56 ± 0.08	0.14 ± 0.07	0.42 ± 0.02	0.77 ± 0.07
0.5mg	2.74 ± 0.02[c]	0.64 ± 0.07[c]	0.16 ± 0.06	0.40 ± 0.03	0.74 ± 0.07
1.0mg	2.76 ± 0.05[c]	0.61 ± 0.16	0.15 ± 0.09	0.36 ± 0.10	0.72 ± 0.34
2.0mg	3.04 ± 0.14[c]	0.68 ± 0.30[c]	0.17 ± 0.13	0.42 ± 0.15	0.82 ± 0.13

a Value are means ± SD.

b Gavage in normal saline without $CdCl_2$.

c Significantly less than normal group.

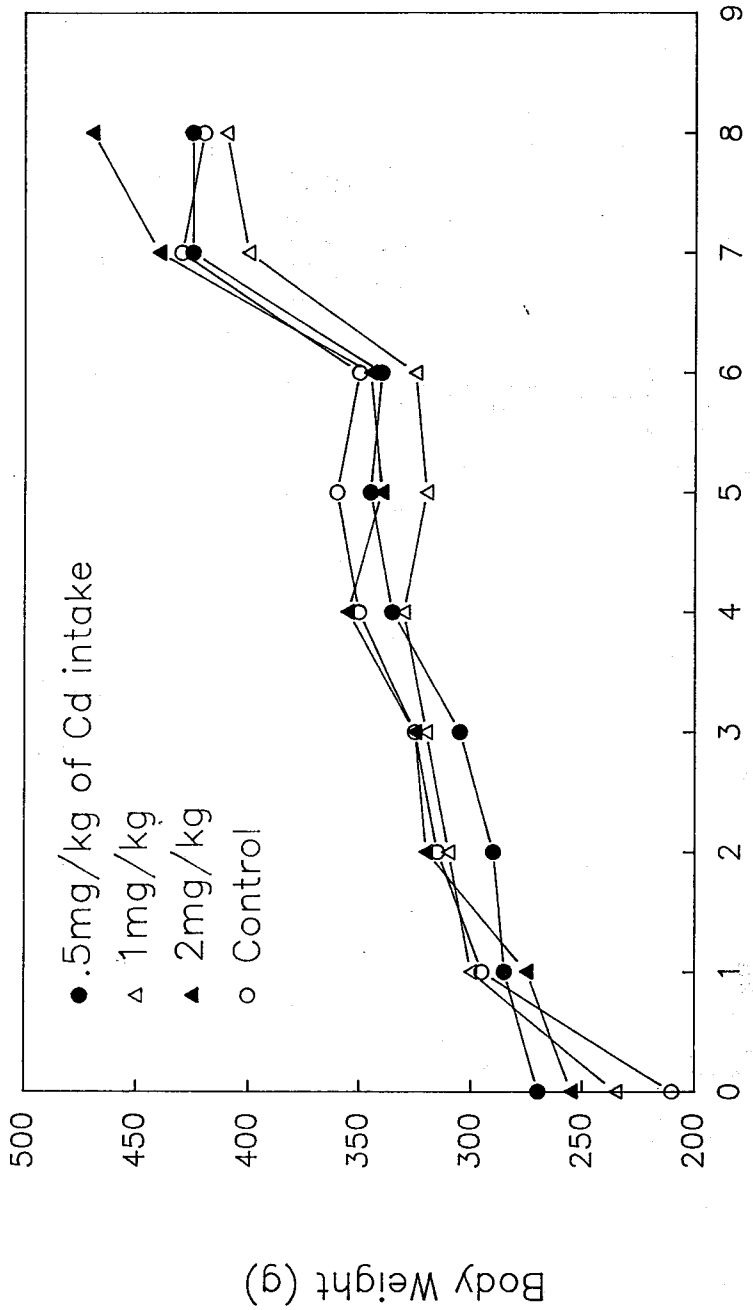

Fig. 1. Body weight of normal and Cd treated rats.

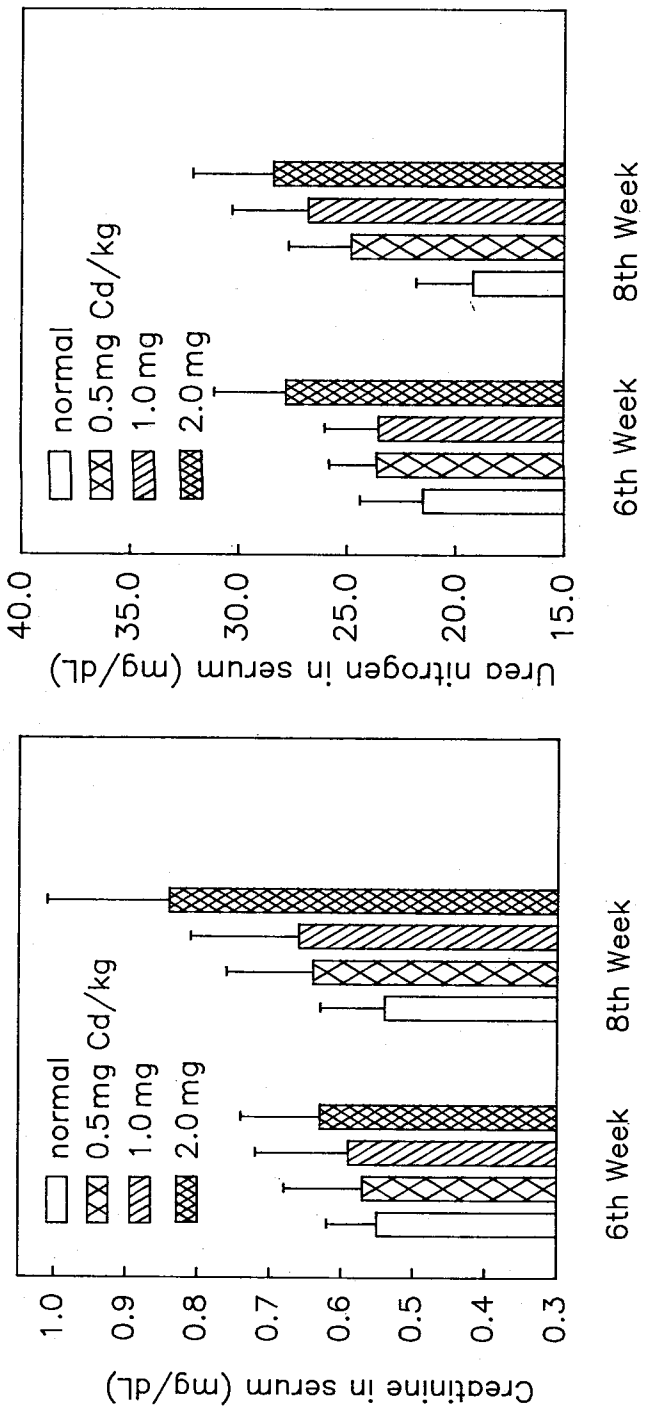

Fig. 2. The concentrations of serum creatinine and blood urea nitrogen in rats exposed to cadmium. The abscissa represants the 6th and 8th week changes after the rats were gavaged with 0, 0.5, 1.0, 2.0 Cd mg/kg.

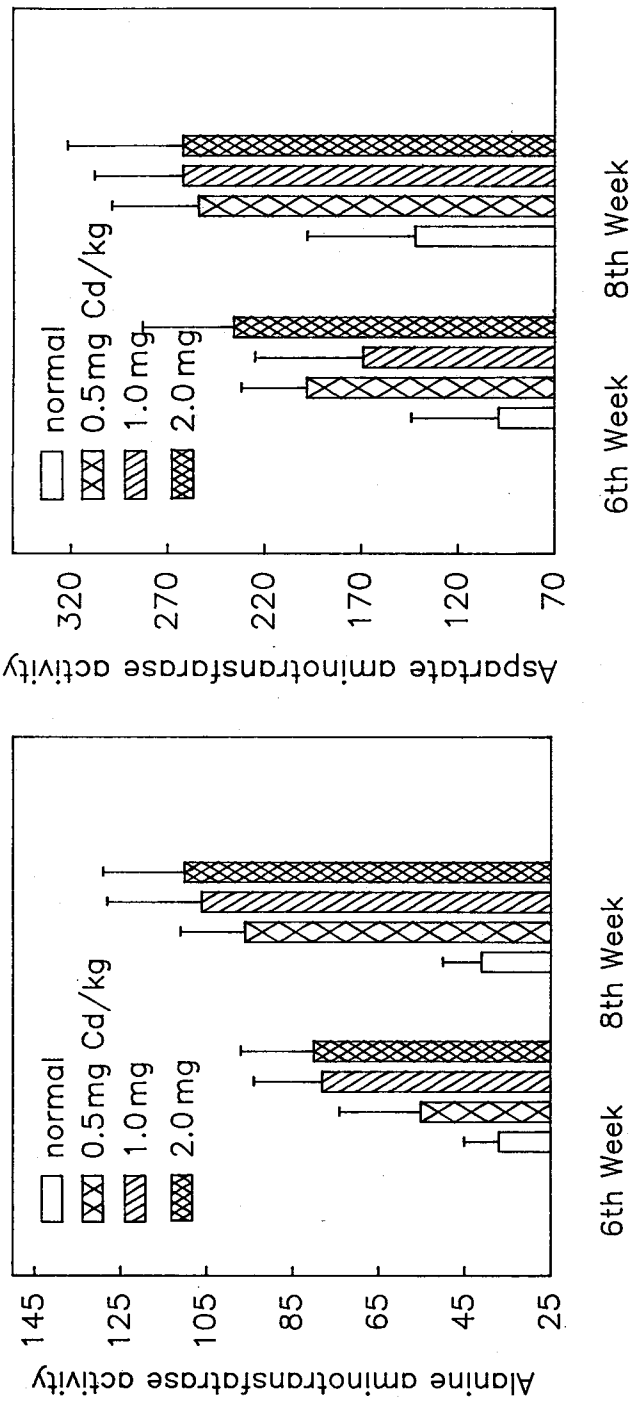

Fig. 3. The activities (IU/L) of serum alanine and aspartate aminotransfarase in rat exposed to cadmium. The abscissa represant the 6th and 8th week changes after the rats were gavaged with 0, 0.5, 1.0, 2.0 Cd mg/kg.

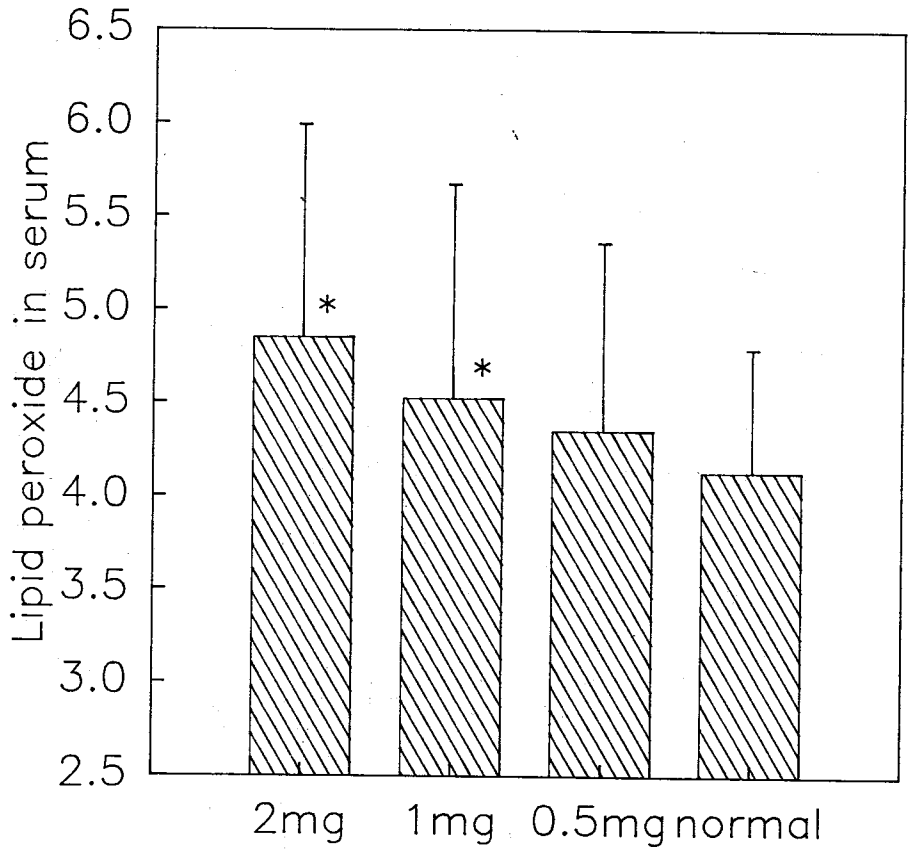

Fig. 4. Serum lipid peroxide value in rats exposed to 8 weeks of Cd intake.

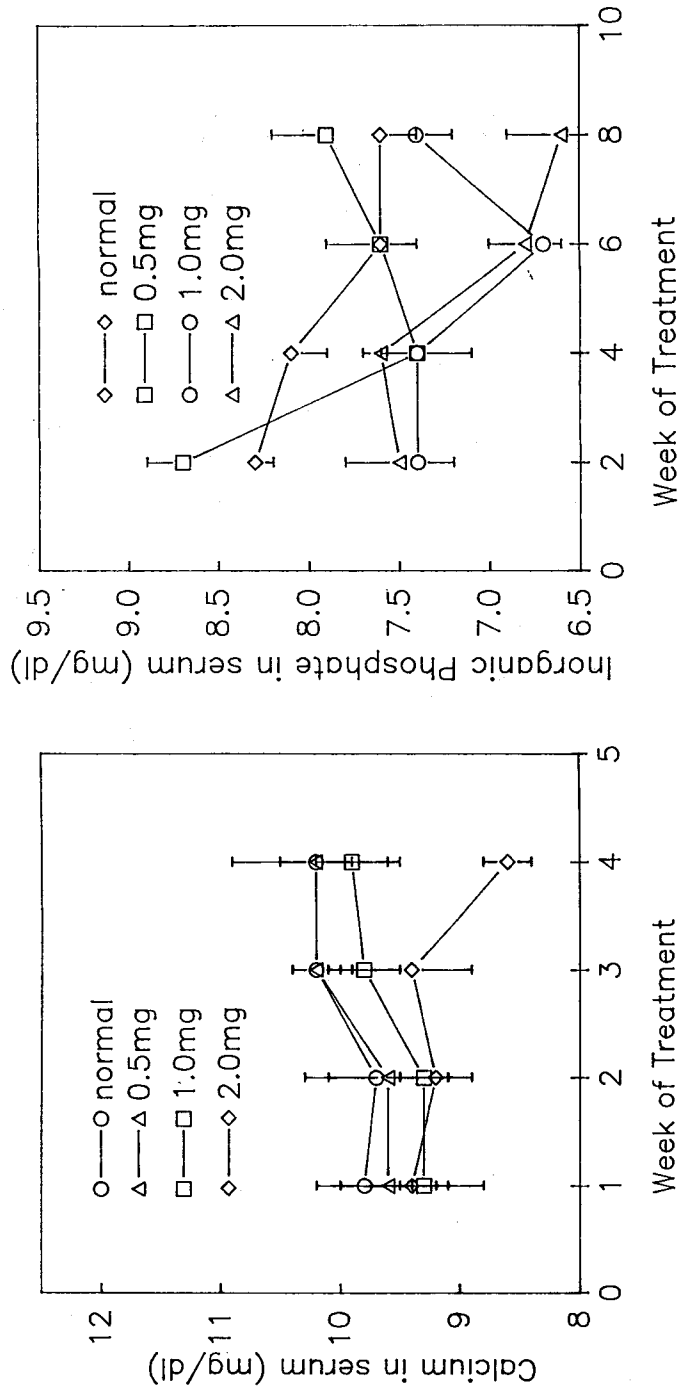

Fig. 5. Serum concentration of calcium and inorganic phosphate on rats exposed to cadmium. The abscissa represant the 2th to 8th changes after the rats were gavaged with 0, 0.5, 1.0, 2.0 Cd mg/kg.

中期鎘中毒鼠體血清生化值的影響

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由於環境鎘污染及職業性鎘暴露日漸嚴重，臨床上急需可偵測鎘暴露量的生化指標以作早期之診斷治療，本研究取雄性大白鼠24隻，分為四組每日由胃管投予不等劑量$CdCl_2$標準溶液（0，0.5，1.0，2.0 mgCd／kg），飼養期間每兩週以心臟取血測定血清Ca^{2+}及無機磷之含量，並於第六週起，偵測肝腎功能之變化，八週後，斷頭取血，測血清中alanine及aspartate aminotransfarase之活性，及BUN， Creatinine， Lipid Peroxide的濃度，結果顯示，六週後，鎘污染之各組其ALT，AST活性有昇高之趨勢，而BUN，Creatinine之濃度，則於第八週才見增加，最高劑量（2.0mgCd／kg）組之血清Ca^{2+}及無機磷濃度於第六週即呈現降低，實驗組其Lipid Peroxide值亦顯著高於正常組（$P<0.05$），結果指出因肝傷害而改變之生化值，將可作為早期偵測鎘毒性之輔助指標。

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