EFFECTS OF CADMIUM ON SOME ENZYME ACTIVITIES OF RAT LIVER—WITH SPECIAL REFERENCE TO DNA AND PROTEIN SYNTHESIS IN THE NUCLEI

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Male Wistar rats were injected intraperitoneally with cadmium acetate (20 μ moles/kg body weight) at two day intervals for six times. The increase of body weight was inhibited by the first injection of cadmium, but subsequent injections did not affect the further increase of body weight.

The weights of liver, kidney and spleen of the cadmium treated rats were not differed from control rats after six intraperitoneal injections. The contents of DNA and protein of livers were also not affected by the administration of cadmium.

The nuclear ^3H -dTTP incorporating activity and the activity of glucose-6-phosphatase of livers were inhibited by the treatment of cadmium, while ^{14}C -leucine incorporating activity of nuclei, acid phosphatase and monoamine oxidase were hardly affected.

It is suggested that cadmium inhibits the DNA polymerase activity directly or by affecting synthesis of the enzyme, or affects the initiation sites on DNA chains. It is also suggested that the enzymes participated in the nuclear incorporation of amino acids are fairly resistant to cadmium. These results might be of value to help explain the carcinogenic or teratogenic action of cadmium.

Cadmium is a relatively rare, physiologically non-essential and toxic element. Recently environmental pollutions due to the frequent employments in industry and hence the chances of human being exposed to cadmium increase substantially. In the meanwhile, biochemical and toxicological studies have shown that the absorbed cadmium tended to be retained for long periods, particularly in the liver and kidney¹⁻³⁾. The discovery of a specific cadmium binding protein in these organs led to a great interest in cadmium toxicology and a very large literatures on this subject has appeared in the past few years⁴⁻⁷⁾. However, the effect of cadmium on the nucleus of cells has received little attention.

The nucleus is known to be an organelle which contains almost all of the genetic information in the cell. Then, if cadmium affects on the mechanism concerning the expression of the genetic information in the nuclei, the influence may be formidable. In fact, developmental abnormalities of embryos have been reported to be induced by the

administration of cadmium to hamster⁸⁾, rat⁹⁾¹⁰⁾ or mouse¹¹⁾. Sarcomas are also induced by cadmium at the injection sites¹²⁻¹⁵⁾. Therefore, an elucidation of the effect of cadmium on the nucleic acid and protein synthesis may be potentially helpful in a basic understanding of the nature of cadmium toxicity in the whole animal.

In this investigation, enzyme activities in the nuclei concerning DNA synthesis and protein synthesis were measured in cadmium treated rats. The activities of acid phosphatase, a marker enzyme in lysosomal fraction, monoamine oxidase, a marker enzyme in outer membrane of mitochondria, and glucose-6-phosphatase, which distributes mainly in microsomal fraction were also assayed.

MATERIALS AND METHODS

Male Wistar rats weighing 120 g were used. Cadmium acetate dissolved in 0.9% sodium chloride was injected intraperitoneally at a dose of 20 μ moles/kg body weight at two day intervals for six times. One day after the last injection of cadmium, rats were killed by decapitation and livers were perfused *in situ* with cold saline *via* portal vein. The 10% liver homogenate in 0.25 M sucrose-3 mM MgCl_2 was prepared in a teflon-glass homogenizer. The homogenate was filtered through four layers of gauze, followed by centrifugation at 1,000 g for 10 min. The pellet was suspended in 2.2 M sucrose-0.3 mM MgCl_2 and centrifuged at 54,000 g for 1 hr. The nuclear pellet at the bottom was washed with 0.25 M sucrose-3 mM MgCl_2 and then finally resuspended in 0.25 M sucrose-3 mM MgCl_2 at a concentration of nuclei equivalent to 1 g tissue per mL.

^3H -dTTP incorporating activity of the nuclei was measured as described by Lynch et al¹⁶⁾. Reaction mixture contained 0.1 M Tris-HCl buffer (pH 7.4), 4 mM MgCl_2 , 8 mM 2-mercaptoethanol, 2 mM ATP, 0.08 mM dCTP, dGTP and dATP, 2 μCi ^3H -dTTP (0.5 Ci/mmole), and nuclear suspension equivalent to 200 μg DNA in a final volume of 0.5 mL. The incubation was carried out at 37°C for 20 min. Reaction was stopped by adding ice cold 10% trichloroacetic acid and the acid-insoluble material was collected on glass fiber disc by filtration. The discs were washed with 5% trichloroacetic acid, ethanol and ether successively. Dried discs were put into toluene-PPO-POPOP scintillator and counted in a liquid scintillation counter.

Amino acid incorporating activity of the nuclei was determined as described previously¹⁷⁾. The incubation mixture contained 0.2 M sucrose, 1 mM MgCl_2 , 40 mM KCl, 20 mM sodium phosphate buffer (pH 6.0), nuclei equivalent to 2-3 mg protein and 0.5 μCi of ^{14}C -leucine (305 μCi /mmole) in a final volume of 1.0 mL. The incubation was carried out at 37°C for 60 min and the reaction was terminated by adding cold 5% HClO_4 -0.25% Na_2WO_4 . Acid insoluble precipitates were collected, washed with 10% HClO_4 and then treated in 5% HClO_4 at 90°C for 15 min. The pellet obtained by centrifugation was successively washed with 10% HClO_4 , ethanol, ethanol-ether (3:1, v/v), and finally dissolved in 85% formic acid and aliquots were plated on stainless steel planchets. Radioactivity was determined by the use of a window-less gas flow counter.

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Another enzyme activities were determined as described below using liver homogenates. The activity of acid phosphatase was assayed by the procedure of DiPietro and Zengerle¹⁸⁾. Monoamine oxidase was assayed according to the procedure of McEwen, Jr.¹⁹⁾ Glucose-6-phosphatase activity was measured as described by Swanson²⁰⁾.

Protein was assayed by the method of Lowry *et al.*²¹⁾ DNA was measured by the diphenylamine method²²⁾.

RESULTS

The increase of body weights of the rats injected with cadmium acetate was stopped or rather decreased after the first injection, as shown in Fig. 1. The average body weight of the rats administered with cadmium was about 120 g (10 rats) at the second injection time, while that of control rats was 150 g (10 rats). However, the increase of body weights of cadmium treated rats was no longer affected by the subsequent injections of cadmium. After six times injections, the average body weight of the experimental group was 190 g and that of the control group was 210 g.

Table 1 shows the wet weights of liver, kidney and spleen of the cadmium treated and control rats. No difference of the wet weight of these organs was observed between two groups.

The amount of DNA and protein per g of liver was not changed after 6th injection

Fig. 1. Changes of body weight of rats administered with cadmium acetate. Cadmium acetate was injected intraperitoneally at a dose of 20μ moles/kg body weight at two day intervals. Control rats were injected with saline. ●—●, control rats (n=10), ×—×, cadmium-injected rats (n=10).

Table 1. Wet weights of liver, kidney and spleen of the rats treated with cadmium acetate as compared with those of the control rats.

	Rat No.	Body weight (g)	Liver (g)	Kidney (g)	Spleen (g)
Control	1	230	12.0	2.2	0.9
	2	180	8.3	1.7	0.7
	3	230	11.6	2.4	0.8
	4	210	10.6	2.0	0.8
	mean±S.D.	210±25	10.6±2.9	2.1±0.3	0.8±0.1
Cadmium-treated	1	220	12.2	2.2	0.8
	2	180	9.5	1.9	1.0
	3	170	9.1	1.8	0.8
	4	220	11.4	2.0	0.9
	mean±S.D.	200±25	10.6±1.5	2.0±0.2	0.9±0.1

Table 2. DNA and protein contents of livers of cadmium treated and control rats.

	Rat No.	Whole liver			Nuclei
		DNA (mg/g of liver)	Protein	Protein/DNA	Protein/DNA
Control	1	2.45	126.0	51	3.4
	2	2.62	129.5	50	3.4
	3	2.68	127.0	48	3.5
	4	2.37	119.5	51	3.6
	mean±S.D.	2.53±0.14	125.5±4.3	50	3.5
Cadmium-treated	1	2.55	127.0	50	3.5
	2	2.52	123.5	49	3.4
	3	2.69	137.0	49	3.4
	4	3.19	136.5	43	3.1
	mean±S.D.	2.74±0.31	129.8±6.9	47	3.4

of cadmium (Table 2). The ratio of protein to DNA in nuclei of the liver was also not affected by the injection of cadmium.

The enzyme activities in livers of the rats treated with cadmium acetate were compared with those of the control rats and the results were summarized in Table 3. The Table shows that: 1) ^3H -dTTP incorporating activity of the nuclei obtained from livers of the rats treated with cadmium was reduced to about 60% (75–50%) of the control. 2) ^{14}C -Leucine incorporating activity of the nuclei was not affected by the administration of cadmium. 3) The activity of acid phosphatase was not affected by the treatment

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Table 3. Enzyme activities of livers of cadmium-treated and control rats.

	Rat No.	Incorporation of ^3H -dTTP ^{a)}	Incorporation of ^{14}C -leucine ^{b)}	Acid phosphatase ^{c)}	Amine oxidase ^{d)}	Glucose-6-phosphatase ^{e)}
Control	1	2260	348	0.99	268	2.02
	2	2260	272	1.05	272	1.98
	3	—†	250	1.16	284	2.29
	4	2280	329	1.40	240	1.87
	mean ±S.D.	2270 ±10	300 ±46	1.15 ±0.18	266 ±19	2.06 ±0.18
Cadmium-treated	1	1720	350	0.99	246	1.28
	2	1070	—†	1.19	227	1.22
	3	1600	294	0.97	230	1.11
	4	1190	302	0.92	174	1.12
	mean ±S.D.	1395 ±310	315 ±30	1.02 ±0.12	219 ±31	1.18 ±0.08

†Not determined.

a) cpm/mg NDA

b) cpm/mg protein

c) μ mole p-nitrophenol liberated/min/mg protein

d) m μ mole benzaldehyde liberated/3 hr/mg protein

e) μ mole Pi liberated/15 min/mg protein

^3H -dTTP and ^{14}C -leucine incorporating activities were assayed using isolated nuclei. Another enzyme activities were measured using homogenates.

with cadmium. 4) The activity of monoamine oxidase was slightly reduced by the cadmium injection. 5) The activity of glucose-6-phosphatase of the cadmium treated rats was reduced to about 60% of the control.

DISCUSSION

It has been reported that cadmium binding protein, metallothionein, is induced in response to cadmium in the liver and kidney⁴⁻⁷⁾, and this protein is suggested to have a protective effect against cadmium toxicity^{23) 24)}. Normal increase of body weight after second injection of cadmium may be related to the induction of cadmium binding protein.

After six intraperitoneal injection of cadmium, gross findings of livers were prominent. Anterior, median and posterior lobes could not be separated each other, and adhesion of liver to peritonealwall was striking. In spite of drastic morphological changes of the liver, the weight of the liver and contents of DNA and protein were not changed by the administration of cadmium.

Some discrepancies were observed in the effect of cadmium on the activity of enzymes in livers. Nuclear dTTP incorporating activity of livers of the cadmium treated rats

was reduced to approximately 60% of the control, while nuclear leucine incorporating activity was hardly affected by the administration of cadmium. In this respect, Stoll *et al.* reported that DNA synthesis in regenerating rat liver was markedly inhibited by a single intraperitoneal injection of cadmium²⁵⁾. Our result on nuclear dTTP incorporating activity seems to be consisted with Stoll's observation. However, it should be pointed out that DNA polymerase present in the nuclei of non-proliferating tissues is reported not to increase significantly during cell proliferation, and is suggested to function in non-replicative polymerization reactions such as DNA repair, while in proliferating tissues such as regenerating liver and hepatoma another DNA polymerase is present in the nuclei and is suggested to function as a replicating enzyme²⁶⁻²⁸⁾. Two possibilities are considered on the mechanism of the inhibiting effect of cadmium on the synthesis of DNA. One is that cadmium may interact with the DNA polymerase or inhibit the synthesis of the enzyme. Another is that cadmium may affect the initiation sites on DNA chains. The later possibility is based upon the ability of cadmium to alter the melting profile of liver DNA²⁵⁾.

The nuclear leucine incorporating activity in livers of cadmium treated rats was not reduced in our experimental condition. Isolated nuclei are able to incorporate ¹⁴C-leucine into their proteins¹⁷⁾. Biological meanings of the nuclear incorporation of amino acid *in vitro* are not yet clear, but *in vitro* and *in vivo* nuclear amino acid incorporating activities of regenerating livers have been reported to be increased in parallel after partial hepatectomy²⁹⁾³⁰⁾. Addition of cadmium acetate at the concentration of 10 ppm to the incubation mixture did not affect the incorporation of ¹⁴C-leucine into isolated nuclei (unpublished data). These results seem to indicate that the enzymes participating the nuclear incorporation of amino acid are fairly resistant to cadmium.

Cadmium was also shown to affect differently marker enzymes representing organelle in liver cells. Glucose-6-phosphatase activity, which distributes mainly in microsomal fraction was reduced to approximately 60% of the control. On the other hand, activity of acid phosphatase or monoamine oxidase, a marker enzyme in lysosomal or mitochondrial fraction respectively, was hardly affected by cadmium. The different effect of cadmium on the activity of enzymes may reflect the different distribution of cadmium in the cells. It may also be related to the nature or structure of enzyme. For instances, acid phosphatase contained in lysosome is resistant to 1 μ M *p*-chloromercuribenzoate¹⁸⁾, a strong inhibitor of SH group. On the other hand, DNA polymerase from rat liver is markedly inhibited by another SH-inhibitor substances, 0.5 mM N-ethylmaleimide or 0.15 mM *p*-hydroxymercuribenzoate³¹⁾.

It has been reported that cadmium either activates or inhibits a number of enzymes of liver. The activities of malic dehydrogenase³²⁾, histidine ammonia lyase³³⁾ and phosphorylase³⁴⁾ are among others enhanced by cadmium, while cytochrome oxidase³⁴⁾, glutamic oxaloacetic transaminase³⁵⁾ and δ -aminolevulinic synthetase³⁶⁾ are inhibited by cadmium. Accurate comparison, however, cannot be made because the conditions of experiments are different with each other. The dose, duration and route of administration of cadmium

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are not same in almost all the experiments.

Considering the present situation concerning the potential environmental impact of cadmium, it is felt that these biochemical investigations provide useful fundamental data which should be of value in regulating the hazard from cadmium exposure. Further, the actions of cadmium which have been demonstrated on nucleic acids and protein synthesis may be of significance to elucidate the mechanism of carcinogenic and teratogenic activities of cadmium.

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