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EFFECTS OF SHORT-TERM INHALATION OF CADMIUM OXIDES ON RABBIT PULMONARY MICROSOMAL ENZYMES

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Abstract—Rabbits, guinea pigs, rats and mice were compared for the activity of benzo[a]pyrene hydroxylase, aminopyrine *N*-demethylase and aniline hydroxylase of pulmonary microsomes. The activity of the microsomal enzymes was highest in rabbits, followed by guinea pigs and then rats and mice. Effects of the inhalation of cadmium oxides (CdO) were studied on the pulmonary microsomal enzymes in male rabbits. Rabbits were exposed for 15 min to air containing microparticles of CdO at four different concentrations ranging from 6.4 ± 0.5 to 22.4 ± 0.4 mg Cd/m^3. The animals were killed 24 hr after the inhalation of CdO. The lung weight of the animals was increased markedly at doses higher than 12.6 mg/m^3, the increase attaining 50 per cent increase at the highest dose. The activity of benzo[a]pyrene hydroxylase was reduced significantly at the higher doses, the reduction being dose-dependent reaching 50 per cent reduction. The activity of aminopyrine *N*-demethylase and aniline hydroxylase was reduced moderately by CdO inhalation but the reduction was not dose-dependent. Time-course effects of CdO inhalation on the pulmonary microsomal enzymes were studied on the rabbits exposed for 15 min to air containing CdO at concentrations of 13.0 ± 0.3 mg Cd/m^3. The animals were killed 0.5, 1, 2, 4, and 6 days after the inhalation. The body weight increase was less in the treated groups than in the controls and edematous lesions in the lung were observed in most of the treated animals. The lung weight was increased after the inhalation, reaching its plateau on day 4. The activity of the microsomal enzymes was reduced throughout the experimental period, the maximum reduction being obtained on day 2 after the inhalation except for aniline hydroxylase that was reduced at the same degree throughout the experimental period.

Cadmium(Cd) has become a widely-spread environmental contaminant and a human being daily takes a certain amount of Cd with food or with inhaled air [1]. Though the food contamination by Cd is still the main problem of Cd contamination, an increasing and general contamination of the atmospheric environment is also of recent concern [2]. Cd-containing microparticles or Cd fumes found in the atmosphere are generated from emissions by automobile engines, tobacco smoking and Cd processing factories [3–5].

Earlier studies on the toxicity of Cd fumes have been done mainly in respect of the pathological disorders induced in the lung and other organs, since the Cd fumes elicited occupational diseases in the mining industries and manufacturing Cd-containing materials [6–9]. Relatively few studies, however, have been done in respect of the biochemical changes of the lung [10, 11], especially the xenobiotic system in the lung exposed to Cd fumes. Though the liver is the most important organ, the ability of the lung to metabolize foreign compounds may be important in controlling the effect of agents. For the lung serves as the primary portal of entry for environmental agents in the forms of gases or airborne particles and it is frequently the target organ for lesions produced by environmental agents, including carcinogenic polycyclic hydrocarbons such as benzo[a]pyrene [12]. It is quite possible that the fate of these compounds is controlled by local metabolism or the pulmonary xenobiotic system.

Cd is known to be one of the potent inhibitors of the hepatic xenobiotic oxidases both *in vivo* and in vitro [13–15], but few studies have been done on the effects of Cd inhalation on the oxidases in the lung.

The present study was undertaken to discover whether Cd fumes, when inhaled through the respiratory system, could modify the activity of xenobiotic enzymes such as benzo[a]pyrene hydroxylase and other drug-metabolizing enzymes in the lung, which might result in the modification of the metabolism or activity of other atmospheric pollutants inhaled simultaneously. Since the activity of the enzymes in the lung is low, rabbits were chosen as experimental animals after having compared four different species of animals. Rabbits were exposed for short period to the aerosol of cadmium oxides(CdO) and acute effects on the pulmonary oxidases were studied to avoid other factors resulting from long-term exposure to Cd fumes such as increased lethality to bacterial challenge [16–18] that might complicate the interpretation of the results.

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MATERIALS AND METHODS

Animals and treatment. For the comparative study of the enzyme activity in the pulmonary microsomes of different species, male rabbits (Fauves de Bourgogne, IFFA-CREDO, Fresnes, France), guinea pigs (Tricolole, E.V.I.C.-C.E.B.A., Blanquefort, France), SPF-rats (Sprague–Dawley, IFFA-CREDO, France) and SPF-mice (OF1, IFFA-CREDO, France) were used.

For the study of the CdO inhalation, male rabbits (Fauves de Bourgogne) were exposed for 15 min in an individual chamber to air containing microparticles of CdO generated by pyrolysis of cadmium acetate solution as described previously [19, 20]. The particles of CdO were of diameter less than 2 μm in their major parts (more than 98%) [20]. CdO microparticles in the inhalation chamber were trapped by the cellulose acetate filter (HAWP 47 mn, pore size: 0.45 μm) fixed at the end of the chamber for Cd determination. The rabbits were killed by injecting air into auricular vein at the indicated time after CdO inhalation as described in the Results section.

Microsome preparation. After the sacrifice of the animals, the lungs were perfused, if necessary, through portal vein, dissected and washed with cold saline. In the comparative study, pooled lungs from 10 mice or 2 rats were passed through a tissue pressor (Poly Labo Paul Block & Cie, Strasbourg, France) and homogenized with 0.25M sucrose (pH 7.4) in a Potter–Elvehjem Teflon–pestle glass homogenizer. The microsomes of the lungs were isolated from the homogenates by conventional centrifugation at 800 g for 5 min, 10,000 g for 15 min and 105,000 g for 60 min and by washing the sediments with 0.15 M KCl–Tris solution (pH 7.6). The microsomes obtained were resuspended in 0.25 M sucrose solution (pH 7.4) and frozen under liquid nitrogen and stored at −40° until use. The microsomal protein was determined by a modification of Lowry's method [21].

Cd *determination and enzyme assay.* The contents of Cd in the inhalation chamber and in the lungs were determined using flame atomic absorption spectrophotometry after digestion of the filter and the pressed pulmonary tissues with hot nitric acid.

Benzo[a]pyrene hydroxylase was assayed on the microsomal preparation by a slight modification of Van Cantfort's methods [22]. The reaction mixture with final volume of 0.5 ml consisted of NADPH-generating system, Tris buffer (0.06 M, pH 8.0), [^{3}H]benzo[a]pyrene (4 μCi, 100 mCi/mmole, purchased from The Radiochemical Centre, Amersham, England and purified as described [22]), and microsomal protein (about 0.3 mg of protein in case of rabbits, 0.8 mg in case of guinea pigs and 1.2 mg in case of rats and mice). Incubation was done at 37° for 20 min for rabbit's and guinea pig's lungs and 30 min for rat's and mice's lungs. After the incubation was stopped by adding 1 ml of KOH (0.15 M)–DMSO (85%, v/v), unmetabolized substrates in the mixture were extracted by 3 ml of hexane three times, remaining aqueous phase was acidified and 0.75 ml of the phase were taken, diluted with 10 ml of Insta Gel (Packard Instruments, S.A., Rungis, France) and counted in a scintillation counter.

The activity of aminopyrine *N*-demethylase and aniline hydroxylase in the microsomes was determined according to the method described by Mazel [23]. The incubation was done for 15 min for aminopyrine demethylase and for 20 min for aniline hydroxylase in case of rabbits and 20 min for aminopyrine demethylase and 30 min for aniline hydroxylase in case of the other animals. The reaction mixture for both enzymes assay contained about 1.2 mg of microsomal proteins in 2 ml of reaction mixture for all the animals used. The activity of the enzymes was expressed as pmoles or nmoles of the substrates metabolized per mg of microsomal protein added in a reaction mixture. The means and the S.E. of the means were calculated and the levels of significance between the treated and the control groups were determined using Student's t-test.

RESULTS

Species differences in the activity of pulmonary microsomal enzymes

The activity of some drug-metabolizing enzymes of pulmonary microsomes of four different species of animals are shown in Table 1. Lungs from mice and rats gave small quantity of the tissue and low activity of the pulmonary microsomal enzymes. The activity of some enzymes like aniline hydroxylase was very low in these animals and in their spectrophotometric measurement, optical density ranged from 0.02 to 0.03. Lungs from guinea pigs were between the rabbit and these two animals in the quantity of the tissue and the enzyme activity. Rabbit's lung was most abundant, among the animals used, in the quantity of the tissue and the activity of the enzymes. Some basic data of the activities of the enzymes are shown in Fig. 1. The results show that benzo[a]pyrene hydroxylase activity was linear up to 1.0 mg of microsomal proteins in an incubation mixture of 1.0 ml containing 80 nmoles of [^{3}H]benzo[a]pyrene substrate and that it increased in proportion to the specific activity of the substrate used (two times higher when a substrate with specific activity of 100 mCi/mmole was used instead of 50 mCi/mmole). For aminopyrine demethylase and aniline hydroxylase, the activity was linear up to 2 mg of microsomal protein per ml of incubation medium. These activities were also linear, as a function of incubation time, until 20 min for aminopyrine demethylase and aniline hydroxylase and 50 min for benzo[a]pyrene hydroxylase.

Dose-related effects of the CdO *inhalation on microsomal enzymes in rabbit's lung*

Rabbits were exposed individually for 15 min to air containing microparticles of CdO generated from the solution containing 1.5, 2.5, 3.5, and 4.5% (w/v) of cadmium acetate. The concentrations of Cd in the inhaled air were 6.4 ± 0.5, 8.8 ± 0.2, 12.6 ± 0.4 and 22.4 ± 0.4 mg Cd/m^3 of air, respectively. As shown in Table 2, the body weight of the treated animals decreased significantly at a dose of 22.4 ± 0.4 mg/m^3 and slightly at lower doses. The lungs of the treated animals demonstrated edematous lesions and increased in its relative weight (wet wt/body wt) at

Table 1. Activity of microsomal enzymes in the lung of different species of experimental animals*

Species (No. of samples)	Body wt (g)	Lung total wet wt (g)	Microsomal protein (mg/g-lung)	Enzyme activity: Benzo[a]pyrene hydroxylase (pmoles/min/mg)	Enzyme activity: Aminopyrine demethylase (nmoles/min/mg)	Enzyme activity: Aniline hydroxylase (nmoles/min/mg)
Mouse (4)	25.2 ± 0.1	0.17 ± 0.01	5.32 ± 0.22	10.8 ± 0.3	1.36 ± 0.12	0.0726 ± 0.0046
Rat (5)	224 ± 7	1.08 ± 0.02	7.93 ± 0.27	11.0 ± 1.9	1.01 ± 0.03	0.0337 ± 0.0007
Guinea pig (4)	378 ± 12	2.72 ± 1.52	7.22 ± 0.05	66.6 ± 3.3	1.72 ± 0.01	0.0651 ± 0.0033
Rabbit (4)	2302 ± 47	10.0 ± 0.1	5.77 ± 0.18	94.3 ± 11.9	2.97 ± 0.21	0.210 ± 0.034

* Values given are the means ± S.E. of 4 to 5 determinations. In parentheses are the number of samples and a sample for mice and rats represents the pool of 10 or 2 animals, respectively. Enzyme activities are expressed as pmoles or nmoles of metabolized substrates per mg of microsomal protein per minute.

Table 2. Dose-related effects of CdO inhalation on microsomal enzymes in rabbit's lung*

Treatment (Cd content in inhaled air) (mg/m^3-air)	Body wt: initial (kg)	Body wt: final (kg)	Lung relative wet wt (g/kg)	Cd content in lung (μg/g-lung)	Microsomal protein (mg/g-lung)	Enzyme activity: Benzopyrene hydroxylase (pmoles/min per mg)	Enzyme activity: Aminopyrine demethylase (nmoles/min/mg)	Enzyme activity: Aniline hydroxylase (nmoles/min/mg)
Control (5)	2.82 ± 0.13	2.84 ± 0.14	3.64 ± 0.12	—	5.52 ± 0.30	76.4 ± 9.5	4.22 ± 0.19	0.311 ± 0.046
Exposed (5) (6.4 ± 0.5)	2.78 ± 0.14	2.72 ± 0.13	3.83 ± 0.56	2.1 ± 0.2	6.29 ± 0.39	78.5 ± 11.5	3.89 ± 0.52	0.247 ± 0.25
Control (4)	2.63 ± 0.04	2.64 ± 0.05	3.59 ± 0.11	—	5.80 ± 2.90	63.0 ± 2.0	2.75 ± 0.25	0.255 ± 0.015
Exposed (6) (8.8 ± 0.2)	2.57 ± 0.05	2.54 ± 0.05	3.45 ± 0.16	2.5 ± 0.1	7.44 ± 0.16†	55.4 ± 5.3	2.51 ± 0.29	0.193 ± 0.014‡
Control (4)	2.18 ± 0.12	2.25 ± 0.11	3.71 ± 0.18	—	5.11 ± 0.22	83.9 ± 6.5	3.53 ± 0.62	0.316 ± 0.041
Exposed (6) (12.6 ± 0.4)	2.17 ± 0.04	2.11 ± 0.05	4.06 ± 0.17	3.3 ± 0.3	4.98 ± 0.21	68.0 ± 2.7‡	3.10 ± 0.33	0.233 ± 0.017
Control (5)	2.49 ± 0.06	2.46 ± 0.06	3.65 ± 0.08	—	5.16 ± 0.09	62.4 ± 5.01	3.95 ± 0.40	0.344 ± 0.020
Exposed (5) (22.4 ± 4)	2.48 ± 0.04	2.26 ± 0.03†	5.49 ± 0.50†	4.6 ± 0.3	5.24 ± 0.06	33.0 ± 6.7†	3.51 ± 0.20	0.254 ± 0.023†

* Male rabbits were exposed in an individual chamber for 15 min to air containing various concentrations of microparticles of CdO and were killed 24 hr after the inhalation. Values represent the mean ± S.E. of 4 to 6 animals as indicated in parentheses. Enzyme activities are expressed as pmoles or nmoles of metabolized substrates per min per mg of microsomal proteins. Values significantly different from corresponding control values at $P < 0.01$(†) and $P < 0.05$(‡).

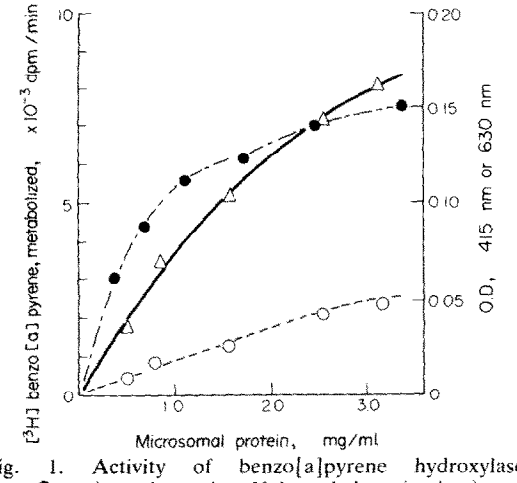

Fig. 1. Activity of benzo[a]pyrene hydroxylase (— - ● - —), aminopyrine *N*-demethylase (—△—) and aniline hydroxylase (- - ○ - -) in pulmonary microsomes in rabbits as a function of the quantity of microsomal protein added to the reaction mixture.

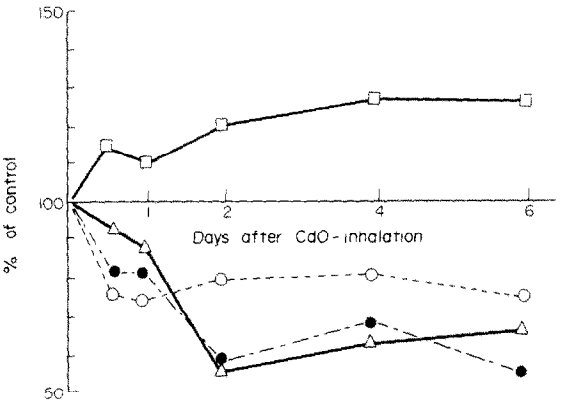

Fig. 2. Time-course effects of CdO inhalation on relative lung weight (—□—), activity of benzo[a]pyrene hydroxylase (— -● - —), aminopyrine *N*-demethylase (—△—) and aniline hydroxylase (- - ○ - -). Male rabbits were exposed for 15 min to air containing microparticles of CdO at concentrations of 13.0 ± 0.3 mg Cd/m^3. Animals were killed at various times after the inhalation. Each point expressed as the percentage of corresponding control values represents the mean of 4 animals.

doses greater than 12.4 mg/m^3, the increase being 50 per cent at the highest dose. There were no significant differences in the contents of either water, or protein of the lung between the control and the treated groups of all the doses studied (i.e. in the 22.4 mg Cd/m^3-groups: control; 82.3 ± 0.2% of water and treated; 82.7 ± 0.3%, while in the protein content; control; 117.6 mg/g-tissue and treated; 115.8 ± 2.5 mg/g-tissue). The concentration of microsomal protein in the lung was increased at the lower doses of the treated groups but remained unchanged in the groups given higher doses.

The activity of the microsomal benzo[a]pyrene hydroxylase was reduced markedly by CdO inhalation and the reduction was dose-dependent, reaching 50 per cent reduction at the highest dose. As for the other enzymes, the reduction of the activity was obtained but was not dose-dependent and approximately the same degree of reduction was obtained at all the doses studied.

Time-course effects of the CdO *inhalation on microsomal enzymes in rabbit's lung*

Rabbits were exposed for 15 min to air containing microparticles of CdO generated from 3.5% (w/v) solution of cadmium acetate and were killed 0.5, 1, 2, 4, and 6 days after the inhalation. Concentrations of Cd in the inhaled air were 15.2 ± 0.2, 12.6 ± 0.4, 12.3 ± 1.3 and 12.3 ± 0.4 mg Cd/m^3 (mean: 13.0 ± 0.3 mg/m^3) and in the lung, 3.97 ± 0.83, 3.31 ± 0.33, 3.07 ± 0.13, 2.97 ± 0.20 and 3.22 ± 0.11 μg/g-tissue for 0.5-, 1-, 2-, 4-, and 6-days groups, respectively. The body weight of the treated groups was lower than that of the corresponding controls and the edematous lesions in the lung were observed in most of the animals. As shown in Fig. 2, the relative weight of the lung increased after the inhalation of CdO, reaching at its maximum around 2 or 4 days after the inhalation. No differences were observed in the contents of water, total protein of the lung tissue, or in the microsomal protein between the treated and their corresponding controls. The activities of the three enzymes were reduced throughout the experimental period and the maximum reduction in the activities of benzo[a]pyrene hydroxylase and aminopyrine demethylase was obtained on 2 days after the inhalation of CdO and the reduction retained substantially the same rate thereafter. However, as for aniline hydroxylase, the reduction was relatively small and not varied throughout the experimental period.

DISCUSSION

Since the lung is the main target organ for lesions induced by atmospheric pollutants inhaled through the respiratory system, studies on the toxic effects of pollutants on pulmonary tissues have recently increased. However, the properties of the microsomal enzymes and the detoxifying mechanism for inhaled chemicals in the lung are not fully elucidated yet. This is partly because that the lung contains large amounts of connective tissues and partly because that the amounts of the enzymes in pulmonary microsomes are very low compared to the hepatic microsomes.

In the present study, we could solve the difficulties of homogenizing the pulmonary tissue rich in connective tissues by passing first through a tissue-pressor and then by homogenizing the pressed tissues by Potter–Elvehjem Teflon–pestle glass homogenizer. In this way the pulmonary tissues could be easily homogenized. To solve the second difficulty, we have compared the amounts of pulmonary tissues and of microsomal enzymes of four different experimental animals. The results obtained largely correspond to those of the other workers [24] who reported a low activity of the microsomal enzymes in the lung of mice and rats, a medium activity in guinea pigs and relatively a high activity in rabbits. These indicate

that as an experimental animal for the study of pulmonary microsomal enzymes, the rabbit is most suitable in that this species provides a large amount of pulmonary tissues and of microsomal enzymes. On the other hand, rats and mice were shown to be not suitable; lungs of these species of animals were so small that lungs from several animals had to be pooled and furthermore, the base line values of the activity of the microsomal enzymes were so low that performance of spectrophotometric and radiometric measurement of the enzymes and interpretation of the results were difficult. In addition, the low activity of the enzymes in these animals would not become measurable merely by increasing the amounts of microsomal proteins added to the assay medium in the usual methods. As shown in the present study (Fig. 1), even with rabbit pulmonary microsomes which contain higher activities than those of mice, rats and guinea pigs, the linearity of the activity of some microsomal enzymes like benzo[a]pyrene hydroxylase was not obtained with high amounts of microsomal proteins added to the present assay system. All these results have shown that except for some minor difficulties in handling a large size of animal and in having relatively homogenous and specific pathogen-free animals, the rabbit might be most suitable for the study of microsomal oxidases in the lung.

The study on the effect of the inhalation of CdO has demonstrated first a dose-related increase in the weight of the lung at higher doses. This increase might be attributed partly to the inflammatory edema accompanied by the increase in the number of migrated cells and proliferated cells and in the exudated serum proteins in the interstitial space of the lung [8, 25, 26]. However, in contrast to the work of Reddy *et al.* [27] who reported an elevated water content in the lung of cadmium chloride ($CdCl_2$)-instilled animals, the contents of water and protein of the lung of CdO-inhaled rabbits did not differ from those of the control animals in the present study. Thus it might be difficult to explain this increased lung weight only by the acute inflammatory reaction. Another possible explanation might be that this increase of the lung weight is due to a temporal adaptation or compensatory reaction of the lung to the inhalation of CdO as in the case of other organs such as the liver under the administration of relatively large amounts of chemicals [28]. However, the increase in the lung weight was observed only in the animals exposed to higher doses and this was always accompanied macroscopically by the edematous lesions. Another explanation for the increase observed 3 days after the exposition might be that this is due mainly to the reparative–proliferative process occurring after the acute inflammatory reaction [25] rather than the temporal adaptation of the lung to the inhalation of CdO.

CdO inhalation revealed a dose-related inhibitory effect on benzo[a]pyrene hydroxylase. On the other hand, the inhibition of the activity of aminopyrine demethylase and aniline hydroxylase was moderate and not dose-dependent. In the time–course study, the activity of aniline hydroxylase was reduced moderately by CdO inhalation throughout the experimental period and the activities of aminopyrine demethylase and benzo[a]pyrene hydroxylase were reduced markedly 2 days after the inhalation of CdO. The different inhibition by CdO of these enzymes might be, in part, related to the differences in the forms of cytochrome P-450s, for the activity of these enzymes is known to depend either on cytochrome P-450 or P-448 and others [29, 30], which has been shown by their differential changes under the influence of various factors [31–33].

The inhibition of benzo[a]pyrene hydroxylase activity by CdO required relatively high doses; furthermore, the inhibition of the enzyme occurred slowly as a function of the time, reaching its maximum inhibition rate 2 days after the inhalation and the inhibition retained the same degree thereafter (Fig. 2). Omaye *et al.* [10] also reported that the maximum changes in biochemical parameters in the lung occurred 3 days after a single instillation of $CdCl_2$. These delayed effects of Cd on the lung might be related to the changes in the ratio of cells types as other workers [25, 26] observed the death of Type 1 cells during the first 24 hr and a marked increase in the number of Type 2 cells after 2 days in the lung of $CdCl_2$-exposed rats. These indicate that the inhibition of the microsomal enzymes by Cd does not represent direct nor primary action site of Cd on the lung. On the other hand, long duration of the inhibition of the microsomal enzymes and the increase in the lung weight observed in the time–course study of CdO inhalation might be, in part, due to the trapped Cd that was retained for long time in the lung. In the present study, the contents of Cd retained in the lung were substantially the same up to 6 days after the inhalation of CdO. This agrees largely with the previous reports on the dislocation of inhaled Cd in the rats [20, 34].

LD_{50} of CdO inhalation on rabbits ranged from 2500 min·mg/m^3 [35] to 8000 min·mg/m^3 [36]; the doses used in the present study (96 min·mg/m^3–336 min·mg/m^3) were relatively high, however, if compared to the actual tolerable dose-limit for Cd in the industrial environment and to the atmospheric contamination levels. But the risk of the inhibition of some pulmonary microsomal enzymes by airborne Cd microparticles might not be excluded, for inhaled Cd is very efficiently absorbed and retained in the body [6, 34–36] and it is slowly excreted from the body so that Cd might accumulate in the lung and other tissues in the repeated long-term exposure to Cd-fumes [37].

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