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BIOCHEMICAL EFFECTS OF INTRATRACHEAL INSTILLATION OF CADMIUM CHLORIDE ON RAT LUNG

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Abstract—Selected biochemical parameters in rat lung tissue were examined after intratracheal instillation of 0.5 μmole/kg of cadmium chloride ($CdCl_2$), which produces levels of approximately 10 μg Cd/g of lung wet weight 1 hr following instillation. Lungs were examined 2 hr, 1 day, 3 days and 7 days after $CdCl_2$ instillation and compared with matched controls receiving saline instillations. Cytosolic lysosomal enzymes, superoxide dismutase (SOD), catalase, and glutathione (GSH) peroxidase-associated enzymes increased significantly 24 hr after $CdCl_2$ insult. Peak increases of enzyme activities occurred about 3 days after $CdCl_2$ instillation. Levels of lung nonprotein sulfhydryl (NPSH) groups, thiobarbituric acid (TBA)-reactive products, protein and DNA increased after $CdCl_2$ instillation in a similar manner. By 7 days most measured biochemical parameters either remained at the peak 3-day values or decreased toward normal levels. The biochemical changes are consistent with known reported $CdCl_2$-induced edema and inflammation accompanied by phagocyte recruitment into lung tissue and reparative proliferation of lung cell types.

Inhalation [1, 2] or ingestion [3] of cadmium (Cd) compounds is known to produce lung damage in man. For this reason and because Cd exposure represents a model of acute lung injury, experimental studies have been undertaken to determine the nature of lung damage in animals exposed to Cd [4–9].

Although the cytodynamic events of Cd-induced lung injury have been well characterized [6, 8–10], less is known about the biochemical events responsible for the injury or accompanying the subsequent proliferative and reparative processes [11, 12]. Palmer *et al.* [6–8] noted that the cytodynamic events and certain biochemical responses of lung cells exposed to Cd resemble the responses produced by O_3-, O_2-, and NO_2-induced lung injury. As part of our overall interest in environmental lung toxicology and especially in biochemical events occurring subsequent to lung injury and reparative processes, selected biochemical parameters, including assessment of lipid peroxide formation (as reflected by TBA-reactive products) and lysosomal enzyme activities, were measured in lung tissues after intratracheal instillation of $CdCl_2$ into rats.

The results of this study are compared with previous reports from this laboratory on effects of O_3, high tensions of O_2, and of paraquat on biochemical measurements of rat lung homogenates [13–15]. In common with the two oxidants and with paraquat, Cd instillation leads to transient or sustained (7 day) increases in some of the biochemical parameters evaluated. Such data are inherently interesting as effects elicidated by $CdCl_2$ instillation and are perhaps even more interesting in that the present results may be compared to biochemical responses elicited by a fairly diverse group of pneumotoxic agents including paraquat and atmospheric oxidants.

MATERIALS AND METHODS

Instillation by intratracheal intubation. Eighty-day-old chronic respiratory disease-free rats of the Sprague–Dawley strain obtained from Hilltop Laboratory Animals, Scottsdale, PA. and weighing 350–400 g were housed in air-filtered, fiberglass cages with free access to rat chow and water prior to and during the experiment. They were injected with atropine (0.05 mg/kg, i.p.) and anesthetized with 50 mg/kg (i.p.) of sodium pentabarbital. An 18-guage (PE-160) catheter was then inserted down the trachea 70 mm from the incisors with the aid of a modified pediatric laryngoscope. $CdCl_2$ solution (91.5 μg $CdCl_2$/kg; 0.5 μmol/kg) in physiological saline was instilled via the catheter in a volume of 1 ml/kg over a period of 15 sec.; controls received 1 ml/kg of saline. At each of 2 hr, 24 hr, 3 days and 7 days following intratracheal instillations, eighteen $CdCl_2$-instilled and twelve saline-instilled rats were anesthetized with 100 mg/kg (i.p.) of pentabarbital and killed by exsanguination. Lungs of half the rats from both treated and control groups were perfused *in situ* with isotonic saline via the pulmonary artery. Lungs of the remaining rats were removed, trimmed of extraparenchymal bronchovascular tissues, blotted with gauze, weighed and divided into two portions, one of which was reweighed and dried at 100° for 48 hr for wet/dry weight determinations and the other used for determination of thiobarbituric acid (TBA)-reactive products, an indicator of lipid peroxide formation.

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Tissue preparation. Excised perfused whole lungs were trimmed of extraparenchymol tracheobronchial and vascular tissue, minced and homogenized with 0.10 M Tris buffer (pH 7.3) and 5 m EDTA. The crude lung homogenate was filtered through two layers of cheesecloth, and an aliquot of the filtered homogenate was used for nonprotein sulfhydryl (NPSH) and DNA determinations. The remaining homogenate was centrifuged at 750 *g* for 20 min and the supernatant fraction was recentrifuged at 100,000 *g* for 60 min. Pellets from each fraction were discarded and the activities of enzymes were determined in the soluble fraction (cytosol).

Enzyme assays. $CdCl_2$-induced lung damage is accompanied by considerable pulmonary edema, hemorrhage, lung phagocyte recruitement, and cellular proliferation [6]. These would be expected to contribute significantly to lung wet or dry weights and to total lung homogenate protein and DNA content. In order to compare the entire biochemical profile of the $CdCl_2$-induced lung with values found in appropriate controls, we chose to express the data per total lung [16]. Enzyme activities were also expressed on a per mg protein and a per mg DNA basis. The activity of cytosolic superoxide dismutase (SOD) was determined by the inhibition of cytochrome *c* reduction by the superoxide anion radical (O_2^-), which was supplied by the xanthine/xanthine oxidase reaction [17]. Catalase (hydrogen-peroxide: hydrogen-peroxide oxidoreductase, EC 1.11.1.6) activity was assayed by the method of Aebi [18]. Glutathione (GSH) peroxide (GSH hydrogen peroxide oxidoreductase, EC 1.11.1.9) activity was assayed by a modification of the method of Little *et al.* [19], with cumene hydroperoxide as a substrate and coupled with added GSH reductase [reduced-NAD(P): oxidized GSH oxidoreductase, EC 1.6.4.2] at pH 7.60. GSH reductase activity was determined by the method of Racker [20] and glucose 6-phosphate dehydrogenase (G-6-P-D) (D-glucose-6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.4.9) was determined by the method of Lohr and Waller [21].

Changes in cytosolic lysosomal enzyme levels were assessed by measuring the following: acid phosphatase activity was determined by the method of deDuve *et al.* [22] and the inorganic phosphate released from β-glycerophosphate was analyzed as described by Fiske and Subbarow [23]. Cathepsins C and D were assayed as described by Misaka and Tappel [24]. β-*N*-acetylglucosaminidase activity was determined by the method described by Dillard *et al.* [25]. Lysozyme activity was measured by the Worthington (Freehold, NJ) assay kit procedure, which is based on turbidometric measurement of the clearing of a *Micrococcus luteus* suspension at pH 6.2. Crystalline egg-white lysozyme was used as a standard.

TBA-reactive products and NPSH groups. Portions of non-perfused lungs were minced and homogenized with 4 ml of isotonic potassium phosphate buffer (pH 7.0) and 0.1 ml of 0.6% *dl-alpha*-tocopherol in 95% ethanol. The homogenate was filtered through two layers of cheesecloth and the filtrate was diluted to a total of 6 ml with phosphate buffer. A 1.5-ml aliquot of homogenate was added to 1.5 ml of 10% trichloroacetic acid (TCA) containing 0.8% TBA. The sample was mixed and placed in boiling water for 10 min. After the sample cooled, it was centrifuged at 1500 *g* for 15 min and the supernatant fraction was assayed spectrophotometrically at 532 nm. The amount of TBA-reactive products was calculated on the basis of the molar extinction coefficient for malonaldehyde (1.56×10^5). Aliquots of the filtered homogenates made from perfused lungs were added to equal volumes of 10% TCA. Ellman's reagent was used to determine NPSH content by the method of Sedlack and Lindsay [26].

Protein and DNA. Protein was determined in the lung cytosol fractions by the method of Miller [27]. DNA was determined in homogenates by its reaction with diphenylamine reagent [28].

Lung Cd contents. To obtain data on the Cd lung burden, twelve rats were instilled intratracheally with 183–440 μg $CdCl_2$/kg (1 to 2.4 μmol $CdCl_2$/kg) in a volume of 1 ml/kg. (A more concentrated solution of $CdCl_2$ was instilled in order to optimize our method for measuring lung Cd concentrations.) After various time intervals, lungs were removed *en bloc*, trimmed of bronchovascular tissue, weighed, homogenized in deionized distilled H_2O, transferred to Erlenmeyer flasks, dried for 24 hr at 105°, reweighed and digested at 60° for 24 hr with 10 ml of 10% aqueous tetramethyl ammonium hydroxide [29]. After dilution with distilled deionized water, samples were analyzed for Cd utilizing a Varian Techtron AA 120 with an air–acetylene flame. The accuracy of this method was demonstrated by an average recovery of 82 per cent for $CdCl_2$ based on an analysis of three separate control rat lungs to which known amounts of $CdCl_2$ were added to lung homogenate preparations. Estimated experimental tissue Cd levels were extrapolated from the measured per cent retentions of Cd at various time intervals (corrected for average recovery).

The relatively low levels of lung tissue Cd concentrations in the present experiments did not produce measurable changes in homogenate enzyme activities, a finding previously noted in the literature for GSH peroxidase activities at levels of Cd up to 1 mM [30].

Calculations and statistics. Biochemical changes were expressed on a per lung basis as per cent increase compared to controls (per cent of control mean activity) and the data were analyzed by Student's '*t*' test. Biochemical changes of treated samples are compared to controls taken at the same respective time periods. Control mean values taken at 2 hr were not significantly different from control mean values taken at 24 hr, 3 days and 7 nights.

RESULTS

Macroscopic lung damage. Intratracheal instillation of $CdCl_2$ into rats provided a fairly uniform pattern of injury (Fig. 1). Multiple petechial hemorrhages and edema were noted throughout the surface of the organ 2 hr after $CdCl_2$ installation. At 24 hr, 3 days and 7 days after $CdCl_2$ installation, lungs showed extensive edema and hemorrhage. Wet lung weights examined 2 hr, 24 hr, 3 days and 7 days after $CdCl_2$ were significantly increased compared with saline-instilled controls (Table 1). Changes in total wet lung weight reached maximal values at 1 day, whereas dry lung weights were increased most markedly 7 days after $CdCl_2$. As a consequence, wet- to dry-weight

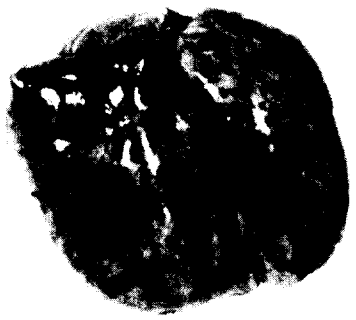

Fig. 1. Multiple petechial hemorrhages in rat lung 2 hr after instillation.

Table 1. Increase in rat lung weights after $CdCl_2$ instillation

Time after treatment	Per cent increase	
	Wet	Dry
2 hr	49 ± 4.5 (< 0.001)	25 ± 5.2 (< 0.05)
1 day	101 ± 17 (< 0.001)	15 ± 4.5 (< 0.02)
3 days	98 ± 14 (< 0.001)	41 ± 10 (< 0.01)
7 days	87 ± 18 (< 0.001)	83 ± 16 (< 0.001)

* Each value shown represents $\bar{x} \pm$ S. E. M. (N = 9). P values compared to matched controls (N = 6) for each time period are as indicated. Lung weights of saline-treated rats at 2 hr (N = 6) were: wet weight, 1.42 ± 0.091 g; dry weight, 283 ± 19 mg.

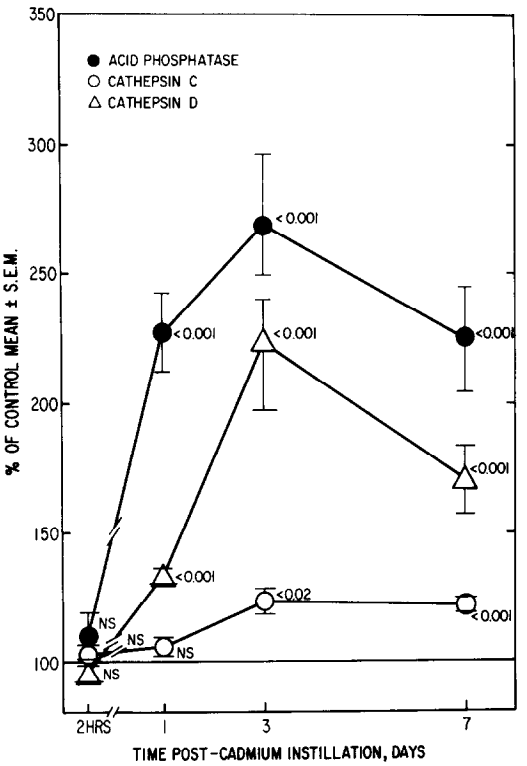

Fig. 2. Effect of $CdCl_2$ instillation on rat lung lysosomal enzymes. Enzyme activities ± S. E. M. for each time (N = 9) are expressed as a percentage increase compared with control mean. Average enzyme activities for control lungs at 2 hr (N = 6) were: acid phosphatase, 0.309 ± 0.008 μmol P_i released min/lung; cathepsin C, 0.839 ± 0.040 μ/mol tyrosyl hydroxamate equiv./min/lung; and cathepsin D, 0.249 ± 0.006 μmol Tyr. equiv./min/lung.

ratios were maximal at 1 day and decreased progressively at days 3 and 7.

Lysosomal enzymes. The changes in the cytosolic activities of acid phosphatase, cathepsin C and cathepsin D in the lungs of rats after $CdCl_2$ instillation are shown in Fig. 2. Two hr after cadmium administration the three cytosolic lysosomal enzyme activities were not significantly different from control levels. At 24 hr all three enzymes increased in activity, with significant increases occurring statistically for acid phosphatase (226 per cent) and cathepsin D (133 per cent). There was an apparent peak increase of all three lysosomal enzyme activities 3 days after $CdCl_2$ instillation, acid phosphatase increasing 268 per cent, cathepsin C 123 per cent and cathepsin D 233 per cent. Seven days after $CdCl_2$ the activities of acid phosphatase and cathepsin D dropped to lower values when compared to day 3, with cathepsin C remaining the same. β-*N*-acetylglucosaminidase activity followed a pattern similar to acid phosphatase and cathepsin D (activities were 222, 300 and 273 per cent of control values at 24 hr, 3 days and 7 days respectively), whereas lysozyme activity increases were similar to cathepsin C (Fig. 3).

SOD. Cytosolic SOD and catalase activities were not significantly increased until 3 days after $CdCl_2$ instillation (Fig. 4), SOD being increased to 142 per cent and catalase to 173 per cent compared to controls. Seven days after $CdCl_2$ exposure, SOD activity decreased to 114 per cent of control whereas catalase increased to 194 per cent of control.

TBA-reactive products and NPSH levels. Two hr after $CdCl_2$ instillation there was no significant increase in lipid peroxidation products, as measured by TBA-reactive products (Fig. 5). However, at 1 and 7 days significant increases to 121 and 154 per cent occurred. Total NPSH levels determined after $CdCl_2$ instillation were elevated to 223, 253 and 334 per cent of controls at days 1, 3 and 7, respectively, after $CdCl_2$ instillation.

GSH peroxidase system. Responses of the GSH peroxidase system to $CdCl_2$ installation are shown in Fig. 6. After exposure to $CdCl_2$, GSH reductase and G6PD activities were not changed significantly from controls in 2 hr. G6PD was increased to 350 and 324 per cent of control values at 1 and 3 days, respectively, then dropped to 208 per cent of control values at day 7. GSH reductase increased to 146, 203 and 181 per cent at 1, 3 and 7 days respectively. Similarly, GSH peroxidase at 1, 3 and 7 days was elevated to 165, 248 and 205 per cent of controls, respectively. At 2 hr after instillation a significant drop in GSH peroxidase

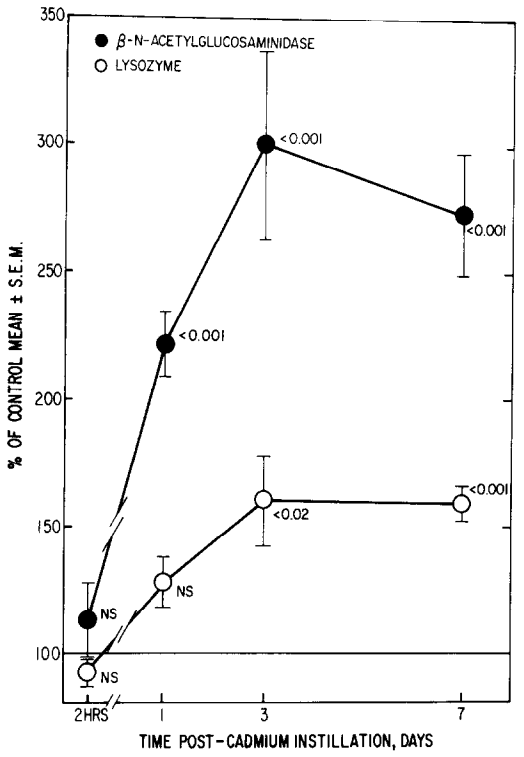

Fig. 3. Effect of $CdCl_2$ installation on rat lung lysosomal enzymes. Enzyme activities ± S.E.M. expressed as in Fig. 2. Average enzyme activities for control lungs at 2 hr (N = 6) were β-*N*-acetylglycosaminidase, 0.131 ± 0.016 μmol *p*-nitrophenol formed/min/lung; and lysozyme, 239 ± 14 μg of lysozyme activity uints/lung.

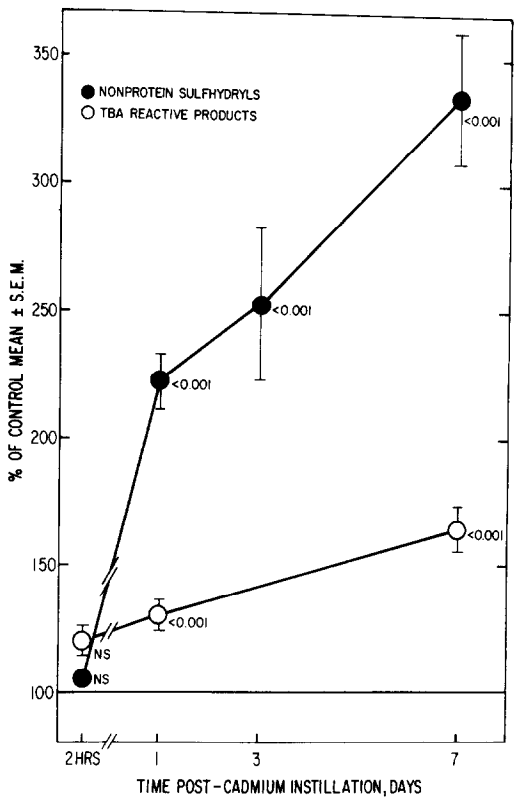

Fig. 5. Effect of $CdCl_2$ instillation on rat lung NPSH and TBA-reactive products. Experimental values ± S. E. M. are expressed as in Fig. 2. Average levels in control lungs at 2 hr (N = 6) were: NPSH, 0.867 ± 0.035 μmol/lung; and TBA-reactive products, 5.68 ± 0.51 μmol/lung. TBA-reactive products at 3 days were not determined.

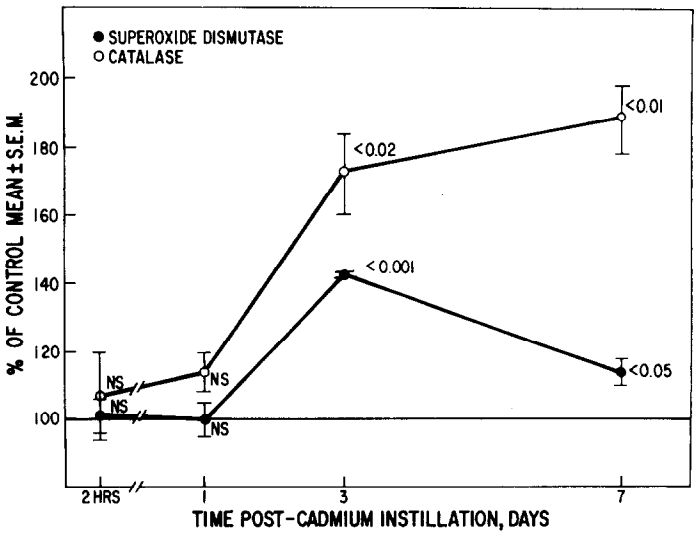

Fig. 4. Effect of $CdCl_2$ installation on rat lung SOD and catalase activities. Enzyme activities ± S.E.M. are expressed as in Fig. 2. Average enzyme activities for control at 2 hr (N = 6) were: SOD, 346 ± 7 units (one unit of activity is defined as one 50 per cent inhibition of the control rate)/lung and catalase 0.702 ± 0.092 μmol H_2O_2 decomposed/min/lung.

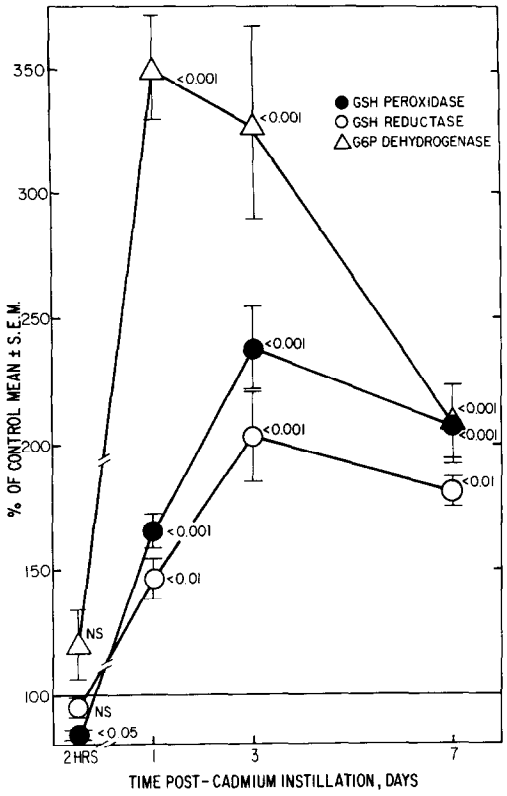

Fig. 6. Effect of $CdCl_2$ instillation on rat lung GSH peroxidase system. Enzyme activities ± S. E. M. are expressed as in Fig. 2. Average enzyme activities for control lungs at 2 hr (N = 6) were: GSH peroxidase, 1.48 ± 0.11 μmol NADPH oxidized/min/lung; GSH reductase, 0.90 ± 0.16 μmol NADPH oxidized/min/lung; and G-6-P-D- 2.60 ± 0.17 μmol NADP + reduced/min/lung.

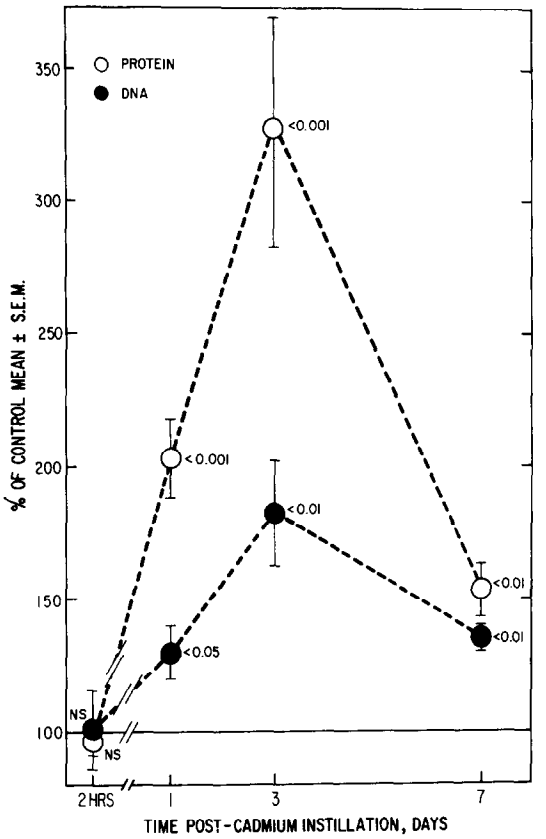

Fig. 7. Effect of $CdCl_2$ instillation on rat lung DNA and cytosolic protein content. Experimental values ± S. E. M. are expressed as in Fig. 2. Average concentrations in control lungs at 2 hr (N = 6) were: DNA, 6.25 ± 0.95 mg/lungs; and protein, 48.4 ± 10.6 mg/lung.

activity (to about 80 per cent of control activity) was observed.

DNA and protein. Cytosolic lung protein and lung homogenate DNA did not significantly increase 2 hr after $CdCl_2$ installation (Fig. 7). Protein significantly increased to 203, 327 and 154 per cent at 1, 3 and 7 days, respectively, whereas DNA increased to 129, 182 and 133 per cent of control values at 1, 3 and 7 days respectively.

Specific activities. If the enzyme activities presented in Figs. 2–4 and 6 are expressed on a per mg protein or per mg DNA basis, as in Table 2, the enzyme activities decrease or increase as the denominator, mg of protein or DNA, fluctuates. There is no way at

Table 2. Enzyme activities after $CdCl_2$ instillation*

Parameter evaluated	Activity† (% of control value)				Activity‡ (% of control value)			
	1 day	3 day	7 day	2 hr	1 day	3 day	7 day	
Acid phosphatase	115	112	82	147	107	175	149	170
Cathepsin C	108	52	38	79	101	81	68	92
Cathepsin D	100	66	68	112	93	103	123	130
β-*N*-acetylglucosaminidase	119	109	92	179	111	171	166	208
Lysozyme	98	65	49	105	91	101	90	121
Superoxide dismutase	106	49	44	75	99	77	79	86
Catalase	113	56	52	124	105	88	95	143
GSH peroxidase	87	82	72	134	81	128	131	155
GSH reductase	101	72	62	118	94	113	112	137
Glucose 6-phosphate dehydrogenase	127	172	100	135	119	269	182	156

* Values were calculated on the basis of Figs. 2–4, 6 and 7.
† Absolute activity is in units/mg of protein.
‡ Absolute activity is in units/mg of DNA.

present to evaluate rigorously the contribution of plasma proteins, inflammatory cell infiltrations or proliferation of endogenous lung cells (although such techniques as ^{131}I labeling of plasma proteins and specific labeling of erythrocytes and leukocytes are available for estimation of blood constituent contaminations in lung homogenate preparations). The data of Table 2 are therefore presented primarily for comparative purposes. As the best means of calculation of such data is an unresolved question at this time, presentation of the same results expressed per lung, per mg protein and per mg DNA appears justified (see Discussion).

Lung Cd *contents.* Following intratracheal instillations of 183–449 μg $CdCl_2$/kg, amounts two to five times greater than were instilled in the studies previously described so as to optimize our measurement techniques, 40–50 per cent of instilled Cd was recovered in the lung homogenates derived from rats killed within 1 hr of instillation (N = 3). One day after instillation, lung retentions were approximately 15 per cent of instilled doses (N = 3), whereas only approximately 10 per cent of the instilled Cd was recovered at days 3 (N = 3) and 7 (N = 3). A somewhat similar time course for lung retention of inhaled lead has been described [31].

DISCUSSION

Inhalation of $CdCl_2$ results in lung damage characterized by early vascular congestion, hemorrhage and inflammatory edema, followed by reparative–proliferative processes. Since the pathology of lung $CdCl_2$ toxicity has been thoroughly described [6–8], similar studies were not repeated in the present investigation; however, gross observations and wet weights of $CdCl_2$-damaged lungs clearly indicated the presence of extensive edema and hemorrhage (Fig. 1). The time sequence and magnitude of the inflammatory–infiltrative and reparative–proliferative responses after instillation of $CdCl_2$, as measured by the increases in lung weights, G-6-P-D activities and DNA contents, are of similar magnitude to changes found following a single $CdCl_2$ exposure which produces a lung tissue Cd content of approximately 1 ± 0.5 μg/g wet weight, a tissue level comparable to our calculated estimate of 2–4 μg/g wet weight at 3 days.

The $CdCl_2$ doses utilized in the current studies (54 μg Cd/kg instilled, 10–2 μg Cd/g wet lung when measured at 1 h–7 days after instillation) can be contrasted with various lung burdens associated with environmental or experimental Cd exposures. Cd can be detected at levels of 1–4 μg/cigarette [32] of which 10–15% reaches mainstream inhalation [33]. Tissue Cd levels are increased several-fold in organs of patients with cigarette-associated lung disease [32, 34, 35], and lung Cd levels are increased in patients with emphysema [36]. Intratracheal instillation of approximately 1000 μg Cd/kg to guinea pigs [10], a dose which causes a fatal massive pulmonary congestion, edema and hemorrhage in most animals, as well as multiple aerosol deliveries to rats, which produce lung tissue concentrations of approximately 6 μg Cd/g wet lung [5], induce extensive lung injury followed by reparative peribronchiolar fibrosis and focal centrilobular emphysema. More recently it has been shown that $CdCl_2$ delivered to rats via one single 2-hr aerosol exposure in concentrations producing lung levels of 1–2 μg Cd/g wet weight causes a marked increase in the number of airway phagocytes [5, 9].

Heavy industrial exposures to Cd also have been implicated as a contributing factor in the genesis of destructive and fibrotic lung diseases [36, 37]. Of interest is the reported Cd level of 2700 μg Cd/g wet weight in a Cd alloy worker who died of acute Cd inhalation associated with emphysema [38]. Industrial exposure to Cd is mostly in the form of CdO as a fume or as a dust; while as a fume the rate of Cd entry into solution is high and therefore relevant to $CdCl_2$, as a dust the rate of solution is extremely low and of questionable biological significance (P. Gross, personal communication).

It should be recognized that the medical literature is replete with attempts to show correlations between various pathologies and trace metal concentrations in tissues. However, such estimates must be tempered with caution. The specific chemical form(s) and location in which the trace metal exists within the tissues are of paramount importance and the measured total concentration in a particular biologic tissue may have little meaning. Little attention has been directed toward possible species variability of Cd burdens. We conclude that the clinical studies taken *in toto* do not indicate that Cd in cigarettes or in Cd workers causes emphysema, although it is valid to speculate that Cd may play a contributing role.

The overall pattern of acute $CdCl_2$-induced structural upset shows similarity to those caused by O_3, NO_2 and high tensions of O_2 [6, 8, 39, 40] and by ingestion of the herbicide paraquat [41]. Like $CdCl_2$, these agents also induce diffuse and multifocal vascular congestion with diffuse edema and hemorrhage, accumulation of neutrophilic polymorphs, and considerable epithelial and endothelial cell damage. Epithelial type II cells and fibroblasts are the major cellular components of the ensuing reparative–proliferative process. Also, it has been pointed out that lung parenchymal injury induced by ionizing radiation, nickel carbonyl, mercury vapors, viruses and anti-lung antibodies shares many cytopathological features and temporal sequences with those induced by the aforementioned insults [42].

As shown in Table 3, the biochemical results of the present investigations, which focused in part on so-called "anti-oxidant" biochemical substances for reasons of comparison, resemble those obtained previously in this laboratory in studies of three other types of lung injury: (1) O_3-induced damage [13]; (2) hyperoxia-induced damage [14]; and (3) paraquat-induced damage [15]. This is not surprising, in view of the fact that many pneumotoxins initiate, in varying degrees, similar sequences of cytodynamic events, depending on the extent of epithelial–endothelial cell damage, the severity of damage to remaining regenerative epithelial, interstitial and endothelial cells, and the nature of most modifying protective and/or amplifying factors [43].

It appears that sequential biochemical changes in $CdCl_2$-exposed lungs share many features with other types of lung injury. Factors presumably accounting for the changes of biochemical parameters in lung

Table 3. Increase in selected metabolic parameters after acute lung injury in the rat*

	Per cent increase					
	NPSH	G-6-P-D	GR	GP	Cat	SOD
O_2 (0.8 ppm × 4 days)	150	180	120	190†	135†	116†
O_2 (90% × 5 days)	250	350	150	380		145
Paraquat [25 mg/kg (i.p.), 4 hr]	200	110	160†	160†	190	115
$CdCl_2$ [0.5 μmol/kg (i.t.), 3 days]	250	325	200	240	175	140

* References are listed in the text except for values marked with a dagger, which represent our unpublished values. $CdCl_2$ values shown are for current study. NPSH represents non-protein sulfhydryl groups; G-6-P-D, glucose 6-phosphate dehydrogenase; GR, GSH reductase; GP, GSH peroxidase; Cat, catalase; and SOD, superoxide dismutase.

homogenates following acute lung injury include: (1) blood contamination factors [44]; (2) phagocyte recruitment from blood into lung tissue; (3) inflammation and reparative–proliferative-associated activities in endogenous lung cells; and (4) an attempt by lung cells to adapt to $CdCl_2$-induced and/or inflammation- and phagocyte-associated metabolic stresses.

Biochemical measurement performed on crude lung homogenates are probably useful qualitative indicators of toxicant-induced changes in lungs. Such analyses, however, cannot presently lead to insights concerning mechanisms of acute lung cell injury, and/or specifically address the question of the unknown mechanism(s) whereby $CdCl_2$ induces lung damage. In the acute phase of such injury, the edema and inflammation cause major perturbations of the protein and DNA content of lung homogenates. As a consequence of these perturbations, expression of data on a per mg of protein or per mg of DNA basis poses a problem in that there is no rigorous way at present to evaluate the contribution of exudative plasma proteins and/or infiltration of phagocytes. Thus, the values in Table 2, which calculate the specific activities of enzymes or enzyme contents "per cell" (the average cell content of DNA in a nucleated diploid mammalian cell is constant at each time point), cannot be rigorously interpreted. For example, it appears that 1–3 days after $CdCl_2$ exposure, significant inhibition of the activities of numerous enzymes, on a per mg protein basis (Table 2), occurs, including the important protective antioxidant enzymes superoxide dismutase, catalase, GSH peroxidase and GSH reductase. It is unclear, however, what proportion of these alterations is due to changes in contaminating exudative plasma proteins and infiltrating inflammatory cells and what proportion is due to changes in endogenous lung cells. Also, the data demonstrating an increase in TBA reactants at 1–7 days after $CdCl_2$ administration lend support to the hypothesis that lipid peroxidation plays a role in $CdCl_2$-induced lung damage. However, it is uncertain what proportion of the increase occurs as a direct result of lung cell damage and inflammation, including that related to the activated oxygen metabolites generated by phagocytes, and what proportion results from lung tissue reparative–proliferative processes. The data concerning cytosolic lysosomal enzyme activities after $CdCl_2$ instillations also follow no readily interpretable pattern. While several investigators have equated release of lysosomal enzymes into the cytosol with lung damage [25], it is recognized that the overall patterns of individual lysosomal enzyme release into cytosol may reflect the subcellular location of the enzymes rather than serving as a quantitative index of injury *per se*.

While the magnitude of biochemical changes may provide useful toxicological information, elucidation of the mechanisms whereby various agents damage the lung will have to await further development of techniques for assessing effects on each of the major lung cell types, fuller understanding of the role of lung inflammation, and further definitions of the role of endogenous protective and amplification factors.

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