

Investigations on the Lung and Kidney Function in Workers Exposed to Cadmium

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The kidney seems more sensitive to the chronic effect of cadmium than the lung. Only minor impairments of lung function (mild form of obstructive lung disease) were found after long-term occupational exposure (< 20 yr) to moderate concentration of cadmium oxide dust and fume. This conclusion, cannot, however be extrapolated to acute or subacute inhalational exposure.

The nephrotoxicity of cadmium consists in a tubular dysfunction characterized by an increased excretion of β_2 -microglobulin and giving rise to the classical tubular proteinuria and in a glomerular dysfunction evidenced by an increased excretion of high molecular weight proteins and increased levels of β_2 -microglobulin and creatinine in plasma and giving rise to a glomerular type proteinuria. These renal changes were mainly found in workers whose cadmium concentration at the time of the survey exceeded 1 $\mu\text{g Cd}/100\text{ ml}$ in blood and 10 $\mu\text{g Cd/g}$ creatinine in urine. It should, however, be stressed that higher levels of Cd in blood and in urine are not necessarily associated with the presence of excessive proteinuria.

In newly exposed workers, the Cd level in blood increases progressively to a plateau after several weeks. Cadmium level in urine fluctuates more. In workers exposed for several months to an airborne concentration exceeding 200 $\mu\text{g}/\text{m}^3$, Cd concentration in urine seems mainly influenced by recent exposure.

During the last 5 years we have undertaken a few epidemiological studies among Belgian workers occupationally exposed to cadmium. We intend to summarize in this paper the main findings of these surveys.

Cross-Sectional Study on the Lung and Kidney Function

In 1973-1974 a first cross-sectional study was carried out to get an estimate of the prevalence of lung and kidney damage in Belgian workers exposed to cadmium (1, 2). A group of 150 workers exposed to cadmium was examined and a control group was selected to match the exposed group according to age, height, smoking habits and duration of work in the same factory. We applied to each control and exposed worker the questionnaire on chronic bron-

chitis of the European Community for Coal and Steel. Maximal expiratory flow-volume curves were obtained by displaying on a storage oscilloscope the flow rate measured with a Fleisch 4 pneumotachograph versus volume obtained by integration of the flow rate. Each worker performed at least four forced vital capacity (FVC) maneuvers. An electronic apparatus enabled the digital readout of the following indices: FVC, forced expiratory volume in 1 sec (FEV 1.0) peak expiratory flow rate (PEFR), maximal expiratory flow rate at 50% (MEFR 50% VC) and at 75% (MEFR 75% VC) of the FVC, and the time required to expire 50% of the FVC (T 0.50). For each worker, we took a postero-anterior chest x-ray in full inspiration. Various biological parameters were also measured in particular: cadmium concentration in blood (Cd-B) and urine (Cd-U), total proteinuria, and aminoaciduria. Electrophoresis of urinary proteins on agarose gel was performed after concentration of the urine.

The workers were attached to four factories: an electronic workshop, a Ni-Cd storage battery factory, and two Cd-producing plants (A and B). The

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total airborne cadmium concentration and its "respirable" fraction were determined at the different work sites. Striking differences between total and "respirable" dust concentration were found. The ranges of total dust concentration found in the four factories at the time of the survey were: electronic workshop, 6.9–18.6 $\mu\text{g}/\text{m}^3$; nickel-cadmium battery factory, 1–465 $\mu\text{g}/\text{m}^3$; cadmium-producing plant A, 3.7–356 $\mu\text{g}/\text{m}^3$; cadmium-producing plant B, 8–27,050 $\mu\text{g}/\text{m}^3$. The highest respirable dust concentration found in the same factories were 4, 88, 21, and 65 $\mu\text{g}/\text{m}^3$, respectively.

Female Workers

In the electronic workshop which employs mainly female workers ($n = 26$; mean age, 29.4 yr; average duration of exposure to Cd, 4.4 yr) no pulmonary and renal disturbances were found. However, the effect of cigarette smoking on pulmonary function was already evident as indicated by a significant reduction of MEF₅₀ VC ($p < 0.05$) and an increase of T 0.50 ($p < 0.05$) in smokers of both the exposed and control groups as compared to the nonsmokers of the same groups. Thus, an occupational exposure of 4 yr to a time-weighted average concentration of about 10 $\mu\text{g}/\text{m}^3$ (total dust) or less than 4 $\mu\text{g}/\text{m}^3$ (respirable dust) has probably no deleterious effect on the lung and kidney function. Since cigarette smoking entails an earlier deleterious effect on maximal expiratory flow rates than does inhalation of Cd dust for 4 yr (at a concentration around 10 $\mu\text{g}/\text{m}^3$), it can be concluded that the pulmonary changes found in smokers is mainly due to compounds of cigarette smoke other than cadmium.

Male Workers

The male workers were divided into two groups according to the duration of cadmium exposure. The first group was composed of 90 workers (75 smokers, mean age 41.5 yr, and 15 nonsmokers, mean age 35.5 yr), all exposed to cadmium dust and fume for less than 20 yr (on the average 7.5 yr). By comparison with a matched control group, the exposed group showed a very slight (but statistically significant) reduction in FVC (–7.2%) FEV_{1.0} (–6%), and PEFR (–6%). No significant difference was found between the control and exposed workers with regard to frequency of cough and/or sputum production, wheezing or shortness of breath. No abnormality was found in chest x-ray. No other significant difference was found between the exposed and control workers except Cd-B ($\bar{X} \pm \text{SE} = 2.5 \pm 0.3 \mu\text{g}/100 \text{ ml}$ in exposed workers versus $0.6 \pm 0.02 \mu\text{g}/100 \text{ ml}$ in control workers) and Cd-U ($\bar{X} \pm \text{SE}: 23.3 \pm 3.3 \mu\text{g}/\text{g}$ creatinine in exposed workers versus $1.7 \pm 0.19 \mu\text{g}/\text{g}$ creatinine in control workers). An abnormal electrophoretic pat-

tern of urinary proteins was found in 9% of the exposed workers versus 5% in the controls but the difference is not statistically significant.

It should be pointed out that Cd-B and Cd-U in the unexposed workers is significantly higher than the levels found in the general population in Belgium (mean Cd-B in 331 nonsmoking and 109 smoking pregnant women were 0.12 and 0.20 $\mu\text{g}/100 \text{ ml}$, respectively; mean Cd-U in 21 adult males and in 45 children were 0.35 and 0.23 $\mu\text{g}/\text{g}$ creatinine, respectively)(2–5). This is due to the fact that although they were not working in direct contact with cadmium, the control workers employed in the same factory as the cadmium workers were exposed to a certain degree of environmental pollution by cadmium. Indeed the total airborne cadmium concentration in the control areas of the factories ranged from 0.03 to 7.3 $\mu\text{g}/\text{m}^3$ (2, 6), whereas the median airborne concentration of cadmium (24 hr averages) in Belgian cities is around 0.010 $\mu\text{g}/\text{m}^3$ (7).

The second group was composed of 25 male workers (mean age 52 yr) exposed for more than 20 yr to cadmium, on the average 27.5 yr. These workers belonged mainly to the cadmium producing plant A. A control group matched with the exposed group was also selected from the same factory. The frequency of cough but not that of sputum production was greater in the exposed workers than in the control. The results of the spirometric tests indicated that, as in the preceding group, the three indices FVC (–12%) FEV_{1.0} (–12%), and PEFR (–9.5%) were moderately reduced in the exposed workers (Table 1). Their lung x-rays were normal. With regard to the biological measurements, several parameters were found to be statistically different between the exposed and control workers such as Cd-B (2.6 ± 0.3 vs. $0.6 \pm 0.02 \mu\text{g}/100 \text{ ml}$), Cd-U (30.7 ± 4.5 vs. $2.2 \pm 0.19 \mu\text{g}/\text{g}$ creatinine) total proteinuria (458 ± 96 vs. $136 \pm 10 \text{ mg}/\text{g}$ creatinine), hematocrit (41.4 ± 0.6 vs. $44.7 \pm 0.6\%$), number of workers with abnormal electrophoretic pattern of urinary proteins (64 vs. 0%). These patterns were classified in four categories according to the following criteria: normal pattern with faint bands of albumin and transferrin, glomerular type pattern with marked

Table 1. Pulmonary function tests in Cd-exposed (> 20 yr) and control workers.

| | Cd-exposed | Controls |
|-------------------------|-------------------|----------|
| N | 25 | 25 |
| FVC, l. | 3.51 ^a | 3.97 |
| FEV _{1.0} , l. | 2.48 ^a | 2.81 |
| T 0.50, sec | 0.39 | 0.39 |
| PEFR, l./sec | 7.22 ^a | 7.97 |
| MEFR 50% VC, l./sec | 3.16 | 3.57 |
| MEFR 75% VC, l./sec | 0.99 | 1.03 |

^a Significantly different from control value.

bands of high molecular weight (HMW) proteins (mainly albumin and transferrin), tubular type pattern with marked bands of low molecular weight (LMW) proteins (β_2 -microglobulin and post- γ -protein), and a mixed pattern which is a combination of the two preceding ones. Of the 16 abnormal electrophoretic patterns, eight were classified as glomerular proteinuria and eight as mixed proteinuria. No pure tubular proteinuria was found by agarose gel electrophoresis (Fig. 1).

The following observations were also made during this study:

The prevalence of kidney lesion (diagnosed on the basis of total proteinuria and electrophoresis) is related to the duration of exposure. It increases markedly after 15–20 yr exposure to cadmium.

The presence of renal lesion associated with an increased Cd concentration mainly in urine but also in blood (1, 8, 9). Usually the workers with excessive proteinuria excreted more than 10–15 $\mu\text{g Cd/g}$

creatinine.

In the control and the exposed workers there was no correlation between Cd-B and duration of employment which suggests that Cd-B does not correlate with body burden but is probably more influenced by recent exposure. In control workers exposed only to slight environmental pollution by cadmium around Cd factories, a low but statistically significant correlation ($r = 0.41$) was found between Cd-U and duration of employment. This suggests that at low Cd exposure as sustained by these workers, Cd-U could mainly be influenced by body burden. Therefore since in this population Cd-B and Cd-U reflect different parameters (exposure or body burden) there was no significant correlation between Cd-B and Cd-U. Contrary to the finding in control workers, no correlation between Cd-U and duration of employment was found in exposed workers without or with kidney lesions. This observation suggests that when exposure has lasted for a certain length of time and has exceeded a certain critical level, Cd-U is probably more a reflection of current exposure than body burden. This conclusion was strengthened by the fact that in workers exposed to cadmium there was a statistically significant correlation between log Cd-U and log Cd-B ($r = 0.53$) (8).

Recently we completed a follow-up study of 11 workers exposed to high levels of cadmium dust and fume ($> 200 \mu\text{g}/\text{m}^3$) in a small plant producing cadmium salts (unpublished results). During this study regular monitoring of the cadmium level in blood and in urine as well as of the airborne cadmium concentration (with the use of personal samplers) was performed. The results tend to support the above hypothesis. However, in newly exposed workers Cd-B does not reach a steady-state level before several weeks (up to 3 months) (Fig. 2). This is in agreement with the findings of Kjellström (10).

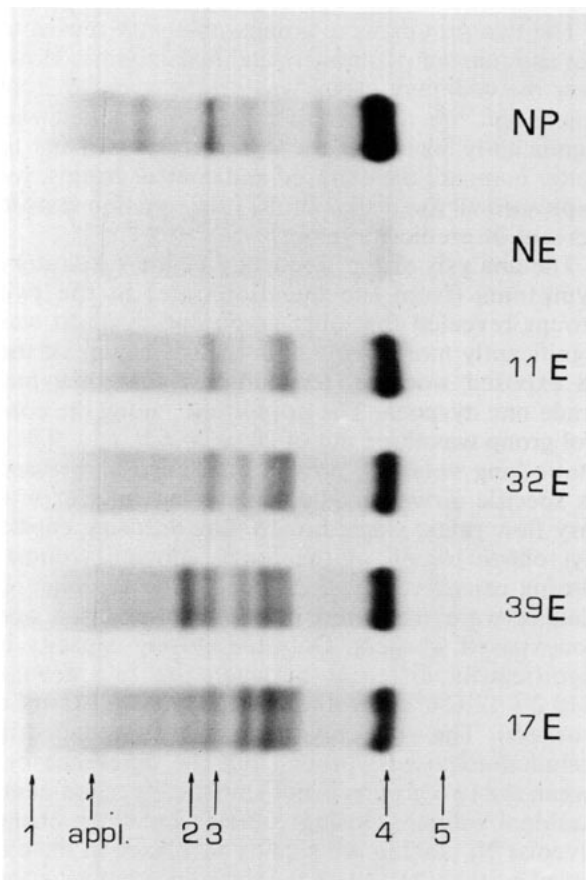


FIGURE 1. Electrophoresis on agarose gel of urinary proteins of one control worker (NE) and four Cd exposed workers (E). The arrows indicate the localization of (1) post- γ -protein, (2) β_2 -microglobulin, (3) transferrin, (4) albumin, (5) prealbumin; (11E, 32E) glomerular type patterns, (39E, 17E) mixed type patterns; (NP) normal plasma.

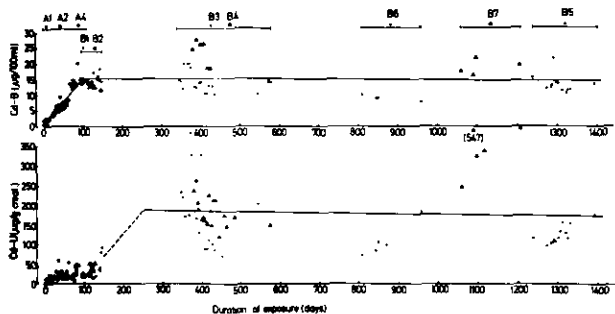


FIGURE 2. Relationship between duration of exposure and Cd level in blood and in urine in workers from the same factory producing various cadmium salts. The airborne cadmium concentration at the various work places exceeded $200 \mu\text{g}/\text{m}^3$. A₁ to A₄ are newly exposed workers, B₁ to B₇ are workers already employed at the start of the survey.

The significance of the cadmium level in blood and urine is now being evaluated by measuring simultaneously the Cd levels in blood, urine, liver, and kidney in 300 workers from two cadmium plants in Belgium.

This is a joint project in collaboration with Dr. T. Harvey and Dr. D. Chettle of the University of Birmingham, who developed a portable neutron activation system for measuring cadmium concentration in liver and in kidney. The results have not yet been statistically analyzed.

Summary

In summary, the results obtained during the first cross-sectional study suggested the following tentative conclusions.

The kidney seems more sensitive to the chronic effect of cadmium than the lung. Only minor impairments of the lung function (mild form of obstructive lung disease) were found, even in the group of workers exposed for more than 20 yr to Cd oxide dust and fumes. However, since only spirometric tests were used during the survey, further investigations with the use of more elaborate lung function tests are indicated to confirm this conclusion.

Cadmium proteinuria is classically considered as a tubular type proteinuria characterized by a predominance of LMW proteins which mainly come from the plasma (β_2 -microglobulin, retinol binding protein, lysozyme, ribonuclease, post- γ -protein, immunoglobulin light chains) but are incompletely reabsorbed by the proximal tubule. Our data indicated that the proteinuria developed by workers excessively exposed to cadmium consists in enhanced excretion not only of LMW proteins but also of HMW proteins like albumin and transferrin. This observation suggests that cadmium can affect not only the reabsorption of LMW proteins by the proximal tubule but also the mechanism regulating in the glomerulus or the tubule the excretion of HMW proteins. However, this conclusion was based on a simple qualitative analysis of urinary protein patterns obtained by agarose gel electrophoresis.

We have attempted in subsequent investigations to further document these preliminary conclusions by investigating lung function in more detail and by characterizing further the proteinuria of workers exposed to cadmium.

Second Clinical Investigation of Lung Function

To test the first conclusion regarding the moderate lung toxicity of chronic exposure to cadmium

oxide dust and fume, we studied in detail the lung function of 18 workers exposed for more than 20 yr (average 32 yr) to cadmium in one cadmium producing plant, and compared them with 20 nonexposed workers from the same factory (11). The exposed group comprised all the workers still in contact with cadmium and also seven workers who had been reassigned to another working place within the last 2 yr because they had developed proteinuria. During the last few years the total airborne cadmium concentration at the different work places of the plant was between 50 and 350 $\mu\text{g}/\text{m}^3$. At the time of the survey the total airborne cadmium concentration in the exposed area ranged from 3 to 67 $\mu\text{g}/\text{m}^3$ but before 1970 a few workers who took part in the survey may have been exposed to higher levels of Cd dust and fumes. We used chest x-rays, lung tomograms, and several lung function tests, including indices which today are considered to reflect destruction of the lung parenchyma.

The two groups were comparable with regard to age and number of smokers and nonsmokers. However the cadmium group was on the average 2 cm taller, and the number of cigarettes smoked was significantly higher in the control group. Thus to better compare the exposed and control groups, we expressed all the results of the lung function tests in percent of predicted values.

The analysis of the frequency of the respiratory symptoms (from the questionnaire) in the two groups revealed that only grade one dyspnea was significantly more frequent in the Cd group. Of the 18 exposed workers, ten admitted that they had grade one dyspnea. The proportion among the control group was three out of 20 ($\chi^2 = 6.9$, $P < 0.01$). Static lung volumes, airway resistance (expressed as specific airway conductance), maximal expiratory flow rates, single-breath lung diffusing capacity, elastic recoil of the lungs, closing volume, closing capacity, and the slope of the alveolar N_2 plateau were measured in cadmium-exposed and nonexposed workers. Only the closing capacity is significantly different between the two groups (110.2% TLC in control workers, 119.9% TLC in Cd workers). The vital capacity is slightly reduced in cadmium-exposed workers, but the difference between the two groups is not statistically significant. Residual volume, closing volume, and slope of the alveolar N_2 plateau are slightly increased in the exposed workers but again the difference between the two groups is not significant. All the spirometric indices (i.e., FEV 1.0, FEV 1.0/VC, PEFR, MEFR 50% VC, MEFR 75% VC) are lower in the cadmium-exposed group but none of the differences are significant. However maximal expiratory flow rates at 50 and 75% VC were drastically decreased

Table 2. Single breath lung diffusing capacity, static lung compliance, and static recoil of the lung at different lung volumes in Cd exposed and control workers (% of predicted value except for lung compliance).

| | Cd-exposed | Controls |
|---|------------|----------|
| N | 20 | 18 |
| DL _{SB} | 105.2 | 113.6 |
| PeI 100% TLC | 100.5 | 111.6 |
| 90% TLC | 102.3 | 109.1 |
| 80% TLC | 96.8 | 102.6 |
| 70% TLC | 88.4 | 94.4 |
| 60% TLC | 72.7 | 84.4 |
| Static lung compliance at FRC, l./cm H ₂ O | 0.294 | 0.325 |

in both Cd-exposed and nonexposed workers. Furthermore, in both cadmium-exposed and nonexposed workers the specific airway conductance was about half the normal value. These changes are probably due to cigarette smoking.

The results of the lung diffusing capacity and of the elastic properties of the lung are summarized in Table 2. Single breath lung diffusing capacity and elastic recoil of the lung measured at different lung volumes were somewhat lower in cadmium workers but none of the differences between the two groups was significant. Among the 18 workers exposed to cadmium 7 had kidney impairment (proteinuria > 400 mg/l.), but their lung function indices were not significantly different from those measured in the other workers. These results confirm our previous findings. In the absence of manifest acute overexposure to cadmium fume mainly a mild form of obstructive lung disease is found in some workers chronically exposed to cadmium. This functional impairment is rather slight by comparison with that caused by smoking. Furthermore we were unable to find evidence of pulmonary emphysema.

Recently Chowdhury and Louria (12) have reported that among several trace metals, cadmium was the only one that reduced *in vitro* in a dose-related fashion the α_1 -antitrypsin content of human plasma and its trypsin inhibitory capacity (TIC). These authors suggested that their observation may offer an explanation for the emphysema reported in workers exposed to cadmium. However, we could not corroborate their findings; furthermore, there was no difference in the α_1 -antitrypsin content and TIC in the peripheral blood between the control workers and those with chronic cadmium intoxication (13).

It can be concluded that the critical organ following long-term exposure to cadmium is usually not the lung but the kidney. This conclusion cannot, however, be extrapolated to acute or subacute inhalational exposure.

Second Epidemiological Study on Kidney Function

Other studies were carried out to elucidate further the effect of cadmium on the renal handling of proteins and enzymes. Since during the first survey cadmium proteinuria had been characterized only by means of a qualitative method, i.e., agarose gel electrophoresis, we confront first the results obtained by this method with those obtained by quantitative determination of urinary proteins of different molecular size: β_2 -microglobulin (MW 11800), orosomuroid (MW 44000), albumin (MW 69000), and transferrin (MW 76500) (6).

In all, 39 workers were examined: 18 male workers exposed on average for 28 yr to cadmium in a cadmium producing plant and 21 nonexposed workers of approximately the same age (54 and 52 yr, respectively).

Ten of the cadmium exposed workers had an abnormal electrophoretic pattern. Eight of them excreted larger quantities of high and low molecular weight proteins. By comparison with the control group the urinary concentration of albumin, transferrin, orosomuroid, and β_2 -microglobulin was increased 8.5, 7, 17, 67-fold, respectively. The other two workers with an abnormal electrophoretic pattern showed only an increased excretion of high molecular weight proteins. However five workers classified as normal on the basis of the electrophoretic pattern exhibited already an increased excretion of β_2 -microglobulin (10 times the control value) (Table 3). This indicates that agarose gel electrophoresis of urinary proteins (about 100 fold concentrated) cannot pick up a slight increase in β_2 -microglobulin. We have confirmed a previous observation, i.e., in the majority of the cadmium-exposed workers with an abnormal electrophoretic pattern or an increased total proteinuria, not only LMW proteins are excreted in greater amount but also HMW proteins.

Table 3. Validity of electrophoretic classification.

| Electrophoretic pattern | Transferrin-U ^a | | Albumin-U ^a | | β_2 -Microglobulin-U ^a | |
|-------------------------|----------------------------|---|------------------------|---|---|---|
| | + | - | + | - | + | - |
| Normal n = 8 | 0 | 8 | 0 | 8 | 5 | 3 |
| Glomerular n = 2 | 2 | 0 | 2 | 0 | 0 | 2 |
| Mixed n = 8 | 8 | 0 | 6 | 2 | 8 | 0 |

^a Values are classified as positive if they exceed mean + 2 SD found in the group not exposed to cadmium; values falling below this threshold are classified as negative.

Table 4. Prevalence of abnormal levels of various biological parameters in the control and cadmium group.

| Parameters ^a | Control group, n = 77 | | Cadmium group, n = 42 | | χ^2 | P |
|---|--------------------------|---------|--------------------------|-----------|----------|----------|
| | - | + (%) | - | + (%) | | |
| Plasma creatinine (a) | 74 | 3 (3.9) | 32 | 10 (23.8) | 11.07 | < 0.001 |
| Plasma β_2 -microglobulin (a) | 74 | 3 (3.9) | 34 | 8 (19.0) | 7.44 | < 0.01 |
| Plasma β -galactosidase (a) | 76 | 1 (1.3) | 38 | 4 (9.5) | 4.57 | < 0.05 |
| Creatinine clearance (a) | 76 | 1 (1.3) | 37 | 5 (11.9) | 6.39 | < 0.025 |
| Aminoaciduria rate (a) | 75 | 2 (2.6) | 32 | 10 (23.8) | 13.5 | < 0.0005 |
| Proteinuria rate (b) | 73 | 4 (5.2) | 35 | 7 (16.7) | 4.26 | < 0.05 |
| β -Galactosidasuria rate (b) | 77 | 0 (0) | 36 | 6 (14.3) | 11.58 | < 0.001 |
| $C_{\beta_2\text{-micro}} \times 10^5/C_{cr}$ (b) | 77 | 0 (0) | 33 | 9 (21.4) | 17.85 | < 0.0005 |
| $C_{Oroso} \times 10^5/C_{cr}$ (b) | 74 | 3 (3.9) | 32 | 10 (23.8) | 11.07 | < 0.001 |
| $C_{Alb} \times 10^5/C_{cr}$ (b) | 73 | 4 (5.2) | 34 | 8 (19.0) | 5.75 | < 0.025 |
| $C_{Trans} \times 10^5/C_{cr}$ (b) | 76 | 1 (1.3) | 35 | 7 (16.7) | 10.24 | < 0.005 |
| $C_{IgG} \times 10^5/C_{cr}$ (b) | 76 | 1 (1.3) | 30 | 12 (28.6) | 20.8 | < 0.001 |

^a $C_{\beta_2\text{-micro}} \times 10^5/C_{cr}$, $C_{Oroso} \times 10^5/C_{cr}$, $C_{Alb} \times 10^5/C_{cr}$, $C_{Trans} \times 10^5/C_{cr}$ and $C_{IgG} \times 10^5/C_{cr}$ are the relative clearances of β_2 -microglobulin, orosomuroid, albumin, transferrin, and IgG respectively. Classified as positive or negative values if higher or lower, respectively, than the arithmetic (a) or geometric (b) mean \pm 2 arithmetic (a) or geometric (b) standard deviations as calculated in the control group.

Third Epidemiological Study on Kidney Function

Since the majority of the workers were exposed for more than 20 yr, it was not possible to decide whether one specific protein was excreted before the other. Thus another more extensive investigation was carried out among 148 workers exposed mainly to cadmium oxide dust and fumes and 108 exposed simultaneously to lead and cadmium. This study is still under way; only the data collected among 42 Cd workers have been statistically analyzed (14). Their age ranges from 41.8 to 64.3 yr. They were exposed to cadmium for 2.3 to 47.1 yr. A control group of 77 workers was also examined. From each worker, 25 ml of venous blood was taken, and urine was collected over a known period of time. Therefore it was possible to estimate not only the urinary concentration of specific proteins but also their renal clearances. Five proteins were studied: β_2 -microglobulin, orosomuroid, albumin, transferrin, and IgG. The urinary excretion rate of total proteins and of five enzymes was also measured: β -galactosidase, lactate dehydrogenase, alkaline phosphatase, total and tartrate resistant acid phosphatase, and catalase. By comparison with the control group, the proteinuria rate, the rate of excretion of β -galactosidase, and the relative clearance of the low molecular weight protein (i.e., β_2 -microglobulin) as well as that of the high molecular weight proteins (i.e.) orosomuroid, albumin, transferrin, and IgG) were significantly increased in the group exposed to cadmium (Table 4). Thus, these results on protein clearances confirmed our previous findings (1, 6, 9). We found also that in the cadmium group, an increase of the relative clear-

ance of β_2 -microglobulin is not more frequent than the increase of the relative clearances of the HMW proteins. Furthermore, an increase in the relative clearance of β_2 -microglobulin is not necessarily associated with an increase in those of the HMW proteins (Table 5). Among the workers exposed to cadmium, three had an increase of the relative clearance of albumin without concomitant change in that of β_2 -microglobulin, whereas four workers had an increased relative clearance of β_2 -microglobulin without change in that of albumin. The same was observed for the relative clearance of IgG. The latter was increased in five workers without alteration in β_2 -microglobulin excretion, whereas two workers had an increased excretion of β_2 -microglobulin without change in IgG excretion. The marginal chi-square tests performed on these values were not

Table 5. Marginal χ^2 tests between the relative clearance of β_2 -microglobulin and that of orosomuroid, albumin, transferrin, and IgG in the group exposed to cadmium.

| Parameter ^a | $C_{\beta_2\text{-micro}} \times 10^5/C_{cr}$ | | Significance ^b |
|--------------------------------|---|---|---------------------------|
| | - | + | |
| $C_{Oroso} \times 10^5/C_{cr}$ | 31 | 1 | $\chi^2 = 0.33$, NS |
| $C_{Alb} \times 10^5/C_{cr}$ | 30 | 4 | $\chi^2 = 0.14$, NS |
| $C_{Trans} \times 10^5/C_{cr}$ | 32 | 3 | $\chi^2 = 1.0$, NS |
| $C_{IgG} \times 10^5/C_{cr}$ | 28 | 2 | $\chi^2 = 1.3$, NS |
| | 5 | 7 | |

^a $C_{\beta_2\text{-micro}} \times 10^5/C_{cr}$; $C_{Oroso} \times 10^5/C_{cr}$; $C_{Alb} \times 10^5/C_{cr}$; $C_{Trans} \times 10^5/C_{cr}$, $C_{IgG} \times 10^5/C_{cr}$ are the relative clearances of β_2 -microglobulin, orosomuroid, albumin, transferrin, and IgG, respectively. They are classified as positive or negative for values higher or lower respectively, than the geometric mean \pm 2 geometric SD as calculated in the control group.

^b NS = statistically not significant ($p > 0.05$).

significant which confirms that in cadmium nephrotoxicity the β_2 -microglobulin relative clearance is not more frequently increased than HMW protein clearances. Immunological methods were used for determining high molecular weight proteins concentration. Thus in order to ascertain that fragments of the HMW proteins (orosomuroid, albumin, transferrin, and IgG) were not simultaneously measured by the immunonephelometric technique—which could lead to an erroneous interpretation of the results—a concentrated urine from a worker with proteinuria was chromatographed on Sephadex G-75 and the HMW proteins were measured in the collected fractions. IgG was eluted at the void volume and was followed by transferrin, albumin, and orosomuroid. None of these proteins was detected in the LMW fractions (Fig. 3).

We found again that increased proteinuria rate and increased relative clearances of LMW and HMW proteins are mainly observed in workers exposed to cadmium for more than 25 yr. These data suggest that the increased relative clearance of β_2 -microglobulin does not necessarily occur earlier than increased relative clearances of HMW proteins. When the excretion of LMW and HMW proteins is found to be enhanced, the cadmium level in urine usually exceed 10 $\mu\text{g/g}$ creatinine (Fig. 4) and that in blood 1 $\mu\text{g}/100$ ml. However, the threshold level of cadmium in blood must be considered as tentative in view of the great difficulty of the precise determination of low concentration of cadmium in this biological material (15). Highly significant correlations were observed between the proteinuria rate and the relative clearances of β_2 -microglobulin ($r = 0.75$), orosomuroid ($r = 0.75$), albumin ($r = 0.89$), transferrin ($r = 0.73$, and IgG ($r = 0.90$). The proteinuria rate and the relative clearances of all proteins were significantly correlated with Cd-U

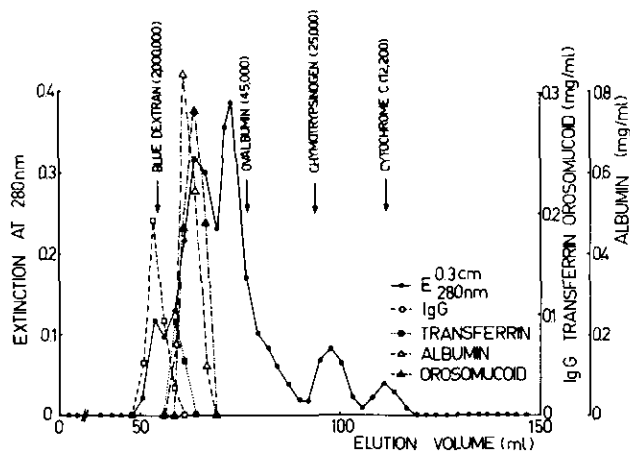


FIGURE 3. Fractionation on Sephadex G 75 of a concentrated urine (about 100 fold) from a Cd-exposed worker.

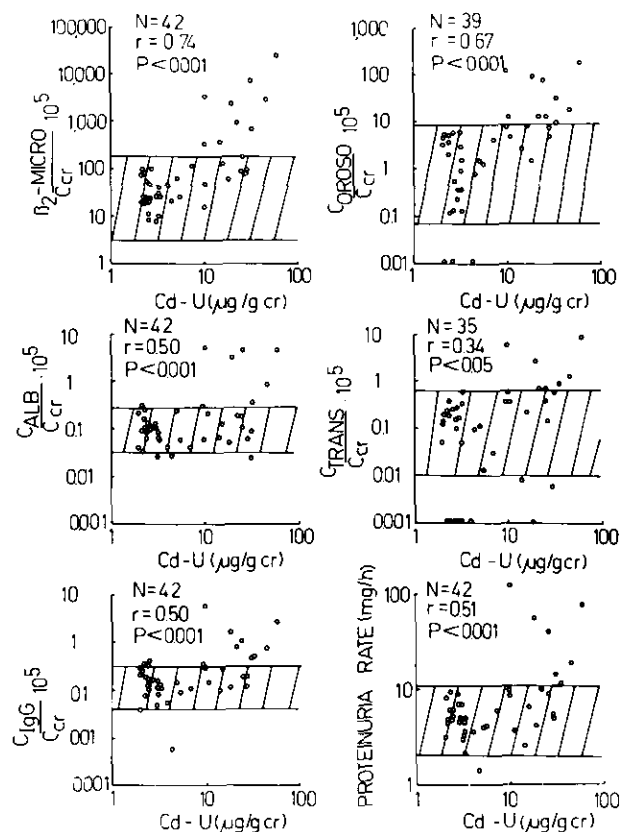


FIGURE 4. Relationship between the concentration of cadmium in urine (Cd-U) and the proteinuria rate, the relative clearances of β_2 -microglobulin, orosomuroid, albumin, transferrin, and IgG. The interval between the two bars represents the range (geometric mean \pm 2 geometric SD) of the values found in the control group.

and Cd-B.

This study confirms that the proteinuria developed by workers with prolonged exposure to cadmium has two components: a tubular type proteinuria characterized by an enhanced excretion of β_2 -microglobulin and a glomerular type proteinuria with an increased excretion of HMW proteins such as albumin, transferrin, and IgG. Although both components can appear independently, they are often associated giving rise to a mixed type proteinuria. These observations on workers exposed to cadmium are confirmed by the results of recent animal experiments. Female rats injected intraperitoneally with CdCl_2 (1 mg Cd/kg five times a week) developed after 2 months a mixed type proteinuria with an increased excretion of HMW and LMW proteins (16). Concomitantly with proteinuria the chronic exposure to cadmium can cause a moderate increase in the urinary excretion of β -galactosidase. β -Galactosidase is a lysosomal enzyme; its increased release suggests that cadmium interferes with the lysosomal system in tubular cells (Table 4). The increased excretion of β_2 -

microglobulin which freely filters through the glomerulus is considered as indicative of a defect in the reabsorption by the proximal tubule at least when there is no marked reduction of the glomerular filtration rate. Among the biological parameters measured in this study, the relative clearance of β_2 -microglobulin was the most affected by the exposure to cadmium. The mean relative clearance of β_2 -microglobulin in the control group was 38.3×10^{-5} , whereas in the cadmium group it rose to a value of 1190×10^{-5} , which corresponds to a 30-fold increase. The mean relative clearances of all the HMW proteins (IgG, transferrin, albumin, and orosomucoid) were in the cadmium group increased about 5-fold as compared with the control group. However, although the excretion of β_2 -microglobulin is proportionally more increased than the excretion of the HMW proteins, on a quantitative basis, the HMW proteins remain the most important components of the proteinuria induced by cadmium. On an average, the cadmium group excreted per hour 0.208 mg of β_2 -microglobulin and 1.35 mg of albumin. The glomerular type of proteinuria can have different origins. It can result from an increased permeability of the glomerulus, an incomplete reabsorption by the proximal tubule or a release in urine of proteins synthesized in the kidneys (e.g. IgG).

That cadmium can affect the glomerular function is supported by the finding of increased levels of β_2 -microglobulin and creatinine in plasma of some workers exposed to cadmium concomitantly with a decreased creatinine clearance. In 1950, Friberg (17), later in 1961 Ahlmark et al. (18), and in 1969 Adams et al. (19) also reported a decrease of the glomerular filtration rate in workers exposed to cadmium. In Japan, a decrease in creatinine clearance accompanied by an elevated plasma level of creatinine was also observed in people suffering from proximal tubular dysfunction induced by environmental cadmium pollution (20). Little attention has been paid to these observations and the glomerular dysfunction has generally been regarded as a late perturbation appearing only in severe cases of cadmium intoxication. Our investigations demonstrate that in the cadmium group there were as many workers with a significantly increased plasma level of β_2 -microglobulin ($n = 8$) and significantly increased relative clearance of albumin ($n = 8$) as subjects with significant increased relative clearance of β_2 -microglobulin ($n = 9$). The number of subjects with significantly decreased endogenous creatinine clearance was lower ($n = 5$), but it is well known that the endogenous creatinine clearance can overestimate the glomerular filtration rate in renal injury. Correlations between the clearance of

creatinine and plasma β_2 -microglobulin or plasma creatinine indicate that the increased levels of these two plasma components result from a reduction of the glomerular filtration rate.

In summary, the results of the various studies carried out by our laboratory on the renal function of workers exposed to cadmium suggest that the nephrotoxicity of cadmium consists in (1) a tubular dysfunction characterized by an increased excretion of β_2 -microglobulin and giving rise to the classical tubular type proteinuria, (2) a glomerular dysfunction evidenced by an increased excretion of HMW proteins and increased levels in plasma of β_2 -microglobulin and creatinine and giving rise to a glomerular type proteinuria, and (3) cellular lesions at the epithelium of the urinary tract as evidenced by an increased release in the urine of β -galactosidase. These effects appeared mainly in workers exposed to cadmium for more than 25 yr and whose cadmium concentration at the time of the survey exceeded $1 \mu\text{g Cd}/100 \text{ ml}$ in blood and $10 \mu\text{g Cd/g}$ creatinine in urine. However it should be pointed out that the 42 cadmium workers for whom biological data have been summarized in this paper were mainly employed in one Cd-producing plant (plant A) (1). This may explain the long latency period before the occurrence of kidney lesion. Recently we examined 11 workers from a small factory producing cadmium salts. The airborne cadmium concentration at the various workplaces exceeded $200 \mu\text{g}/\text{m}^3$, and the median airborne cadmium concentration measured with personal air samplers was around $500 \mu\text{g}/\text{m}^3$. The critical biological threshold of 10-15 $\mu\text{g Cd/g}$ urinary creatinine was reached rapidly (Fig. 2) and the three workers (B5, B6, B7 on Fig. 2) employed for more than 3 yr in this factory (3 to 5 yr) already had signs of kidney damage (one pure glomerular proteinuria, one pure tubular proteinuria and one mixed proteinuria).

Other Human Studies on Cadmium

Our laboratory has also carried out studies on the exposure of the general population to cadmium. A survey carried out among 500 pregnant women in Belgium showed that cadmium is less easily transferred from the mother to the fetus than lead and organic mercury. At term, cadmium concentration in the placenta reaches a value on the average 10 fold higher than that found in maternal blood. Smoking has a statistically significant influence on cadmium concentration in maternal blood and in placenta (3, 4, 21). The degree of exposure to cadmium of children (10 yr) attending schools situated

at less than 1 km and 2.5 km from a lead smelter was compared with that of children living in a rural area (5). The average cadmium concentration in blood and urine of the children living near the factory is significantly higher than that of the children at 2.5 km or of the rural children. The group at 2.5 km exhibited a similar cadmium level in blood as the rural group but excreted three times more cadmium in urine (mean Cd-U in the rural area and in the groups living at 2.5 km or at less than 1 km from the factory was 0.23, 0.76, and 1.06 $\mu\text{g/g}$ creatinine, respectively). The exposure had no effect on urinary β_2 -microglobulin excretion. A recent investigation suggests that ingestion of dirt represents an important source of cadmium exposure for the children living close to the plant.

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REFERENCES

1. Lauwerys, R. R., et al. Epidemiological survey of workers exposed to cadmium. Effect on lung, kidney and several biological indices. *Arch. Environ. Health* 28: 145 (1974).
2. Materne, D., et al. Investigations sur les risques résultant de l'exposition au cadmium dans deux entreprises de production et deux entreprises d'utilisation du cadmium. *Cah. Méd. Travail* 12: 1 (1975).
3. Lauwerys, R., et al. Placental transfer of lead, mercury, cadmium and carbon monoxide in women. I. Comparison of the frequency distributions of the biological indices in maternal and umbilical cord blood. *Environ. Res.* 15: 278 (1978).
4. Buchet, J. P., et al. Placental transfer of lead, mercury, cadmium and carbon monoxide in women. II. Influence of some epidemiological factors on the frequency distributions of the biological indices in maternal and umbilical cord blood. *Environ. Res.* 15: 494 (1978).
5. Roels, H., et al. Lead and cadmium absorption near a non-ferrous metal plant: a follow-up study of a test case. *Environ. Res.* 15: 290 (1978).
6. Bernard, A., et al. Characterization of the proteinuria in cadmium exposed workers. *Int. Arch. Occup. Environ. Health* 38: 19 (1976).
7. Kretzchmar, J. G., et al. The Belgian network for the determination of heavy metals. *Atm. Environ.* 11: 263 (1977).
8. Lauwerys, R., Buchet, J. P., and Roels H. The relationship between cadmium exposure or body burden and the concentration of cadmium in blood and urine in man. *Int. Arch. Occup. Health* 36: 275 (1976).
9. Roels, H., et al. Study on cadmium proteinuria. Glomerular dysfunction: an early sign of renal impairment. In: *Proceedings, International Symposium-Recent Advances in the Assessment of the Health Effects of Environmental Pollution*, CEC, EPA, WHO, Luxembourg, 1975, p. 631.
10. Kjellström, T. Accumulation and renal effects of cadmium in man. A dose-response study. Thesis, Department of Environmental Hygiene, Karolinska Institute, Sweden, 1977.
11. Stanescu, D., et al. Effect on lung of chronic occupational exposure to cadmium. *Scand. J. Resp. Dis.* 58: 289 (1977).
12. Chowdhury, P., and Louria, D. P. Influence of cadmium and other trace metals on human α_1 -antitrypsin: an *in vitro* study. *Science* 191: 480 (1976).
13. Bernard, A., et al. α_1 -Antitrypsin level in workers exposed to cadmium. In: *Clinical Chemistry and Chemical Toxicology of Metals*, S. S. Brown, Ed., Elsevier-North Holland, Biomedical Press, Amsterdam, 1977, p. 161.
14. Bernard, A., et al. Renal excretion of proteins and enzymes in workers exposed to cadmium. *Eur. J. Clin. Invest.*, in press.
15. Lauwerys, R., et al. Intercomparison program of lead, mercury and cadmium analysis in blood, urine and aqueous solution. *Clin. Chem.* 21: 551 (1975).
16. Bernard, A., et al. Experimental confirmation in rats of the mixed type proteinuria observed in workers exposed to cadmium. Paper presented at XIX International Congress on Occupational Health, 25-30 September 1978, Dubrovnik, Yugoslavia.
17. Friberg, L. Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning. *Acta Med. Scand.* 240: 1 (1950).
18. Ahlmark, A., et al. Further investigations into kidney function and proteinuria in chronic cadmium poisoning. In: *Arch. 13th Int. Cong. Occup. Health*, New York, 1961, p. 201.
19. Adams, R. G., Harrison, J. F., and Scott, P. The development of cadmium induced proteinuria, impaired renal function and osteomalacia in alkaline battery workers. *Quart. J. Med.* 38: 425 (1969).
20. Saito, H., et al. Cadmium induced proximal tubular dysfunction in a cadmium polluted area. *Nephrol.* 6: 1 (1977).
21. Roels, H., et al. Placental transfer of lead, mercury, cadmium and carbon monoxide in women. III. Factors influencing the accumulation of heavy metals in placenta and in maternal and cord blood. *Environ. Res.* 16: 236 (1978).