Urinary β_2 microglobulin in the biological monitoring of cadmium workers

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ABSTRACT Urinary β_2 microglobulin (β_2m) estimation has been added to an existing pre-employment and periodic medical surveillance programme for cadmium workers. Pre-employment values were measured in 203 men not occupationally exposed to cadmium. The overall geometric mean was 76 $\mu\text{g/l}$ (adjusted to specific gravity 1016): a significantly higher level of 96 $\mu\text{g/l}$ was found in the specimens stored continually at -20°C after voiding, compared with 73 $\mu\text{g/l}$ in specimens that thawed during transport. Sodium azide had been added to all specimen bottles. Employees exposed to cadmium pigments at various stages of their manufacture had no evidence of raised urinary β_2m despite exposures above 50 $\mu\text{g/m}^3$ for up to 11 years. This is believed to be due to the insolubility of these compounds. Five known cases of cadmium induced proteinuria whose exposure ceased up to 15 years ago had raised urinary β_2m concentrations. Moderately raised concentrations were found in seven others with a history of cadmium oxide exposure and in whom proteinuria has never been detected. The place of urinary β_2m in the health care of cadmium workers is discussed and the question of correct management of a cadmium worker with a high β_2m is raised.

β_2 microglobulin (β_2m) is a plasma protein having an approximate molecular weight (MW) of 11 800 and a molecular radius (MR) of 15 Å.¹ It is one of the low molecular weight proteins filtered through the glomerulus and is normally almost completely reabsorbed in the proximal tubule. The proteinuria of renal tubular disorders is characterised by an increased excretion of low molecular weight plasma proteins including β_2m , whereas glomerular disease is characterised by an increased excretion of albumen, a relatively large molecule (MW 69 000, MR 35 Å).^{1,2} The term "tubular proteinuria" has been coined for low molecular weight proteinuria.² Tubular proteinuria occurs in the various congenital and acquired causes of the Fanconi syndrome and in other conditions such as acute renal failure, Balkan nephropathy, and chronic potassium depletion.^{3,4} Friberg⁵ described proteinuria after long-term cadmium exposure, and this has been shown to have the characteristics of tubular proteinuria.^{6,7}

The mechanism whereby cadmium induces tubular proteinuria is unknown. Raised serum β_2m in the absence of glomerular dysfunction has been reported in people occupationally exposed to cadmium,⁸ and this may itself be toxic to the renal

tubule.⁹ An anti-enzymatic action has been postulated: when more cadmium accumulates in the kidney than can be bound to the metal-binding protein metallothionein, it may exchange with zinc in enzyme systems necessary for protein reabsorption and catabolism.¹⁰ Animal work suggests that the cadmium-metallothionein complex is itself toxic to the renal tubule.¹¹

Raised urinary β_2m has been used in epidemiological studies as a criterion of cadmium effect. Kjellstrom *et al*^{12,13} found a dose-effect relationship between duration of occupational and environmental cadmium exposure and the prevalence of raised urinary β_2m . In these studies "raised" was defined as a urinary β_2m exceeding the upper 95th percentile of a reference group without unusually high cadmium exposure. This upper level is variously reported, from studies in Sweden, Japan, and Peru,¹⁴ to be between 200 and 700 $\mu\text{g/l}$, thus any study must establish the range in a comparable group. We are not aware of a published "normal range" in Britain.

Early in 1979 we decided to include urinary β_2m in our health surveillance programme for cadmium workers, and we report the results to the end of 1979. The objectives were:

- (1) to define an upper limit of urinary β_2m in

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non-cadmium workers as a comparison for the health assessment of cadmium workers;

(2) to investigate the relationship between raised urinary β_2 m and past cadmium exposure;

(3) to investigate the relationship between raised urinary β_2 m in cadmium workers and the results of biological monitors already in use; and

(4) to identify workers with raised urinary β_2 m for future follow-up.

Material and methods

Only men are employed as cadmium workers in this company. The largest group, about 170, works at a pigment factory in Staffordshire, where exposure is to cadmium pigments at various stages of their manufacture, from soluble cadmium sulphate to insoluble pigmentary cadmium sulphide. There has been no general exposure to cadmium oxide at this factory since 1969 when its manufacture and subsequent delivery to reaction vessels were totally enclosed.

A smaller number of employees at two other sites are potentially exposed to cadmium oxide fume and dust during intermittent processing of cadmium-bearing material; such material was also processed at a third small site until 1967.

Cadmium workers have had pre-employment and periodic health surveillance, including physical and biological monitoring, since the mid-1960s. Blood and urine specimens are collected every three months, and during the period reported blood and urine were collected from new employees before starting work. Blood cadmium concentrations were measured by the National Occupational Hygiene Service.

Urine specimens were spot samples collected in 125-ml screw top plastic containers to which 200 μ l of 10% sodium azide solution had been added; buffer was not added. Urine analyses were undertaken in the analytical laboratory at the pigment factory; specimens from employees were frozen to -20°C soon after collection and remained at this temperature until analysed. Specimens from other sites were stored frozen in that factory's medical department until transport, during which they were unfrozen for about four days. On arrival at the laboratory they were frozen to -20°C for storage. The following tests were performed on each specimen:

- measurement of cadmium by atomic absorption spectrophotometry;
- specific gravity (SG) by refractometer;
- total protein using Gallenkamp proteinometer standards;
- reaction with trichloroacetic acid;

albumen, glucose, and pH using "Labstix"; and β_2 microglobulin using the Phadebas® β_2 -micro test.¹⁵ Duplicate analyses are performed on specimens and standards. A batch of 51 specimens and standards taken at random had a within duplicate variance of 2.2%.

Urinary cadmium and β_2 m concentrations are adjusted to SG 1016, the average found in the workforce.

There has been regular atmospheric monitoring since the mid-1960s. Concentrations quoted in this communication refer to personal samples taken with a Casella radio-active head and glass fibre (GF/A) filter, unless otherwise stated. At the pigment factory every man has a personal sample taken monthly on a random basis. This is believed to give a good estimate of average exposure and of excursions above and below this average. At one of the sites with potential oxide exposure, air sampling tends to be used as a monitor of control measures, rather than to estimate a man's total "dose." At the other, operatives regularly wear personal samplers for a whole shift, and environmental surveys are made from time to time with a *BNF* metal fume detector.

Results

URINARY β_2 M IN PEOPLE NOT OCCUPATIONALLY EXPOSED TO CADMIUM
 β_2 m was measured in the urine of 203 men before they started work in two factories between April and November 1979. Their ages ranged from 18-55, mean 30 years. Those who had previously encountered cadmium at work, including former employees of the factories concerned, are excluded. Thirty-five specimens came from the pigment factory and were frozen within an hour or two of collection, remaining at -20°C until analysed. The remaining 168 specimens came from a factory in North London and were frozen, thawed, and frozen again before analysis.

The results ranged from less than 10 to 2080 $\mu\text{g/l}$, arithmetic mean 130 $\mu\text{g/l}$. The distribution of results was rendered more normal by logarithmic transformation, giving a geometric mean of 76 $\mu\text{g/l}$.

When the results for the two factories were considered separately, there was a clear difference between them. The geometric mean of results from the pigment factory was significantly higher ($p < 0.001$) than that from the London factory. The two groups are of similar age range and the difference cannot be accounted for by a higher proportion of acid urines in the group with the lower level (table 1).

Table 1 *Urinary β_2m in non-occupationally exposed men at two factories*

	No of subjects	% pH (≤ 5.5)	Geometric mean ($\mu\text{g/l}$)	Upper 95% tolerance limit ($\mu\text{g/l}$)	Upper 97.5% tolerance limit ($\mu\text{g/l}$)
Pigment factory	35	63%	96	546	765
London factory	168	48%	73	418	585
Both factories	203	51%	76	441	617

URINARY β_2m IN WORKERS AT CADMIUM PIGMENT FACTORY

Figure 1 shows the urinary β_2m results from the 173 employees at the pigment factory; the highest concentration from each individual is recorded. Results of pre-employment β_2m concentrations at this factory are also shown.

Fig 1 *Urinary β_2m in employees at cadmium pigment factory.*

A raised urinary β_2m is defined as one in excess of the upper 97.5 percentile of the pre-employment results (765 $\mu\text{g/l}$). By this criterion, nine had a "raised" level. Four were already known to have clinical proteinuria and had ceased occupational exposure to cadmium at the time of diagnosis, one to 15 years previously, and now have repeated β_2m concentrations greater than 20 000 $\mu\text{g/l}$. The other five did not have such a grossly raised β_2m (table 2). Results of other biological monitors for these nine people are summarised in table 2. The

urinary cadmium record in cases 5, 6, 8, and 9 and the blood cadmium of cases 8 and 9 include results identical to those found in non-exposed subjects.

The air sampling data from the factory since 1964 are summarised in fig 2. Anecdotal evidence leads

Fig 2 *Atmospheric cadmium levels at pigment factory 1964-79: geometric mean $\pm$ one standard deviation.*

us to believe that the concentrations encountered before 1964 were at least as high as those found that year, when over 80% of breathing zone samples showed a cadmium in air exceeding 220 $\mu\text{g/m}^3$. Men employed before 1960 worked in an old factory, since demolished, where exposures are believed to have been still higher. The four with β_2m over 20 000 $\mu\text{g/l}$

Table 2 *Biological monitoring in men with raised urinary β_2m —pigment factory*

Case No	Year of start	Maximum β_2m $\mu\text{g/l}$	Reaction with TCA	Albumen	Total urinary protein (mg%)	Cadmium in blood ($\mu\text{g}/100\text{ ml}$)	Cadmium in urine ($\mu\text{g/l}$)
1	1947	>20 000	Trace	+	35	1.5-3.4	11-38
2	1955	>20 000	Trace	+	30	1.4-1.6	12-53
3	1957	>20 000	+	+	15	3.6-4.1	40-42
4	1958	>20 000	Trace	Trace	5	2.8-4.2	39-55
5	1940	3 925	Neg	Neg	< 5	0.6-1.2	7-10
6	1957	7 000	Neg	Neg	< 5	0.9-1.0	2-29
7	1959	2 737	Neg	Neg	< 5	1.0-1.2	24-33
8	1959	1 531	Neg	Neg	< 5	0.3-0.6	7-9
9	1964	1 203	Neg	Neg	< 5	<0.3-0.4	9-23

TCA = Trichloroacetic acid.

and four of the five other men all started work before 1960. No case of raised β_2m has been found in anyone joining after enclosure of oxide manufacture in the late 1960s (table 3), though mean exposure to cadmium as pigmentary cadmium sulphide and its less insoluble precursors varies between 60 and 140 $\mu\text{g}/\text{m}^3$.

β_2m CONCENTRATIONS IN WORKERS POTENTIALLY EXPOSED TO CADMIUM OXIDE DUST AND FUME

In these workers a raised urinary β_2m is defined as one exceeding 585 $\mu\text{g}/\text{l}$ as specimens thaw during transport.

Table 3 Distribution of raised β_2m according to year of starting employment in pigment factory

Year of start	No still employed	No with raised β_2m
Pre-1960*	14	4 plus 4
1960-68†	13	1
1969-74	41	0
1975-79	105	0
Total	173	9

*Employed originally in old factory (see text).

†Started employment in existing factory before total enclosure of oxide manufacture.

At one site where dust and fume emissions have been controlled below 50 $\mu\text{g}/\text{m}^3$ since the early 1970s, two of the 34 current employees engaged before completion of control measures have a raised β_2m . One worked there as a melter from 1951 to 1966, since when his occupational exposure has been virtually nil. The other was a melter for two years from 1965 to 1967. None of the 24 current employees engaged since 1972 has a raised β_2m concentration. No cases of clinical proteinuria have been found in this work force.

At the other site where cadmium is still encountered, one of the current 11 employees has a raised β_2m concentration. He has worked there since 1954 and had intermittent proteinuria from 1968 to 1976. Present whole-shift exposures are well below 50 $\mu\text{g}/\text{m}^3$. Evidence of past exposure comes from a survey in 1967, when breathing zone cadmium in air levels of 63 and 152 $\mu\text{g}/\text{m}^3$ were recorded over

three-hour sampling periods.

At the melting house where cadmium melting was discontinued in 1967 one of the melters working there at that time is still in employment. He is one of five cases of proteinuria detected there that year. At the same time an environmental survey recorded a personal cadmium in air level in a melter of 330 $\mu\text{g}/\text{m}^3$ over three-and-a-half hours.

The results of biological monitoring in these four men are summarised in table 4.

Discussion

The range of urinary β_2m that we have found in men not occupationally exposed to cadmium is among the highest reported and is more similar to Japan than to Sweden.¹⁴

In the pigment factory no cases of raised urinary β_2m were found in workers potentially exposed to cadmium compounds other than oxide, although mean cadmium in air levels have always exceeded the threshold limit value of 50 $\mu\text{g}/\text{m}^3$. Our findings differ from those of Kjellstrom *et al.*,¹² who found a 19% prevalence rate of increased urinary β_2m among workers exposed to cadmium oxide dust and fume at a concentration of about 50 $\mu\text{g}/\text{m}^3$ for 6-12 years. When we define "raised" as they do—a urinary β_2m above the 95% tolerance limit—one (2.4%) of our 41 men engaged between 1969 and 1974 had a raised β_2m . While the difference in these two reports may in part be due to differing practice with regard to personal protection, we believe it is also due to the differing chemical form in which cadmium was encountered: cadmium oxide on the one hand, pigmentary cadmium sulphide and its precursors on the other.

Cadmium-induced proteinuria can regress after cessation of exposure.¹⁶ Nevertheless, the five old cases of proteinuria in our work force whose exposure has ceased still have raised urinary β_2m , and in three of these exposure ceased at least 12 years ago.

Pertinent to the health care of cadmium workers is the question of the management of the worker with a moderately raised urinary β_2m . More work is necessary to evaluate whether there is a long-term health benefit in removing him from exposure.

Table 4 Biological monitoring in men with raised urinary β_2m ; past or present cadmium oxide exposure

Case No	Year of start	Current exposure	Maximum β_2m ($\mu\text{g}/\text{l}$)	Reaction with TCA	Total urinary protein (mg %)	Cadmium in blood ($\mu\text{g} \%$)	Cadmium in urine ($\mu\text{g}/\text{l}$)
10	1951	Minimal since 1966	994	Neg	<5	NA*	<3.9
11	1965	Minimal since 1967	1460	Neg	<5	<0.3	<3
12	1954	<50 $\mu\text{g}/\text{m}^3$	8764	Neg since 1976	<5	2.6	7-12
13	1962	Nil since 1967	1611	Neg since 1969	<5	NA*	3-20

*Not available.

TCA = Trichloroacetic acid.

It has not been shown that raised urinary $\beta_2\text{m}$ per se has an effect on morbidity and mortality, and against this must be balanced the consequences to the individual of a medical recommendation to change job. Also to be considered is the management of an individual whose pre-employment urinary $\beta_2\text{m}$ is above an arbitrary limit.

The place of $\beta_2\text{m}$ estimation in the health care of cadmium workers has yet to be established. If a moderately raised concentration is an indicator of effect, but not damage, then $\beta_2\text{m}$ may be of use in conjunction with blood and urine cadmium, the indices of absorption and body burden most widely used. If any rise of urinary $\beta_2\text{m}$ indicates permanent damage, then its usefulness in routine health surveillance is limited although it may still have a place in the pre-placement medical examination and in the surveillance of long service employees with old high exposure.

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