LEAD FILE

LEAD POISONING IN AUSTRALIAN CHILDREN MAY HAVE BEEN CADMIUM POISONING

Renal Damage After Prolonged Exposure to Cadmium

An Experimental Study

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THAT POISONING from long exposure to cadmium can give rise to renal damage and proteinuria has been demonstrated in man and laboratory animals.[1-7] Exposure to cadmium results in functional disorders in the renal tubules; whether it is the proximal or distal segment that is the main site of impairment has not been established. In electrophoretic examinations of urinary proteins the electrophoretic pattern was characterized by a fairly low albumin content and high α_2-, β-, and γ-fractions.[4] The proteinuria is a tubular type resembling that in a number of metabolic disorders involving impairment of tubular function. Further evidence of tubular proteinuria was presented by Kazantzis et al.[6]

No consistent connection between the examined renal functions and presence of proteinuria has been found,[5] but tubular changes seem to provide the most plausible explanation of the proteinuria. It is conceivable that there is either defective tubular reabsorption of proteins normally occurring in the glomerulus filtrate, or a leakage or secretion of proteins from damaged tubule cells; a combination of these mechanisms is conceivable.

With the object of examining problems arising from clinical study, the following animal experiments were designed. A serum study was also carried out, a report of which is given elsewhere.[8]

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The problems given consideration were the following:

1. In which part or parts of the nephron is the damage located?
2. Is the proteinuria a symptom of tubular damage?
3. Where in the kidney is the cadmium deposited?
4. Is the amount deposited a measure of the degree of poisoning?
5. Does the cadmium excretion provide a measure of the toxic effect?

Material and Methods

Experimental Conditions.—The experiments were performed on 83 Belgian Giant rabbits (weight 3.3-4.9 kg). These were divided into two series, A and B, and a pilot group of 15 animals, with a control group of ten. Series A, which constituted the chief material for the study of renal function, was divided into four groups, of seven rabbits each and one control group of ten. Series B, on which most urinary and serum studies were performed, was divided into one group exposed to cadmium and a control group, each of which contained ten rabbits. Mean weights of the various groups at the beginning of the experiments were about the same. The grouping is shown in Table 1 with the exposure time for each group.

Cadmium was administered as a subcutaneous injection of 0.5 ml/kg of body weight (corresponding to 0.25 mg of cadmium per kilogram) of an isotonic solution with the following composition: 82 mg of cadmium chloride and 720 mg of sodium chloride in 100 ml of sterile water. Injections were given five days a week for the period specified in Fig 1. To minimize the risk of untoward tissue reactions the site of injection was varied as much as possible.

The study was started simultaneously for the various groups. Group 4 in series A, ten rabbits in series B, and the 15 in the pilot group were given cadmium chloride and the others physiological

TABLE 1.—*Number of Rabbits and Exposure Time in Different Groups*

	Number of Rabbits	Exposure Time (Weeks)
Pilot group	15	24
Controls	10	0
A. Group 1	7	11
" 2	7	17
" 3	7	23
" 4	7	29
Controls	10	0
B. Controls	10	0
Exposed group	10	24

saline by subcutaneous injection. Cadmium chloride was administered to groups 3, 2, and 1 of series A in turn, at intervals of five weeks. The pilot group was used to determine the time at which the animals exposed longest (group 4) could be expected to display observable functional and morphological renal damage, apart from the proteinuria.

Once a month two exposed rabbits were taken from the pilot group and one from its control group for examination of renal function. When these tests disclosed functional and morphologic tubular damage and an appreciably increased excretion of protein in the urine (>150 mg of protein daily), the exposure of groups 1-4 was discontinued in turn, at intervals of one week. The renal function tests on exposed groups (1-4) were performed simultaneously with those on two or three rabbits from the control group.

For series B the exposure was discontinued after 24 weeks, when the protein excretion was considerably increased. Serum and urine specimens were taken weekly during the first three weeks of the experiment, and then every two to three weeks. Examinations were continued for 25 weeks after exposure had ceased.

Seven days after the renal function had been examined the rabbits in series A were sacrificed by bleeding, a heart puncture being made to obtain a blood sample. The puncture was performed under pentobarbital (Nembutal) anesthesia.

Renal Function Tests.—The glomerulus filtration rate was examined by means of a creatinine clearance test, performed largely as described by Josephson and Kallas.[9]

Under shallow pentobarbital anesthesia 50 ml of tepid water per kilogram of body weight was given through a stomach tube to obtain good diuresis, and a Foley catheter No. 16 or 18 was introduced into the bladder.

The rabbit was then placed in a wooden box designed so that the rabbit was seated in a natural posture with the head passing through a hole in the lid large enough not to prevent head movements. The bladder catheter left through another hole under the hindquarters of the animal. The rabbit was given 50 ml of freshly prepared 5% creatinine solution by subcutaneous injection. It usually recovered from the anesthetic after about half an hour, and after one hour the effect of the anesthesia had worn off. The animal usually did not display nervousness.

Blood specimens were obtained by puncturing an ear vein. The first specimen was taken about two

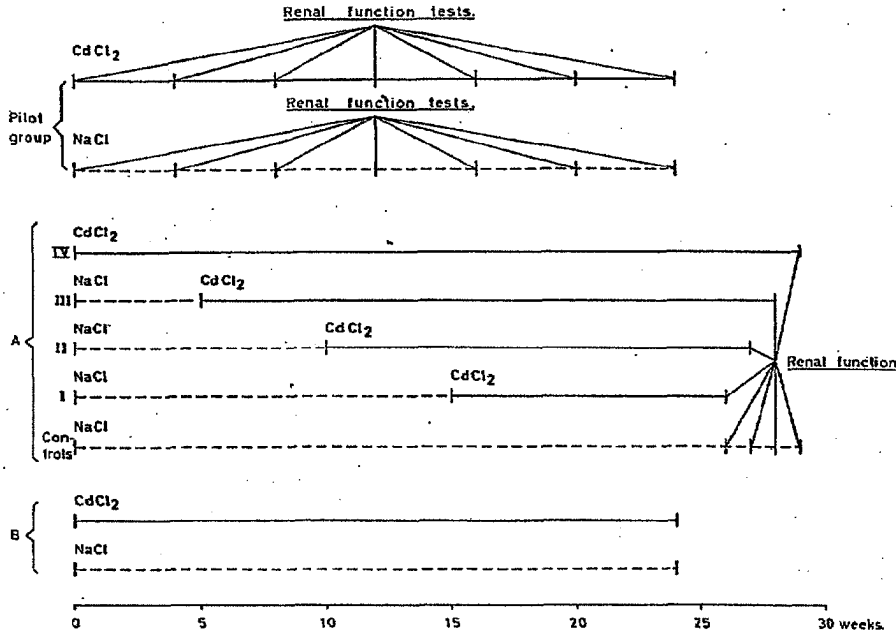

Fig 1.—Plan of the exposure experiment.

hours after the creatinine injection. The urine flowed down through the catheter into a dish and was collected over three periods, of 20-40 minutes each. At the end of each period the bladder was flushed twice with 20 ml of water and twice with air.

The creatinine was determined by the method described by Heyrowsky, and used as a routine method at St. Erik's Hospital, Stockholm.

The clearance value reported is the mean for the three periods.

The function of the proximal tubules was examined by the glucose reabsorption test. The examination was performed at the same time as the creatinine clearance, and the three periods in the two tests were coincident.

Thirty percent glucose solution was given by continuous infusion (0.375 ml/min) through a polyethylene catheter inserted in an ear vein. Just before the infusion was begun 20 ml of a 50% solution of glucose was given through the catheter.

Blood and urine specimens were taken at the same time as those for the creatinine clearance determination, and the former specimens were invariably taken by puncturing a vein of the ear not used for the glucose infusion. This infusion technique gave blood sugar values that usually exceeded 300 mg/100 ml.

The blood and urinary sugar analyses were performed by the routine method used at St. Erik's Hospital, Stockholm.[10] The results showed that with the sugar levels used, the upper limit for tubular reabsorption of the glucose could not be determined and the glucose during concurrent glucosuria increased with the amount of glucose filtered. This is consistent with results reported by Decsi for a study on the rabbit.[11] The reason why the rabbit differs in this respect from other species and man is obscure. Since the reabsorption capacity is a function of the amount of glucose filtered the results of the study are reported as the relationship between the reabsorbed and filtered amounts.

Urine Examinations.—The 24-hour volumes of urine were collected in metabolic cages with separation of feces. Urine was centrifuged and filtered before the various tests were performed. Glucose was assayed with diagnostic tests for glucose in urine (Clinistix and Clinitest).* Total protein was determined by the biuret method after precipitation of the urinary proteins with Tsuchiya reagent.[12]

The excretion of α-amino acid-N was determined in series B by a Ninhydrin method.[13] For the control group it was performed only after 41 and 49 weeks.

For preparation of urine colloids a method described earlier[4] was used, involving concentration by ultrafiltration through a dialysis tube,† dialysis, and freeze drying. The urine colloids were then examined by paper electrophoresis at pH 8.6, and in some cases also with starch gel electrophoresis with various buffer systems.[14,15]

* Ames Co., Inc., 819 McNaughton Ave, Elkhart, Ind
† Visking dialysis tubing, 24/32 inch

For fractionation of urine colloids the pooled urine colloids from series B were gel filtered on Sephadex G-200 ‡ with 0.1 M NaCl as the eluent. An account of the theory and range of application of gel filtration has been published by Flodin.[16] A fraction collector § was used and the fractions were read at 250 and 280 mμ in a spectrophotometer.‖ Pooled fractions were dialyzed, freeze dried, and examined by paper and starch gel electrophoresis. Cadmium was estimated with a dithizone method.[17]

Other Analyses.—Alkaline phosphatase activity in the renal cortex was determined. Dry weight, used as a reference standard in reporting enzyme activity, was determined on specimens of renal cortex from areas adjacent to those from which autopsy specimens for enzyme analysis were taken (the upper pole of the right kidney). The enzyme activity was measured by a method reported by Holmgård[18] as the amount of P-nitrophenol formed by P-nitrophenylphosphate through the action of alkaline phosphatase present in the homogenate. P-nitrophenylphosphate is colorless, and in the hydrolysis of the phosphate group P-nitrophenol it is liberated as a yellow salt. Color was read in a spectrophotometer ¶ at 410 mμ. The substrate was 0.42% sodium P-nitrophenylphosphate # and the standard 1 mM P-nitrophenol.** Results are expressed in moles of substrate split per kilogram of dry weight per hour.

The cadmium in the kidney and liver was estimated. Specimens were taken at autopsy of about 100 mg of cortex, 100 ml of medulla, usually from the upper right pole, and about 100 ml of liver tissue. On these specimens activation analyses of cadmium were performed by a method reported by Westermark and Sjöstrand.[19] †† Because of the fairly high cost of the activation method, assays on renal medulla and liver were performed only on some animals.

Cadmium in urine and feces was determined by the polarographic method of Cholak and Hubbard,[20] which, with minor modifications, is routine at this Institute. Mineralization was effected with nitric acid and hydrogen peroxide. The determination was made on total urine. For series B it was performed only after 18 and 49 weeks; on the latter occasion it was also performed on filtrate obtained after ultrafiltration through a dialysis tube.

A dry weight determination of renal cortex tissue was performed for presence and extent of renal edema and to obtain a reference standard for reporting the activity of alkaline phosphatase.

Histological examination of renal and liver tissue was performed on specimens stained with hematoxylin and eosin and by the method of Timm.[21]

‡ Pharmacia, Uppsala, Sweden.
§ Radi-Rac, LKB Produkter, Stockholm, Sweden
‖ Beckman DB
¶ Beckman B
# Sigma 104, Sigma Chemical Co.
** Sigma Chemical Co.
†† The determinations were performed by T. Westermark and B. Sjöstrand, Royal Institute of Technology, Stockholm, Sweden.

the latter to locate deposited metal. To precipitate all metals as sulfides specimens were fixed in alcohol saturated with hydrogen sulfide, after which they were treated with silver nitrate solution. In the vicinity of metal sulfide particles silver is then precipitated in the presence of hydroquinone; the sites at which metals were originally deposited can be identified histologically from the presence of the black particles.

Since marked hypochromic anemia with a hemolytic component developed in the course of the experiment, and iron was conceivably stored in the kidney and liver, specimens were stained for iron. A more detailed description of methods used in the histological examination is given elsewhere.[22]

Examination of renal proteins: From two rabbits in series B that died during the study the kidneys and liver were removed. Cadmium was assayed by the dithizone method. The organs were frozen and later homogenized in 0.1 M phosphate buffer (pH 7). After centrifugation, the soluble fraction was filtered through Sephadex G-25 in distilled water; the protein fraction was then freeze dried. The proteins were examined by paper and starch gel electrophoresis. In addition to protein staining, the Prussian blue reaction was used for ferritin.

Muramidase activity was determined qualitatively by eluting 5 mm of segments from electrophoresis strips with phosphate-chloride buffer at pH 6.2 and changes in the transmission of a suspension of muramidase substance §§ were measured. Egg white muramidase ¶¶ was used as a standard.

As a general check of the condition of the animals a regular record of weight was made. It has been shown that rabbits exposed to cadmium for a long period develop anemia,[1,23,24] so hemoglobin levels were determined and red cell counts performed. Results are reported elsewhere.[8]

§§ Difco Laboratories
¶¶ Sigma Chemical Co., St. Louis

Results

Series A.—In series A two animals died—one after eight weeks and one after 14 weeks of exposure. Both are excluded from the following results.

Creatinin Clearance (Fig 2): Reduction in mean clearance was not significant, but might be responsible for some effect.

Reabsorption of Glucose (Fig 2): The decrease in the ratio of reabsorbed to filtered amounts of glucose from 23 weeks exposure was statistically significant ($F = 8.75+$).

The Activity of Alkaline Phosphatase in the Renal Cortex Homogenate (Fig 2): Reduction in mean alkaline phosphatase activity in the renal cortex homogenate from 17 weeks of exposure was highly significant ($F = 8.52+++$).

Cadmium in the Kidney and Liver (Fig 3): Between 11 and 17 weeks of exposure deposition of cadmium in the renal cortex and liver reached a "saturation value." As to distribution in the kidney, the renal cor-

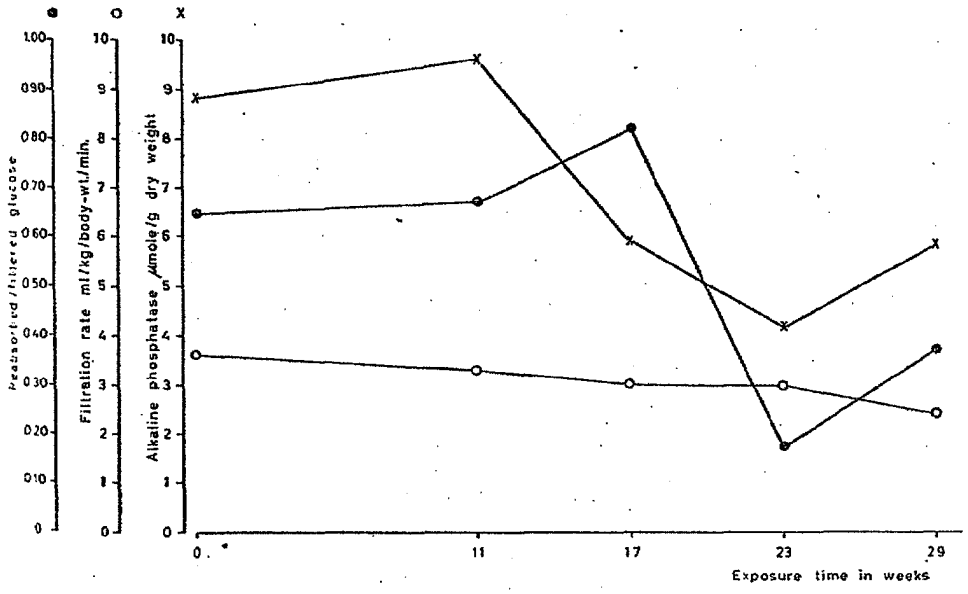

Fig 2.—Filtration rate, ratio of reabsorbed to filtered glucose and activity of alkaline phosphatases in the renal cortex homogenate (series A).

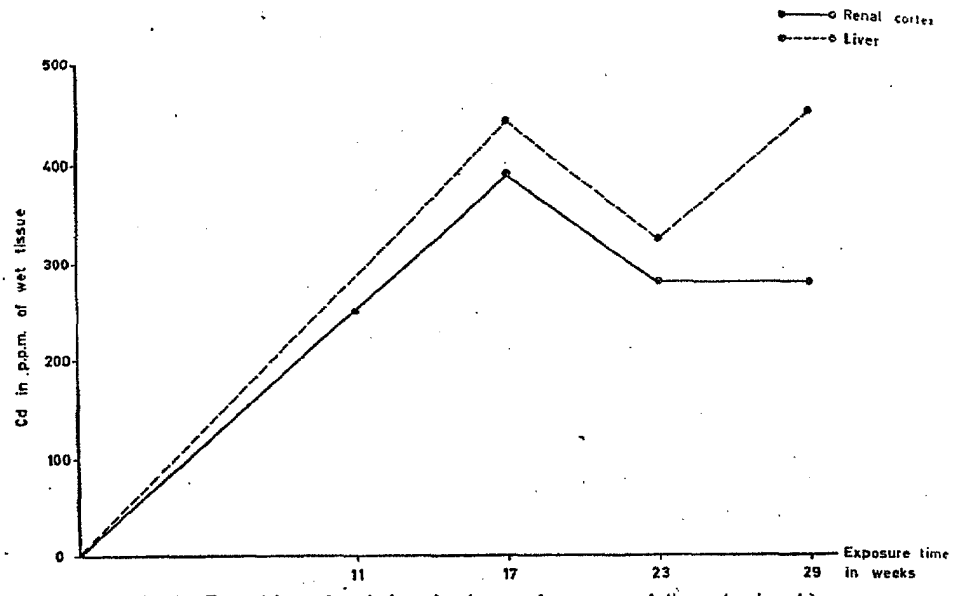

Fig 3.—Deposition of cadmium in the renal cortex and liver (series A).

tex contained two to nine times more cadmium by weight than the medulla (Table 2). There was no definite change in distribution with exposure time.

Cadmium in the Urine (Fig 4): For the animals exposed for 23 and 29 weeks there was a marked increase in urinary cadmium excretion. At 23 weeks the rabbits were given about 1 mg of cadmium daily but they excreted an average of 1.2 mg in the urine.

Excretion of Protein in the Urine (Fig 4): For 23 and 29 weeks, for the animals exposed, there was a marked increase in amounts of protein excreted in the urine.

Excretion of Glucose in the Urine: In the group exposed for 23 weeks, glucosuria was found in two rabbits, and the same signs were found in four of those exposed for 29 weeks. For shorter exposure times no glucosuria was detected.

Dry Weight Determination of Renal Cortex: The increase in the ratio of wet to dry weight of renal cortex with exposure time was highly significant ($F=12.22^{+++}$); this indicates that the administration of cadmium resulted in an increase in the fluid content of the renal cortex. This is consistent with the gross observations at autopsy; the kidneys of rabbits receiving cadmium were strikingly pale and swollen compared with controls.

Histological Picture: At least two kidney sections from each animal were examined. The pathologist was not informed which series of rabbits had been exposed nor for how long the exposed series had received cadmium injections. The results will be reported in detail; in the present connection only a brief survey of results will be presented. There was some overlapping between the various cadmium exposure groups regarding degree of morphological

TABLE 2.—*Amounts of Cadmium in the Rabbit Kidneys After Various Periods of Exposure (series A)*

Duration of Exposure (Weeks)	Renal Cadmium (PPM of Wet Tissue)	
	Cortex	Medulla
Control	<2	<1
Control	<2	<1
11	194	77
11	249	37
11	261	29
17	344	68
17	577	146
23	237	70
23	231	68
29	281	72
29	192	23
29	361	178

alteration; the description applies to the material as a whole.

Control Group. No pathological changes in the kidneys nor pathologic deposits of pigment or metal particles were found.

Exposure for 11 Weeks. Mild, largely degenerative changes were found in the proximal tubules. There were distinct deposits of metal in the cells of the proximal tubules. The glomeruli were well preserved and displayed no abnormal alterations. There was no evidence of inflammatory reaction.

Exposure for 17 Weeks. In principle the picture resembled that of the 11-week group. Deposition of metal in the tubule cells was more pronounced and degenerative changes in the tubules more severe.

Exposure for 23 and 29 Weeks. There were fairly severe pathological alterations. Tubule cells in the proximal segments were largely exfoliated; residual cells contained vacuoles and were granulated and partly disrupted, the nuclei being destroyed or absent. In this group the changes were not restricted to the proximal tubules but were found also in other segments; apart from epithelial fragments and cell detritus in place in their lumina, the collecting tubules were unaffected.

Most animals had no pathological alterations of glomeruli. In some rabbits, chiefly those exposed for 29 weeks, there was slight thickening of the capsule and hyaline fibrotic thickening of the basal membrane.

Special staining methods disclosed abundant deposits of metal particles in the tubule cells, especially in the proximal segments. In the glomeruli, collecting tubules and renal medulla there were no pathological accumulations of metal. In some animals hemosiderin staining showed an increase in iron deposits chiefly in the proximal segments.

Series B.—1. Urine Examinations: There was no difference in 24-hour volumes of urine from the exposed and control rabbits.

Protein excretion had increased significantly after 23 weeks of exposure (Fig 4). The same results for series B are seen in

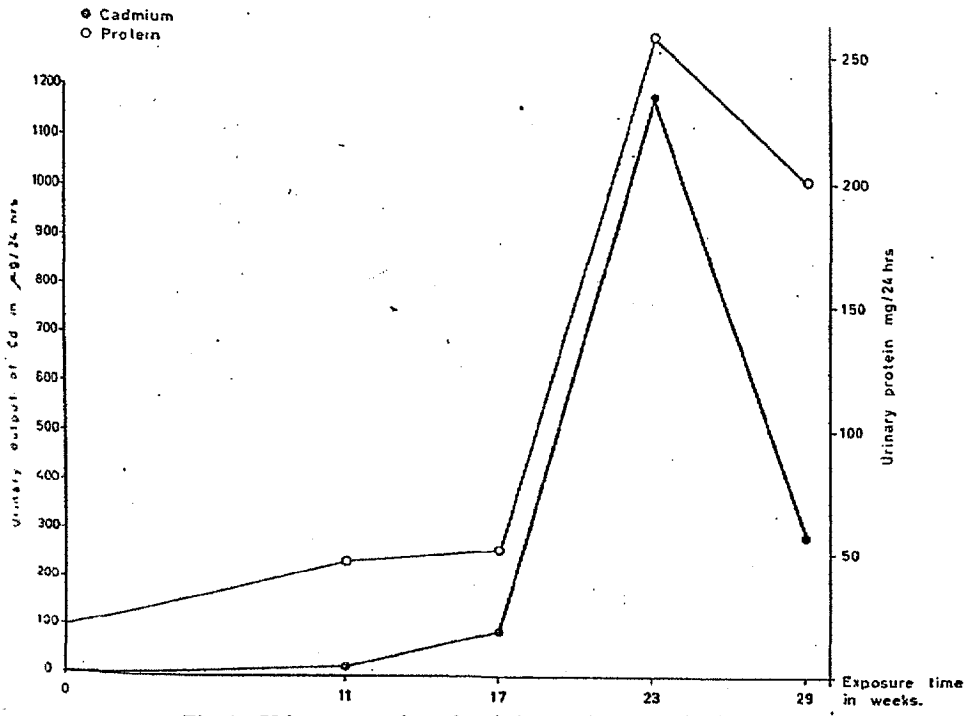

Fig 4.—Urinary excretion of cadmium and protein (series A).

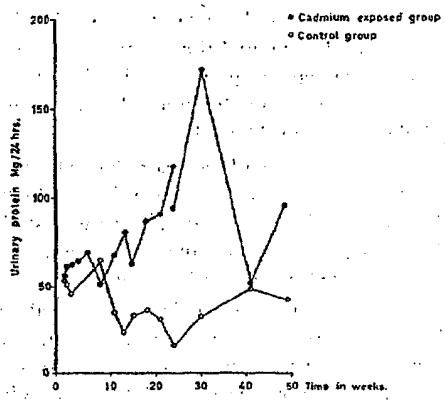

Fig 5.—Urinary excretion of protein (series B). Exposure was discontinued at 24 weeks.

Fig 5, which shows the means for protein excretion at various times. The line representing exposed animals is interrupted at 24 weeks when exposure was discontinued. Compared with controls there was a definitely greater increase in protein excretion after 18 weeks of exposure (Fig 5). There was a significant difference after only 12 weeks but this must be interpreted with reserve in view of the reduction in protein excretion by the controls compared with the initial values observed after ten weeks. The line showing the situation after exposure was discontinued constitutes the mean for five rabbits remaining after three had died

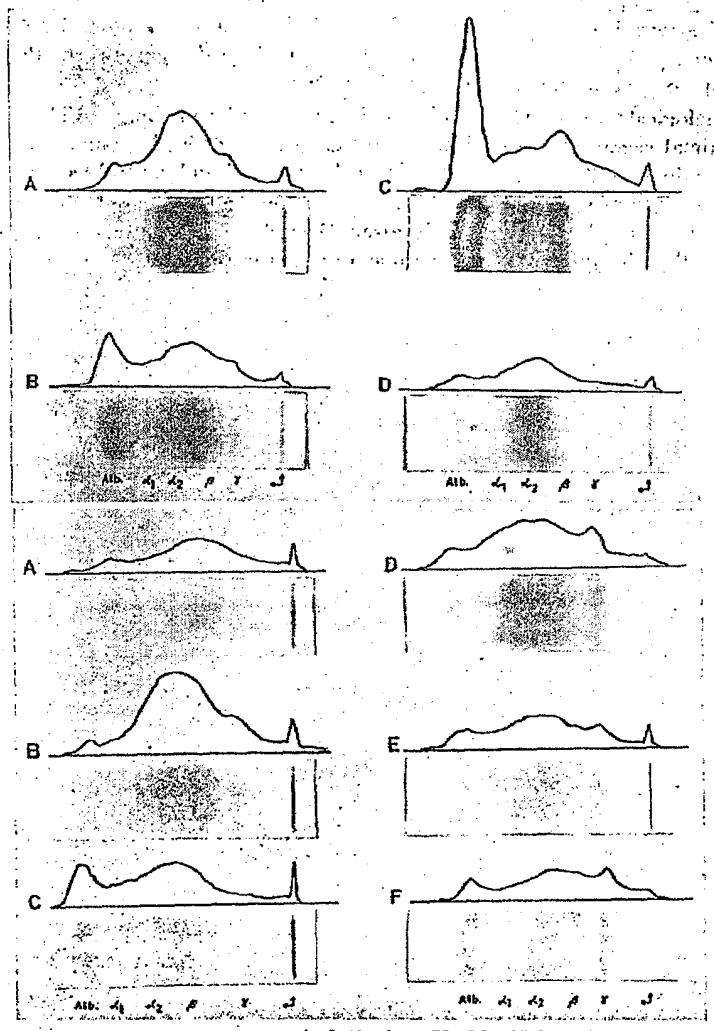

Fig 6.—Electrophoretic pattern for urinary protein from controls (series B). (*A*) Time, 3 days, 64 mg protein per day; (*B*) time, 10 days, 124 mg protein per day; (*C*) time, 8 weeks, 236 mg protein per day; (*D*) time, 11 weeks, 23 mg protein per day.

Fig 7.—Electrophoretic pattern for urinary protein from a rabbit exposed to cadmium (series B). (*A*) Before exposure, 49 mg protein per day; (*B*) exposed 10 days, 39 mg protein per day; (*C*) exposed 5 weeks, 54 mg protein per day; (*D*) 6 weeks after exposure: 327 mg protein per day; (*E*) 17 weeks after exposure, 59 mg protein per day; (*F*) 25 weeks after exposure, 225 mg protein per day.

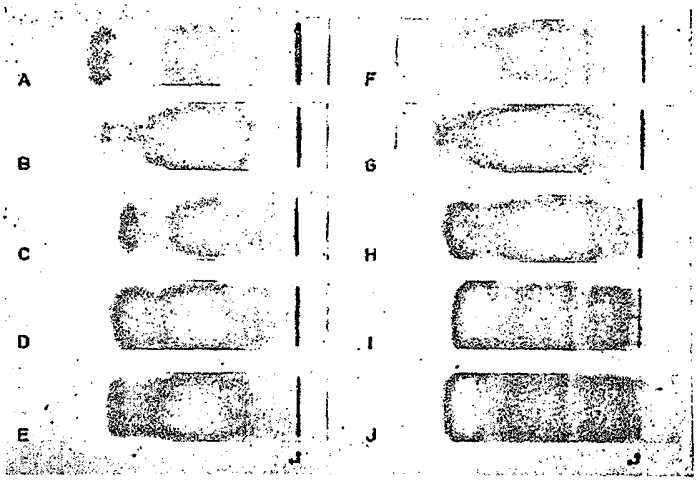

Fig 8.—Electrophoretic pattern for urinary protein from a rabbit exposed to cadmium (series B). (*A*) Before exposure, 114 mg protein per day; (*B*) exposed 10 days, 115 mg protein per day; (*C*) exposed 5 weeks, (*D*) exposed 13 weeks, 150 mg protein per day; (*E*) exposed 18 weeks, 64 mg protein per day; (*F*) exposed 21 weeks, 42 mg protein per day; (*G*) exposed 24 weeks, 77 mg protein per day; (*H*) 6 weeks after exposure, 363 mg protein per day; (*I*) 17 weeks after exposure, 372 mg protein per day; (*J*) 25 weeks after exposure, 850 mg protein per day.

between 24 and 28 weeks [8] and when excretion values had been deducted for the animal that later developed nephrosis with an extremely high protein excretion (more than 500 mg/day). Inclusion of this animal in the account of protein excretion up to 24 weeks does not affect the mean excretion.

Protein excretion increased during the first six weeks after exposure had been discontinued, and ten weeks later had fallen to the level of the controls. The increase after 49 weeks is not significant.

The electrophoretic pattern for urinary proteins excreted by the controls is characterized by a fairly small albumin fraction (Fig 6). The mean for 98 determinations was 22%. In a few controls with low protein excretion (< 20 mg/day) extremely weak staining was obtained, and in some rabbits the albumin was not clearly separated. During the first weeks there was a tendency for an increase in excretion of α-proteins (Fig 6*A*).

Most rabbits in the control and exposed groups sometime during the first months of the experiment displayed a high albumin level and a relatively high daily excretion of protein (100-300 mg/day) (Fig 6 *C*); proteinuria was then assessed as orthostatic. During preparation of the urine colloids a mucoid substance was precipitated which stained positive for protein but did not migrate in electrophoresis. No correction

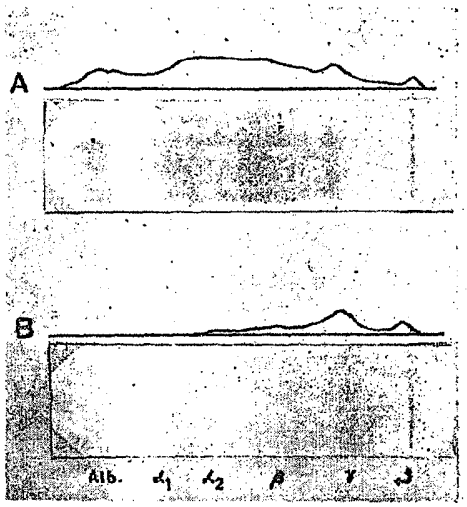

Fig 9.—Electrophoretic pattern for pooled urinary protein after 24 weeks of exposure (series B). (*A*) Original pattern. (*B*) Fraction found after gel filtration through Sephadex G-200.

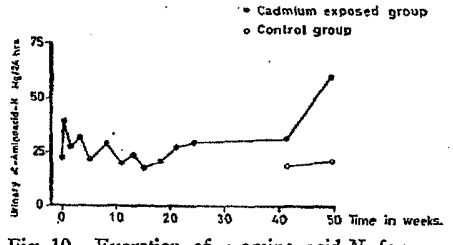

Fig 10.—Excretion of α-amino acid-N for exposed rabbits (series B). Values for controls at 41 and 49 weeks.

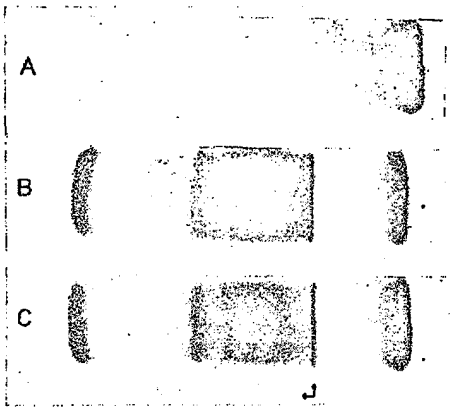

Fig 11.—Electrophoretic pattern for muramidase and kidney protein from one exposed rabbit that died after 32 weeks (series B). (*A*) Muramidase; (*B*) kidney protein; (*C*) kidney protein with added muramidase.

for this mucoid was made in the urinary protein determinations.

The electrophoretic pattern for urinary proteins from exposed rabbits showed an initial increase in the α-fraction (Fig 7 *B*); a protein band then appeared also in the anterior γ-region. This was found also for the controls at the same time (Fig 6 *A, B*; Fig 7 *B*). The pattern was then normalized (Fig 7 *C*); after 15 weeks of exposure the γ-fraction reappeared to remain for the rest of the experiment (Fig 7 *D-F*). The albumin fraction was consistently small and the maximum of proteins was in the α_2- β-region.

Protein excretion varied considerably after exposure was discontinued, being high after 6 weeks, normal after 17, and high again after 25 weeks while the electrophoretic pattern was unchanged throughout.

The other rabbits displayed largely the same changes in electrophoretic pattern, with a single exception when it was not found on any occasion, and there was no increase in protein excretion. Mean albumin content ranged from 10% to 20%. The lowest values were obtained during the first weeks of the experiment.

In one rabbit the electrophoretic pattern was as for the other exposed rabbits up to 41 weeks, but thereafter a picture of nephrosis developed (Fig 8).

While in the majority of exposed rabbits the typical fraction in the anterior γ-region was found toward the end of the exposure period or after discontinuance it was not found at this stage in any control.

With starch gel electrophoresis a post-γ-protein was found in one rabbit six weeks after exposure had ceased, and in two of series A after 29 weeks of exposure.

With gel filtration the distinct fraction in the γ-region was enriched. It was the last protein to appear on the Sephadex G 200 column (Fig 9). Cadmium was present in all protein fractions. The enriched γ-protein contained about 5% of totally protein-bound cadmium.

The mean daily excretion of cadmium in series B was 53μg after 18 weeks of exposure (range 16-117μg) and 160 (20-400μg) after 49 weeks—25 weeks after exposure had been discontinued. On this last occasion less than 20μg was obtained in the ultrafiltrate in all six rabbits.

After an initial transient increase in excretion of α-amino-acid-N the level was fairly constant to the end of the exposure period when there was a further increase. At 41 and 49 weeks, the only times when determinations were made for the control group, excretion by the cadmium group was significantly higher (Fig 10).

Six weeks after exposure had been discontinued three exposed rabbits exhibited glucosuria but this was temporary and 17 weeks after exposure ceased glucosuria was no longer detectable.

Examination of Renal and Liver Proteins: These tests were performed on two rabbits in series B that died after 25 and 28 weeks, after exposure had terminated. In the soluble protein fraction a protein was found that in paper electrophoresis migrated toward the cathode at pH 8.6. A muramidase activity was recorded in this area and the basic protein migrated as muramidase (Fig 11). Paper electrophoresis did not disclose the protein in the kidneys of normal rabbits nor in the liver of exposed ones.

Out of the 10 mg constituting the total cadmium in one liver, 8.7 mg was found in the soluble protein fraction; in two kidneys with totals of 2.3 and 2 mg this fraction contained 1.3 and 1.5 mg, respectively.

With paper and starch gel electrophoresis ferritin was found in both liver and kidney extracts from exposed rabbits but in no control.

Correlations Between the Various Tests (Series A): No relation was found between urinary protein excretion and creatinine clearance ($r=-0.26$ for the whole material).

There was a significant association between glucose reabsorption and protein excretion ($r=-0.65$ for the whole material); this indicates that a low ratio between reabsorbed and filtered amounts of glucose was usually associated with a high excretion of protein.

Here, too, there was a significant correlation between the activity of alkaline phosphatase and the excretion of protein ($r=-0.56$ for the whole material). This indicates that a low enzyme activity was usually associated with a high excretion of protein.

No association was found between the amount of cadmium deposited in the renal cortex and results of renal function tests nor between cadmium content and alkaline phosphatase activity.

The amount of cadmium deposited was not associated with urinary excretion of the metal. Thus, early in the exposure period, there were high levels of cadmium in the renal cortex with little if any excretion of metal.

Protein excretion was positively correlated with cadmium excretion but apparently not linearly, for extremely high cadmium excretion values were not accompanied by a proportionate increase in protein excretion.

Excretion of cadmium in the urine was significantly correlated with glucose reabsorption ($r=-0.50$, $n=18$) and activity of alkaline phosphatase ($r=-0.41$; $n=24$).

There was no significant correlation between cadmium excretion and creatinine clearance.

Comment

From the reduction in glucose reabsorption and activity of alkaline phosphatase it is evident that in the rabbit exposure to cadmium gives rise to functional disorders of the tubules.

Examination of creatinine clearance disclosed no definite effect on glomerular function. In some rabbits exposed for the longest period mild morphologic changes were noted in some glomeruli, but whether these and the evidence of reduction in creatinine clearance with exposure time reflect glomerular impairment is not certain. There was probably no serious functional disorder of the glomeruli. Nevertheless, if the exposure had been continued, significant glomerular damage would probably have been found with the method used.

These results are consistent with earlier observations that the rabbit suffers renal damage in cadmium poisoning.[2] No direct comparison can be made, however, since there is no earlier study in which renal function and proteinuria were examined simultaneously.

If the various function tests were equivalent from all aspects the results would indicate that the functional disorders were located at first, and chiefly, in the proximal tubules. If exposure had been continued it is probable that definite interference with glomerular function would also have developed. Histological examination indicates that this is the probable chronological order of functional disorders.

According to earlier studies on proteinuria in cadmium poisoning in the rabbit the chief component is not albumin but a α_1-globulin[1] and that α- and β-globulins are predominant.[25] It has recently been found that rabbits exposed to cadmium excrete a protein of low molecular weight.[26] In none of the studies mentioned was the normal excretion of urinary protein given special attention. In the present study, normal excretion of protein and the electrophoretic pattern varied considerably. There were orthostatic reactions with "albuminuria," and even in the controls there were transient increases in α-fraction at the beginning of the experiment—apparently initial reactions to the injection to which the animal later became adapted. Minden et al[25] reported in rabbits exposed to cadmium that

after seven weeks the albumin constituted 30% of the total protein and the globulins 70%. In the present study a mean of 22% of albumin was obtained for a large number of determinations on the controls whereas the exposed animals gave a mean of 10%-20%. The lowest values were recorded during the first weeks of exposure, that is during the acute phase of the poisoning. In an examination of urinary proteins from workers exposed to cadmium the mean albumin content was 15% against 24% for the controls.[4]

When, toward the end of the exposure time, the excretion of protein began to increase, the pattern in general became slightly more distinct than in the controls, with the appearance of several peaks in the α- and β-region. The most marked difference was the distinct fraction in the anterior γ-region.

By and large, however, the differences between the electrophoretic patterns of the controls and the cadmium group were qualitative, being more distinct for the exposed animals. It is notable that in the case of workers exposed to cadmium no quantitative difference in the electrophoretic pattern was found between those with low and high protein excretion. There was, however, a qualitative difference, the latter groups having the more distinct peaks, especially in the β-region.[4]

Certain similarities were thus apparent between proteinuria in the cadmium-exposed rabbit and man. In the latter there is a so-called tubular proteinuria, which is ascribed to defective reabsorption of proteins in the glomerular filtrate.[4,6] Many of the proteins excreted are of low molecular weight and occur normally in the serum.[27] To judge from the results of the gel filtration experiment the distinct fraction in the anterior γ-region was probably of low molecular weight, and presumably identical with the one recognized by Kench et al[26] by ultracentrifugation. The typical β-peak in the urine from the exposed worker was found to be largely due to a low molecular weight protein,[17] about 13,500; and it is possible that it corresponds to the typical fraction in the rabbit. The fact that a similar protein has also been found in the human serum suggests that in the rabbit serum a low molecular weight protein may be present in extremely low concentration and owing to defective reabsorption will appear in the urine.

No explanation can be offered of the appearance of a similar component in both controls and exposed animals during the first weeks of the experiment; it is not known whether they are identical.

A further similarity between the tubular proteinuria in exposed man and the proteinuria found in the rabbit is the presence of a post γ-protein which in man has always been associated with impairment of tubular function.[4,6,28]

Of the other metals that cause renal damage uranium[1,29] and mercury salts[30] have been found in animal experiments to produce a picture reminiscent of nephritis, and exposure to cadmium thus gives a different picture. According to results of the present renal function studies and earlier electrophoretic studies of urinary proteins the demonstrated proteinuria appears to be due to tubular dysfunction.

On discontinuing exposure the maximum protein excretion was recorded, and this was followed by a reduction. In a few rabbits the typical electrophoretic pattern persisted, and in one of them features of nephrosis developed, including massive proteinuria and histologic evidence of glomerulus alterations. This may be consistent with experience from cadmium workers, some of whom exhibited no changes in renal function or protein excretion even after quite long exposure while others showed obvious signs of renal damage after only a short time. In the present study there was one rabbit in which no pathological signs were found in the urine at any time.

Further evidence of tubular damage was found in an increase in excretion of α-amino acid-N, which occurred later than the proteinuria and was less pronounced. This is consistent with the fact that the excretion of amino-acids by cadmium workers is usually normal or moderately increased.[27] Glucosuria, too, was found on discontinuing exposure or just afterwards. In cadmium

workers there was no apparent association between excretion of glucose and protein.[27] The most striking finding of the preliminary studies of renal proteins from animals exposed to cadmium was a protein that migrated toward the cathode in paper electrophoresis. Muramidase activity was demonstrated in this fraction and the protein migrated as egg-white muramidase. An increase in muramidase in the kidneys has recently been found in experimentally induced tumors.[31,32] This would be due to an increase in production of muramidase by the reticuloendothelial system and subsequent increase in its tubular reabsorption.[33]

Where Is the Cadmium Deposited in the Kidney? Is the Amount Deposited a Measure of the Degree of Poisoning.—The renal cortex of exposed rabbits contained from two to nine times as much cadmium as the medulla. The deposits were found histologically chiefly in the proximal segment and, to a lesser extent, in the distal, while the collecting tubules and medulla as a rule contained hardly any metal. Using an autoradiographic method on the rabbit, Friberg and Odeblad [34] also found that the deposits were located chiefly in the cortex. On injecting ^{115}Cd in the rat, Gunn and Gold [35] found a selective accumulation in the cortex, the amount being related to the number of nephrons. They considered that cadmium has a physiological role in renal function and proposed that it is in some way associated with reabsorption of water in the tubules. Berlin and Ullberg,[36] found by an autoradiographic technique that injected cadmium was deposited in the proximal tubules. Using a clearance method and stop-flow analyses, Vander [37] found that cadmium increased reabsorption of sodium in the proximal tubules.

The location of pigment just below the basal membrane of the proximal tubules in histological sections fixed in alcohol saturated in hydrogen sulfide is in agreement with Timm and Neth's [38] findings for heavy metals occurring normally in kidney. Part of the pigment certainly indicates the location of other metals than cadmium. A special staining method showed that in some animals iron could occur at the same sites. The amounts were small, however, compared with the deposit of metal and had no evident relationship to them. In view of this, the great difference in amounts of pigment in exposed and control rabbits, and the presence of cadmium in the renal cortex demonstrated by activation analysis, it is reasonable to suppose that the pigment is a fairly reliable indication of cadmium deposition. This is discussed in greater detail elsewhere.[22] The action of deposited cadmium is not fully understood. The evidence suggests that the element exerts an inhibitory effect, as measured on the living organism, organ functions, and enzyme systems due to a reversible formation of mercaptan with SH groups in the protein component of cell enzymes.

The extent to which tubular function was impaired apparently had no relationship to size of cadmium deposits. One possible explanation of this is that once the critical toxic amount of cadmium has been exceeded further increase has little effect.

The significance of the deposition of iron in the kidneys so far as damage is concerned is unclear. The advanced structural alterations in the absence of iron deposits suggest that this metal is probably of little importance compared with cadmium.[8]

Does the Cadmium Excretion Provide a Measure of the Severity of the Toxic Effect.—The deposit of cadmium in the kidneys and liver was practically the same after 11 weeks of exposure as after 29 weeks, although there was no appreciable increase in metal excretion until 23 weeks of exposure. At this time the kidneys excreted more cadmium than was being supplied. Parallel with this the cadmium level in the kidneys and liver was lower in these rabbits than in those exposed for 17 weeks. At 29 weeks the excretion roughly balanced the supply and there was again an increase in cadmium content of the liver while renal deposits remained largely constant. The excretion in the urine appears to play a considerably more important role than in the feces.

Urinary excretion of cadmium correlated significantly with the capacity for glucose reabsorption and alkaline phospha-

tase activity, that is, with the function of the proximal segment of the tubules, cadmium excretion increasing as function deteriorated. There was no correlation between cadmium excretion and creatinine clearance. The results suggest that during exposure fairly large amounts of cadmium are not excreted until renal damage has been caused, and that the amount then increases with the degree of damage.

The fact that cadmium excretion also correlated significantly with protein excretion is consistent with results reported by Friberg.[39] Ultrafiltration experiments performed 25 weeks after exposure had been discontinued, following a 24-week period of exposure, showed that at least 95% of the excreted metal was colloid- or cellbound. Previous dialysis experiments[39] yielded varying percentages for dialysable cadmium. This may be due to differences in pH and the use of different types of dialysis membrane.

It appears probable that excreted cadmium is largely bound to urinary proteins. It is logical to suppose that part of the excretion is due to desquamated tubule cells containing cadmium, evidence for which is found in results of histological examination.

In a fairly advanced stage of poisoning the urinary excretion of cadmium during exposure can provide at least an approximate measure of tubular damage in the rabbit.

This study has not provided a definitive answer to the problem of the mechanism underlying renal damage. In fact, so far as the rabbit is concerned, the problem is further complicated in that account must also be taken of hemolysis and the possible effect of hemosiderosis on the kidney, as well as a direct effect of cadmium on the tubules. The experiments have shown, however, that quite a large amount of cadmium can accumulate in the rabbit kidney without producing more than relatively mild alterations.

Summary

Rabbits have been exposed for 11, 17, 23, and 29 weeks to 0.25 mg of cadmium per kg of body weight, given as subcutaneous injections of cadmium chloride. One group of animals was exposed for 24 weeks and then followed for a further 25 weeks.

To ascertain the effect of exposure on the kidneys and to examine the mechanism underlying the proteinuria, experiments were performed on renal function and urinary and renal proteins. Determinations were also made of cadmium in the kidneys and liver, excretion of cadmium in urine and feces, and the cadmium level in the various urinary protein fractions. Histological examination was performed by the usual methods, hemosiderin staining and, to study the deposition of metal in the kidneys, also by special staining methods.

Results.—1. Damage to the renal tubules was found localized to the proximal segment. In a number of rabbits exposed for long periods small histological alterations were seen in the glomeruli. There was no definite impairment of filtration rate.

2. Proteinuria developed characterized by a low albumin level and fairly large α- and β-fractions, and a distinct fraction in the anterior γ-region. In some rabbits there was also a post γ-protein.

3. The results suggest that proteinuria in chronic cadmium poisoning is a sequel of impairment of the tubules.

4. Cadmium is deposited chiefly in the cortex where, to judge from histochemical examination, it is localized mainly in the proximal segment of the tubules. Metal deposits were found also in the distal tubules but not in collecting tubules, glomeruli, or stroma.

5. The amount of cadmium deposited was not correlated with the severity of functional impairment.

6. The excretion of cadmium in the urine, as the proteinuria, increased greatly after renal damage had been produced and was significantly correlated with the function of the proximal tubules.

7. A basic protein in the renal cortex of the exposed rabbits was found by electrophoresis. It migrated as muramidase; mura-

midase activity was demonstrated in this fraction.

8. Ultrafiltration experiments showed that after exposure had been discontinued the greater part of excreted cadmium was bound to colloids or cells.

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