Hypocalcaemia in Experimental Cadmium Poisoning

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Renal stones, hypercalciuria, and radiological bone changes have been found in workers exposed to compounds containing cadmium. In the present experiments the effect of cadmium chloride injections on the serum calcium levels of rabbits has been studied. Repeated subcutaneous injections over a period of two weeks at a dosage of up to 0.04 mmole/kg. body weight produced a slight but consistent fall in the serum calcium level by about 2 mg./100 ml. Vacuolation of the parathyroids occurred in a few animals.

Single intravenous injections of cadmium chloride, at dosages of 0.01, 0.015, and 0.02 mmole/kg. body weight, produced falls of between 2 and 4 mg./100 ml. in the serum calcium level measured six hours later. In animals which survived the level was still low at 24 hours, after which the serum calcium returned to normal levels. The degree of hypocalcaemia was proportional to the dose of cadmium used. It is suggested that this fall in the serum calcium level may have been due to increased renal excretion.

Industrial workers exposed to cadmium (Cd) have a high incidence of renal calculi; Friberg (1959) estimated that about one quarter of the men engaged in the Swedish accumulator industry had been found to have a history of urinary stones. A more exact estimate of the incidence of nephrolithiasis was given by Ahlmark, Axelsson, Friberg, and Piscator (1960) who showed that the incidence of nephrolithiasis rose with the duration of exposure to cadmium. In men exposed to cadmium for between six and 10 years, 12% had renal stones, but in those exposed for more than 15 years the incidence rose to over 40%.

Quite apart from cadmium poisoning, renal calculi are common, and hypercalciuria has been recognized as one of the causes of calculi since the observations of Flocks (1939). However, the only detailed study of the urinary calcium excretion of men exposed to cadmium is that of Kazantzis, Flynn, Spowage, and Trott (1963), who found levels in excess of 300 mg./day in seven out of 12 subjects; the serum calcium levels were normal. Previous to this there is no published account of disorders of calcium metabolism in cadmium poisoning although radiological changes in bone resembling those of Milkman's syndrome have been found (Nicaud, Lafitte, Gros, and Gautier, 1942). These French workers described the serum calcium levels as being normal but in one case they gave a figure for the urinary calcium of 334 mg./l., a level which suggests

that hypercalciuria may have been present. Cotter and Cotter (1951) also studied the serum calcium levels in five cases of cadmium poisoning but found no evidence of hypocalcaemia.

There appears to be no published account of calcium metabolism in experimental cadmium poisoning although the literature on this subject is large. This paper is an account of some changes in the serum calcium level of the rabbit which occurred when cadmium chloride was given experimentally.

Methods

Two series of experiments were performed using adult rabbits of between 2.0 and 2.5 kg. in weight; the animals were fed on diet SG1 (Oxoid Ltd.). Cadmium chloride was injected as a 0.02 molar sterile solution in water.

Subcutaneous Injections Six rabbits were given 0.02 mmole Cd/kg. body weight on alternate days for one week. During the following week this dose was raised to 0.04 mmole/kg. Another three rabbits served as controls and were given equivalent volumes of 0.9% saline. Venous blood samples were obtained from all the rabbits before the injections; further specimens were obtained two, four, nine, and 16 days later. After the last specimen had been collected the animals were killed and their parathyroids were removed for histological examination. Although the number of experiments was small it was large enough to provide results which were statistically significant.

Intravenous Injections Four groups, each consisting of five rabbits, were used. Group I, the controls,

were each injected with 0.9% saline at a dose of 1 ml./kg. body weight. Group II were given cadmium chloride at a dose of 0.02 mmole/kg., group III 0.015 mmole/kg. and group IV 0.01 mmole/kg. Immediately before the injections, venous blood samples were collected from all the animals and further blood samples were taken six hours later. During this period of six hours the rabbits were deprived of food so that there could be no difference between the calcium intakes of the control group and of the experimental groups. Blood samples were obtained, from those rabbits which survived, 24 hours and five days after the injections.

Technical Methods All blood samples were collected from the ear veins without anaesthesia. The serum calcium was measured by the method of Clark and Collip (1925), serum inorganic phosphorus by the method of Gomori (1942), total serum protein by the method of Reinhold (1953), and urea by the urease-nesslerization method as described by Varley (1962). The significance of the results was determined by Student's 't' test.

Results

The mean serum calcium levels of the rabbits injected subcutaneously are shown in Table I. In

TABLE I
MEAN SERUM CALCIUM LEVELS (MG./100 ML.) OF RABBITS GIVEN SUBCUTANEOUS INJECTIONS OF CADMIUM CHLORIDE

Group	Before Cd Injection	Duration of Experiment (days)			
		2	4	9	16 n = 5
Cadmium- treated n = 6	14.6 (±0.53)	13.1 (±0.083)	12.6 (±0.74)	12.5 (±0.27)	11.0 (±2.1)
Controls n = 3	14.7 (±0.46)	14.5 (±0.28)	14.1 (±0.025)	14.5 (±0.44)	14.2 (±0.81)
Significance of the difference		P < 0.001	P < 0.05	P < 0.01	N.S.

The standard deviations are given in brackets.
N.S. = not significant.

contrast to the control group, which showed little change in the serum calcium levels, the treated group showed a progressive hypocalcaemia. The mean serum fell from the initial level by 1.5 mg./100 ml. calcium at two days ($P < 0.02$), by 2.0 mg./100 ml. at four days ($P < 0.001$), by 2.1 mg./100 ml. at nine days ($P < 0.001$), and by 3.6 mg./100 ml. at 16 days, by which time one of the rabbits had died. As shown in Table I, the difference in the mean serum calcium levels of the experimental group and of the controls was statistically significant.

TABLE II
MEAN SERUM INORGANIC PHOSPHORUS LEVELS (MG./100 ML.) OF RABBITS GIVEN SUBCUTANEOUS INJECTIONS OF CADMIUM CHLORIDE

Group	Before Cd Injection	Duration of Experiment (days)			
		2	4	9	16
Cadmium- treated n = 6	5.6 (±0.98)	4.8 (±0.74)	5.1 (±0.77)	4.4 (±0.48)	n = 5 8.0 (±4.1)
Controls n = 3	6.2 (±0.21)	6.1 (±1.28)	5.8 (±0.78)	5.2 (±0.28)	6.7 (±0.47)

Significance of the
difference

N.S. N.S. P < 0.01 N.S.

The standard deviations are given in brackets.
N.S. = not significant.

The serum inorganic phosphate levels given in Table II show a slight fall in both the treated group and the controls but the difference between them was not statistically significant except at nine days when the level of the treated group was 0.8 mg./100 ml. lower than that of the controls. However, by 16 days one of the treated group was uraemic with a blood urea level of 270 mg./100 ml., and in this case the serum inorganic phosphate level was grossly raised at 11.8 mg./100 ml.; with this one exception the phosphate levels tended to fall. This was the only rabbit to show any rise in the blood urea level but its influence was sufficient to raise the mean phosphate level of the group to 8.0 mg./100 ml. at 16 days.

Histologically, the most interesting findings were in the parathyroid glands. Four of the six rabbits given cadmium injections showed the presence of clear spherical vacuoles in the parenchyma (Figs 1 and 2); these vacuoles occur only to a very minor extent in normal rabbit parathyroids. The vacuoles do not stain either with fat stains or by the P.A.S. method.

The only significant finding in the remaining organs was a variable degree of patchy necrosis in the proximal convoluted tubules of the kidney. No lesions were found in the three rabbits of the control group.

Intravenous Injections None of the control rabbits died nor did any of those in group IV. In group II, three rabbits died within 24 hours and a fourth died on the second day. In group III only two animals died within 24 hours, one survived for 48 hours, and the remaining two survived until the end of the experiment.

The serum calcium levels are shown in Table III. In group I, the controls, the serum calcium level fell

FIG. 1. Rabbit parathyroid gland in experimental cadmium poisoning by daily subcutaneous injection. The vacuoles do not stain by the P.A.S. method nor with Sudan dyes. H. and E. $\times 350$.

TABLE III

SERUM CALCIUM LEVELS (MG./100 ML.) FOLLOWING THE INTRAVENOUS INJECTION OF CADMIUM CHLORIDE

Group	Before Cd Injection	Time After Injection		
		6 hours	24 hours	5 days
I (Controls)	14.6 (± 0.51)	13.7 (± 0.63)	14.2 (± 0.51)	14.3 (± 0.68)
II	14.8 (± 0.14)	11.0 (± 0.96)	10.8 (± 1.09) ($n = 2$)	15.1 ($n = 1$)
III	14.7 (± 0.39)	11.9 (± 0.75)	11.8 (± 1.22) ($n = 3$)	13.5 (± 0.22) ($n = 2$)
IV	14.5 (± 0.30)	12.4 (± 1.34)	13.0 (± 1.34)	14.7 (± 0.46)
Significance of differences between	I and II I and III I and IV	$P < 0.01$ $P < 0.01$ N.S.	$P < 0.001$ $P < 0.05$ N.S.	— $P < 0.05$ N.S.

The standard deviations are given in brackets.
N.S. = not significant.

by $0.9 (\pm 0.4)$ mg./100 ml. at six hours but returned almost to the baseline by 24 hours. Such a fall was to be expected for stress has been shown to lower the serum calcium level of the rabbit by as much as 10% of the initial value regardless of the substance injected (Natelson, Pincus, and Rannazzisi, 1963).

In group II, the mean serum calcium level fell by $3.8 (\pm 0.7)$ mg./100 ml. in six hours. At this time the mean serum calcium level of the group was 11.0 mg./100 ml., a level which is significantly lower than that of the controls (Table III). By 24 hours only two rabbits were alive and these were still hypocalcaemic, their serum calcium levels being 10.0 and 11.5 mg./100 ml. When fed on the diet used for this experiment, rabbits do not normally have a serum calcium level below 12.8 mg./100 ml. One of these two rabbits survived until the end of the experiment when its serum calcium levels had returned to normal levels.

In group III, the serum calcium level fell by 2.8

FIG. 2. Normal rabbit parathyroid from a control animal. Very few vacuoles are present. H. and E. $\times 560$.

(± 0.59) mg./100 ml. to a level that is significantly different from that of the controls ($P < 0.01$). The three rabbits which were alive at 24 hours were still very hypocalcaemic, but by five days the two survivors had restored their serum calcium levels to within the normal range.

In group IV, the serum calcium level had also fallen after six hours but the fall of $2.1 (\pm 1.5)$ mg./100 ml. was less than in group II. Comparing the serum calcium levels of groups IV and I at six hours, the difference is not statistically significant although group IV did show a fall which was twice as great as that of the controls. At 24 hours the serum calcium level had started to recover and by five days it was normal. No deaths occurred in this group. However, two rabbits in this group did show severe falls in their serum calcium levels: one had a serum calcium level of 10.4 mg./100 ml. at six hours, the other had a level of 10.9 mg./100 ml. at 24 hours. Two other rabbits reached levels of 12.1 and 12.4 mg./100 ml. at six hours.

In groups I and II, the serum protein and inorganic phosphate levels were measured; the protein levels are shown in Table IV. The controls showed

TABLE IV
SERUM PROTEIN LEVELS (MG./100 ML.) FOLLOWING THE
INTRAVENOUS INJECTION OF CADMIUM CHLORIDE

Group	Before Cd Injection	6 Hours after Injection
I	7.3 (± 0.54)	7.0 (± 0.37)
II	7.3 (± 0.97)	6.7 (± 0.66)

The standard deviations are given in brackets.

a fall of only 0.3 (± 0.22) mg./100 ml. at six hours but in group II there was a fall of 0.6 (± 0.26) mg./100 ml. In neither group was there any significant change in the serum phosphate levels.

In this experiment, no constant histological changes were found in the parathyroid glands; in particular, no evidence of vacuolation was found. This may have been because the hypocalcaemic rabbits died so quickly that there was insufficient time for histological changes to appear.

In those animals which died within the first day of the experiment, the organs other than the parathyroid showed intense congestion and occasional petechial haemorrhages. These changes were particularly marked in the lungs, and it was noticed that the animals which died were dyspnoeic before death.

Discussion

In previous work it was found that in a series of 52 male rabbits the mean serum calcium level was 14.4 (± 0.7) mg./100 ml. Slightly lower and more varied results were found in a small series of females (Kennedy, 1965). If two standard deviations on either side of the mean are allowed, few normal rabbits on this diet should have a serum calcium level below 13.0 mg./100 ml.; in fact some female animals do have levels as low as 12.8 mg./100 ml. but this is the lowest level for healthy adult rabbits that has been found in this laboratory. On this basis the levels of serum calcium found in these experiments must be classed as hypocalcaemic.

In the experiments in which the cadmium was given subcutaneously, the hypocalcaemia could perhaps be attributed to a fall in the rabbits' food consumption, a factor that was not measured. However, changes in food consumption were eliminated in the series of experiments in which intravenous injections of cadmium were given. The fall in the serum calcium level could not have been due to changes in the serum protein levels for these (Table IV) were much too small to explain a fall of 3.8 mg. in the serum calcium level at six hours.

Cadmium is known to damage the renal tubules, and in these experiments histological evidence of this was obtained. I have found that, in rabbits which were made uraemic by the ligation of both ureters, profound hypocalcaemia appeared within 48 hours; however, in the present experiments, only one of the rabbits which were given subcutaneous injections became uraemic and this occurred only at the end of the experiment. Uraemia cannot explain the hypocalcaemia demonstrated in the acute experiments, for in these the serum calcium level fell within six hours. The mean fall in the serum calcium level at six hours was 3.8 mg. in group II, 2.8 mg. in group III, and 2.1 mg. in group IV; this indicates that the hypocalcaemia was not only due to the injection of cadmium but that its severity was related to the dose employed. The doses of cadmium used in these acute experiments were considerably greater than those encountered in occupational exposure in which the cadmium is absorbed by inhalation over a number of years. In acute cadmium poisoning in man the actual dosage has rarely been determined but death has been recorded after doses of 8.9 g. cadmium chloride orally and 0.2 g. cadmium bromide intravenously (U.S. Public Health Service, 1942), doses equivalent to at least 7 mmole/kg. and 0.014 mmole/kg. respectively. This intravenous dose of 0.014 mmole/kg. is similar to that used in group III of the present animal experiments.

At present it cannot be explained why cadmium causes hypocalcaemia in the rabbit. An attractive hypothesis would be that cadmium increases the renal excretion of calcium. This idea is supported by the findings of Kazantzis and his colleagues (1963), who found high urinary calcium excretions in men exposed to cadmium. Their subjects also had aminoaciduria and proteinuria indicating disturbed renal tubular function. It is well known that cadmium is toxic to the renal tubules, and Vander (1962) has shown that cadmium enhances the tubular reabsorption of sodium in dogs. Under conditions of high urine flow in normal dogs, Walser (1961) found that the clearances of sodium and calcium were proportional to one another and suggested that this was brought about by the tubular reabsorptions of the two ions being inversely proportional. Thus the action of cadmium in enhancing the tubular reabsorption of sodium may be coupled with a decrease in the reabsorption of calcium. In normal circumstances the tubular reabsorption of calcium is so complete that quite a small change in tubular reabsorption can make a very large difference to the amount of calcium excreted. Kazantzis and his colleagues (1963) also found some evidence to suggest that the mechanism of urinary acidification was impaired in their subjects. This is not only a further sign of a renal tubular defect but it suggests that the increase of urinary calcium excretion could be a result of a form of acquired renal tubular acidosis. If this explanation—that the hypocalcaemia results from increased renal excretion—is correct, the changes in the parathyroid may be secondary to the hypocalcaemia rather than primary, borne out by the fact that they occurred only inconsistently. However, the severe hypocalcaemia which occurs in rabbits made uraemic by ligation of the ureters does not result in vacuolation of the parathyroids.

A second possibility is that cadmium either interferes with the secretion or action of parathyroid hormone or, possibly, promotes the secretion of calcitonin. If the hypocalcaemia was due to an induced state of hypoparathyroidism the serum inorganic phosphate level might have been expected to rise; but it has been shown that, if anything, the phosphate level tended to fall. This suggests that cadmium does not have a direct action on the parathyroid. This evidence is, however, weak, as in hypoparathyroidism the changes in the serum calcium may precede the changes in the phosphate level.

Rabbits have a higher serum calcium level than other mammals but, provided the dietary conditions are standardized, the levels remain within fairly narrow limits. The evidence available indicates that

this high serum calcium level includes a relatively high diffusible fraction (Kennedy, 1965). Partly as a result of this the rabbit is capable of excreting large amounts of calcium via the kidney. On one diet, which was not used in the present experiments, excretions of over 1 g. per day may be found. This ability to excrete large quantities of calcium may explain the rapid development of hypocalcaemia in the present experiments although the urinary calcium excretions in acute poisoning have not yet been measured. There is also evidence that in rabbits the pituitary may be involved in the maintenance of the serum calcium level: Natelson and his colleagues have shown that commercial ACTH contains a substance that can cause acute hypocalcaemia and tetany in the rabbit (Natelson *et al.*, 1963). This does not necessarily mean that hypocalcaemia in the rabbit is a non-specific reaction, for in the present experiments it did not occur in the controls, and in other experiments it did not occur after the injection of such substances as dimercaprol, cysteine, and zinc salts.

A final consideration is whether the occurrence of hypocalcaemia contributed to the death of the animals in the fatal experiments. It is hoped to present some detailed observations on this point in another paper but it has been found that prevention of the hypocalcaemia by giving calcium gluconate did not reduce the mortality of experimental cadmium poisoning.

The photomicrographs are the work of Mr. David Williams.

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