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# Hypertension following Chronic, Very Low Dose Cadmium Feeding (39900)[1]

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Schroeder observed (1-3) and we have confirmed (4, 5) that chronic ingestion of small amounts of cadmium can induce hypertension in rats. We found an average increase in systolic pressure of 15 to 20 mm Hg which, although less than Schroeder reported (6), was highly significant statistically. Schroeder's standard procedure used weanling Long-Evans rats for which the only drinking water contained 5 parts per million (ppm) of cadmium. Although this cadmium exposure produced no overt illness, either acute or chronic, it resulted in an intake of nearly 300 μg/kg/day (5); whereas the diet of the average American adult has been estimated to contain 0.5 to 1 μg of cadmium/kg/day (7). On the other hand, the level of cadmium in the kidneys (where it is strongly concentrated) of rats exposed to 5 ppm of cadmium for 24 months approximates that of the average American adult with no known specific exposure: 45 μg/g in the rats (5) versus 30 μg/g in man (8).

Although Schroeder did not investigate the effects of lower cadmium exposures, we tested the effects of a range of concentrations, including both 1 and 2.5 ppm of cadmium (4, 5). We found that 2.5 ppm of cadmium behaved very similarly to 5 ppm but that 1 ppm of cadmium seemed to require somewhat longer to induce hypertension. Once it had appeared, however, the hypertension induced by 1 ppm of cadmium was as marked as that produced by larger doses. We therefore investigated the effect on systolic pressure of long-term exposure to 0.5, 0.25, and 0.1 ppm of cadmium in drinking water. When even the lowest of these exposures induced increased systolic pressure within 6 months, we tested the effect of exposure to 0.03 and 0.01 ppm of cadmium. This report considers the results of a range of cadmium exposures below 1 ppm.

*Methods and materials.* The methods were those we had used in prior experiments involving larger amounts of cadmium (4, 5), and they approximated Schroeder's standard procedure (1) very closely. They can be summarized as follows: Weanling female Long-Evans rats, obtained from Blue Spruce Farms, Altamont, New York, were raised in a special low-contamination area with extensive precautions to minimize exposure to trace metals and particularly to subgroup II metals. They were fed *ad libitum* with a special "rye-based" diet containing less than 14 μg of cadmium/kg of food. Their only drinking water was doubly deionized and then had five essential trace elements (manganese, cobalt, copper, zinc, and molybdenum) added, as per Schroeder's report (9). During the two experiments considered here, the animals were subjected to only two procedures: weekly group weighing and individual blood pressuring in triplicate at 6, 12, 15, and, in the first experiment only, 18 months.

The systolic pressure was measured indirectly, as previously described (5), by inflating a tail cuff to a pressure above systolic and then letting it deflate slowly until the pulse distal to the cuff reappeared; both the distal pulse and the pressure in the tail cuff were simultaneously recorded. Twenty minutes before a measurement, each animal was minimally anesthetized with 25 mg of sodium pentothal/kg of body weight, given by intraperitoneal injection. During the 10 min immediately before a measurement, the animal was kept in a warming box at 39°.[2]

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[2] In our initial cadmium feeding experiment, the temperature of the warming box was 37°, but in later experiments we have used 39° because it makes the tail pulse more easily observable.





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Prior work has indicated that the systolic pressure, indirectly measured in this manner, correlated very well with directly measured values (4).

Cadmium assay was performed, as previously described (5), on aliquots of renal cortex weighing approximately 100 mg. These were wet ashed with nitric acid and hydrogen peroxide and then assayed for cadmium by atomic absorption, using a Perkin-Elmer model 403 spectrophotometer with an attached deuterium arc for background correction.

In the first experiment, there were 60 control rats which received no added cadmium, and there were three cadmium-exposed groups, each with 20 rats. For these three groups, 0.10, 0.25, or 0.50 ppm of cadmium, i.e., 0.5, 0.25, or 0.1 mg of cadmium ion as the acetate, was added to each liter of drinking water. This experiment was continued for 18 months, when four rats in each group were sacrificed, the kidneys were weighed, and an aliquot was taken for cadmium assay by atomic absorption spectrography. In the second experiment, there were 40 control rats which received no added cadmium, and four cadmium-exposed groups of 20 rats each which received water containing 0.01, 0.03, 0.10, or 5.0 ppm of cadmium as the acetate. This experiment continued for 15 months when the kidneys of four rats in each group were weighed and assayed for cadmium.

*Results.* The increases in systolic pressure associated with chronic ingestion of "very low" doses of cadmium (i.e., less than 1 ppm) are cited in Table I. At the four times tested in the first experiment, the "0.1 ppm cadmium group" had average systolic pressures from 14 to 23 mm Hg higher than the average for the control group. This difference was highly significant statistically, with $P < 0.001$ at all four times. Rats receiving 0.25 and 0.5 ppm of cadmium had average increases, ranging from 10 to 22 mm Hg. These increases too were all significant, when compared to the average for the control group, with $P < 0.01$. Thus, these three low levels of cadmium exposure all seemed to induce increases in systolic pressure comparable to those induced by 1 and 5 ppm of cadmium.

With the even lower exposures to cadmium used in the second experiment, the increases in systolic pressure, if present at all, were much less marked. There was no increase in pressure at 6 months, although there was an unusually high average pressure in the control group which might have obscured any trend. The "0.01 and 0.03 ppm cadmium groups" never had more than

TABLE I. AVERAGE SYSTOLIC PRESSURE FOR RATS EXPOSED TO "VERY LOW" LEVELS OF CADMIUM IN DRINKING WATER[a]

| Cadmium exposure (ppm) | Number of rats | Average systolic pressure (mmHg) at various ages (months) | | | |
|---|---|---|---|---|---|
| | | 6 | 12 | 15 | 18 |
| First Experiment Using "Very Low" Levels of Cadmium Exposure | | | | | |
| Control, i.e. none | 60 | 99 ± 12 | 102 ± 9 | 101 ± 11 | 101 ± 12 |
| 0.10 | 20 | 115 ± 11*** | 116 ± 15*** | 123 ± 14*** | 124 ± 15*** |
| 0.25 | 20 | 121 ± 12*** | 114 ± 12*** | 113 ± 15* | 120 ± 20*** |
| 0.50 | 20 | 110 ± 14* | 113 ± 13*** | 111 ± 13* | 115 ± 17*** |
| Second Experiment Using Even Lower Levels of Cadmium Exposure | | | | | |
| Control, i.e. none | 40 | 106 ± 8 | 103 ± 13 | 99 ± 11 | |
| 0.01 | 20 | 105 ± 9 | 112 ± 20* | 101 ± 10 | |
| 0.03 | 20 | 106 ± 9 | 111 ± 13* | 102 ± 17 | |
| 0.10 | 20 | 107 ± 10 | 107 ± 14 | 109 ± 13** | |
| 5.0 | 20 | 112 ± 12* | 119 ± 13*** | not done | |

[a] In the first experiment, the average systolic pressures of all "cadmium-exposed" groups of rats differed significantly from the average pressure of the comparable control group, with $P < 0.01$. In the second experiment, the average "control" systolic pressure of 106 mm Hg at 6 months was higher than our usual average for control rats which now approximates 100 ± 2 mm Hg; hence any cadmium-induced increase in pressure may appear to have been diminished. One, two, and three asterisks indicate that the average systolic pressures of "cadmium-exposed" groups of rats differed significantly from the comparable control pressures with $P < 0.05$, 0.005, and 0.001, respectively.

marginally significant increases in pressure, and even the marginal increases were only at one of the three times tested. Moreover, when the experiment was terminated at 15 months, the pressures in these two groups were not different from the control group. In the second experiment, rats receiving 0.10 ppm of cadmium required 15 months to develop a relatively small increase in systolic pressure; that increase, however, was highly significant. The standard exposure to 5 ppm of cadmium produced the usual increase of 15 to 20 mm Hg, with $P < 0.001$, but this degree of significance required a year of exposure. Thus, exposure to levels below 0.1 ppm of cadmium apparently had less hypertensive effect than higher exposures and may have had no hypertensive effect at all.

Renal cadmium concentrations for these rats after 18 (first experiment) or 15 (second experiment) months of exposure are cited in Table II. During the 18-month experiment, each control rat was estimated to ingest 125 $\mu$g of cadmium from food which contained 14 $\mu$g/kg (5). In 18 months, the average rat exposed to 0.1 ppm of cadmium drank about 1480 $\mu$g of cadmium dissolved in its water, making its total cadmium intake about 1600 $\mu$g. The renal cadmium concentration following 18 months of exposure to 0.1 ppm of cadmium averaged about 15 times that of control rats. Since the quantity of both food and water consumed were approximately the same for all groups, the average cadmium intake can be easily calculated for the other groups, and the average renal cadmium concentrations were linearly related to exposure. For purposes of comparison, the average "unexposed" American adult has accumulated approximately 30 $\mu$g of cadmium/kg of kidney (8).

TABLE II. MEAN RENAL CADMIUM FOR RATS EXPOSED TO "VERY LOW" LEVELS OF CADMIUM FOR 15 TO 18 MONTHS[a]

| Cadmium exposure (ppm) | Renal cadmium (Mean ± $\sigma$; $\mu$g Cd/g kidney) |
|---|---|
| Control, i.e. none | 0.069 ± 0.055 |
| 0.01 | 0.28 ± 0.11 |
| 0.03 | 0.64 ± 0.18 |
| 0.10 | 0.99 ± 0.29 |
| 0.25 | 2.06 ± 0.57 |
| 0.50 | 5.48 ± 2.10 |
| 1.0 | 9.86 ± 2.41 |
| 5.0 | 41.1 ± 12.50 |

[a] For the first experiment rats were sacrificed at 18 months and for the second experiment, rats were sacrificed at 15 months. For the 0.10 ppm rats, the 15- and 18-month results were averaged. The average for the 1.0 ppm rats was obtained from animals sacrificed at 18 months in a prior experiment (5) and is cited for comparison.

*Discussion.* As the first experiment indicates, chronic exposure to as little as 0.1 ppm of cadmium in drinking water, i.e., one-fiftieth of the 5 ppm which we accepted from Schroeder as "standard exposure," induced the 15 to 20 mm Hg increase in systolic pressure which we characteristically observe with 5 ppm of cadmium. As the second experiment indicates, however, still lower concentrations have little, if any, effect on pressure. Although only cadmium exposure has been purposely varied, the observed increases in pressure seem likely to be related to complex interactions between cadmium and other trace metals, and probably between cadmium and other substances in addition to metals. The observations reported here indicate that the renal cadmium concentrations which are associated with hypertension in the rat are much lower than the renal cadmium concentration in the average American adult.

The mechanism of cadmium-induced hypertension in animals is not well defined; several possible mechanisms have been suggested. At present, the most likely possibility involves the sodium retention which cadmium induces (10–12). Observations which suggest other possible mechanisms include the following: (a) Intraarterial cadmium apparently increases cardiac output (13); (b) parenteral cadmium has some direct vasoconstrictor effect (14); (c) both parenteral and peroral cadmium are associated with hyperreninemia (15); and (d) "hypertension-sensitive" Dahl rats show a greater pressor response to intraarterial cadmium than "hypertension-resistant" Dahl rats; moreover there is a significant decrease in vascular responsiveness to angiotensin II in the resistant strain (16). In addition, it has recently been observed that very small amounts of cadmium (0.05 to 0.5 ppm) can make growing rats gain weight more rapidly than rats without any cadmium exposure

(17), raising the possibility that cadmium may have some "essential" function; as such, its pressor effect might be related to some unrecognized physiologic effect.

Finally, it should be emphasized that cadmium-induced hypertension in rats, although reproducible, is relatively small in magnitude. That, however, does not preclude such hypertension from being significant or important. Most human hypertension is mild. Of the 25 million Americans who are estimated to have hypertension, more than two-thirds have diastolic pressures which are usually below 105 mm Hg. Nonetheless, in man at least, even such mild elevations in blood pressure double the risk of heart attack and stroke, the major cardiovascular causes of disability and death. Moreover, like cadmium-induced hypertension in rats, mild essential hypertension in man has no obvious associated findings. In rats, the small amounts of cadmium that induce hypertension produce none of the usual toxic manifestations of more extensive exposure to cadmium; while in man, the absence of symptoms is a major part of the problem in identifying mild hypertension and convincing the involved individuals of its ominous prognostic significance.

*Summary.* Exposing female Long-Evans rats to 0.1, 0.25, or 0.5 ppm of cadmium in drinking water for 6 to 18 months from the time of weaning induced a statistically significant average increase in systolic pressure of 10 to 24 mm Hg, and there was no evidence of cadmium toxicity. Comparable exposure to 0.01 or 0.03 ppm of cadmium was much less effective in raising pressure and probably had no pressor effect. After 18 months of exposure, the average renal cadmium level for the "0.1 ppm cadmium group" approximated 1 μg/g in contrast to more than 40 μg/g for the standard "5 ppm group" and less than 0.1 μg/g for the control group.

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