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Electrocardiographical, Biochemical and Morphological Effects of Chronic Low Level Cadmium Feeding on the Rat Heart (40344)

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Cadmium is known to dissociate myocardial excitation-contraction coupling and to depress the excitability of the cardiac conduction system *in vitro* (1–7). Short-term feeding experiments concluding that cadmium increases conduction time through the atrioventricular node-His-Purkinje system have partially extended these *in vitro* observations to *in vivo* systems (8, 9). Although the cadmium content of the heart increases with age and exposure (10–14), the effects of cadmium have not been systematically analyzed for possible significance as a contributing factor to degenerative heart disease. This preliminary study was undertaken to investigate electrocardiographical, biochemical, and morphological changes in mammalian hearts associated with long-term, low-level cadmium feeding.

Methods. Eighteen weanling female rats of the Long-Evans strain were obtained and housed as described (15). They received a special rye-based, low-cadmium diet and deionized water fortified with Mn, Co, Cu, Zn, and Mo, as described by Schroeder and Vinton (16), for a total of 24 months when all were sacrificed. For half of them, cadmium acetate was added to the water to provide a final concentration of five parts per million (ppm) cadmium; for the half which served as controls, no cadmium was added; however, they were otherwise treated identically (16).

For 16 rats (8 cadmium-fed and 8 control), indirect systolic pressures were determined 12, 18, and 21 months after weaning. Eight of the rats (4 cadmium-fed and 4 control) were studied electrocardiographically and their blood pressures determined directly after which they were sacrificed so that the various parts of the heart could be assayed for cadmium and zinc. The phosphate spectrum of hearts from the eight remaining rats (4 cadmium-fed and 4 control) were analyzed by phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR). In addition hearts from a pair of rats not otherwise studied (1 cadmium-fed and 1 control) were examined by electron microscopy.

I. *Indirect systolic pressure determinations.* At 12, 18, and 21 months after weaning systolic pressure was measured indirectly, as described (17), in rats minimally anesthetized with intraperitoneal sodium pentobarbital, 25 mg/kg of body wt. A tail cuff was slowly inflated to a pressure above systolic and then allowed to deflate slowly until the pulse distal to the cuff reappeared; both the distal pulse and the pressure in the tail cuff were simultaneously recorded on the same graph. A rat's systolic pressure was taken as the median of three measurements made within a period of about a minute.

II. *Electrocardiology.* Eight rats, randomly selected for this phase of the study, were anesthetized with intraperitoneally administered sodium pentobarbital, 35 mg/kg. Lead II of the electrocardiogram (ECG) was monitored during surgery from needle electrodes with a Grass model 7 polygraph. Right femoral artery blood pressures were continuously recorded using a Statham P23 pressure transducer connected to the Grass model 7 polygraph. Systolic pressures recorded with this catheterization technique are reported as the 24-month pressures. The left carotid artery was isolated and catheterized with an insulated silver electrode which served as the active electrode for the His bundle electrogram (HBE) recordings. A reference electrode was placed in the right jugular vein to minimize background noise in the HBE. The active electrode was then positioned to achieve optimal His wave amplitude. The HBE was monitored with an Electronics for

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Medicine variable filter amplifier and oscilliscope, model PR6, using standard low and high filter settings, 40 and 500 cycles per sec (cps), respectively (18). Simultaneously, the ECG signal was filtered between 0.1 and 200 cps on a separate channel of the monitor. The electrical events of the heart were recorded with a Tandberg-Honeywell Series 100 FM tape recorder for later replay at one-half speed when they were photographed for analysis on light sensitive paper.

III. *Assay of cadmium and zinc by atomic absorption spectrophotometry.* Following surgery, the hearts from these eight rats were removed, washed in saline, and weighed. The left and right ventricles, upper and lower septum, and atrium were taken. These samples were rinsed in saline, weighed, frozen, and stored in acid-washed plastic containers for later cadmium and zinc assay by atomic absorption spectrophotometry (AAS). Samples were also taken from the left kidney and the liver; these too were weighed, frozen, and stored for metal assay. For the actual assay, aliquots of the heart weighing 0.013–0.770 g, and of kidney and liver, weighing 0.036–0.249 g, were "wet-ashed" by standard techniques, as described (15). The cardiac aliquots were adjusted to final volumes of 2 ml and were then assayed by AAS, using the graphite furnace (19). The renal and hepatic aliquots were adjusted to final vol of 5 ml and were assayed by AAS, using the burner method (15, 19).

IV. *^{31}Phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR).* The eight remaining rats with indirect systolic pressure measurements were heparinized prior to cervical dislocation. The heart from each was then rapidly excised in the cold (0–4°) and immediately immersed in cold Hartmann's modified Ringer solution (4) at pH 7.2. Two 10 ml bolus infusions of this solution, injected through the aorta, washed the remaining blood from the coronary vasculature. After careful dissection to remove connective and adipose tissue from the heart, a sample from the apical region of the left ventricle, was taken for cadmium and zinc assay as described above; samples of the left kidney and the liver were also taken for similar assay. The remaining heart tissue from these rats was weighed and minced while in the cold for ^{31}P NMR analysis.

Since perchloric acid (PCA) extraction increases the resolution of small tissue samples without altering the phosphate spectra (20, 21), this minced tissue was treated with 1/10 w/v of 60% PCA and homogenized in a Virtis "S" 45 homogenizer for 5 min. The resulting suspension was centrifuged at 10,000 rpm (12100*g*) for 10 min in a Sorvall superspeed centrifuge, model RC2-B. The pellet was saved for Biuret protein determination, and the supernatant was neutralized to a pH between 7.0 and 7.5, using 10 N KOH in the presence of EDTA.

The neutralized PCA extract was centrifuged at 10,000 rpm (12100*g*) for 5 min after a 30-min refrigeration period. The supernatant was saved, while the pellet was washed once with 2 ml and thrice with 1 ml of water. The resulting supernatants were combined with the original one and evaporated on a rotary evaporator. Samples were lypholized overnight and then resuspended in 2 ml of 20% D_2O.

Spectronic analysis of these PCA extracts was undertaken using a Bruker HFX-5 nuclear magnetic resonance spectrometer with ^{2}D-stabilization operating at 36.43 MHz for ^{31}P (21 kG magnetic field, ^{1}H frequency 90.000 MHz). This instrument is equipped with facilities needed for all modes of Fourier transform signal averaging and broad band and continuous wave heteronuclear ^{1}H decoupling. Details of the actual conditions and analytical parameters of ^{31}P NMR spectroscopy have been described (20). Samples were scanned for 2.5 hr.

V. *Electron microscopy.* Representative tissue cubes, 1 mm on a side, of sinoatrial and atrioventricular nodes, left ventricle, right atrium, and septum from nembutal anesthetized, 35 mg/kg, animals were fixed by perfusion with 3% glutaraldehyde in Sorensen's phosphate buffer at pH 7.2. This treatment was followed by osmication in Millonig's fluid, dehydration and embedment in Epon-Araldite. After establishing the morphological orientation with semi-thin sections (1.5 μm) stained with methylene blue, ultra-thin sections were prepared for electron microscopic viewing. Electron photomicrographs of the several cardiac regions from control and cadmium-fed rats were compared in search of significant anatomical differences.

VI. *Data analysis.* For all ECG and HBE

recordings, a mean was calculated from ten intervals measured with a Bausch and Lomb ocular micrometer scaled at 0.1 mm graduations. The paper speed of 150 mm/sec permitted resolution to 1 msec. Heart rates were calculated from A-A wave interval measurements of the HBE.

Standard analytical techniques for the identification of compounds and determination of their concentrations were used (20, 21) to analyze the ^{31}P NMR spectra.

Briefly, concentrations of each phosphate compound studied were computed from the ratio of the compound peak area to the phosphate primary standard (1.5 m*M* inorganic orthophosphate, Na form) peak area. Student's *t* test was applied to the statistical means and variances of each phosphate compound analyzed in the control and cadmium hearts to determine the significance of the measured differences in their concentrations. A value of $P < 0.05$ was accepted as significant.

The electrocardiographical, tissue cadmium and zinc concentrations and blood pressure data were similarly analyzed for statistical significance. Student's *t* test was applied to the means and variances of control and cadmium data and a value of $P < 0.05$ was accepted as significant.

Results. I. *Systolic blood pressures.* The averages of the indirectly measured systolic pressures for the eight rats fed 5 ppm cadmium exceeded the averages of the control animals at 12, 18, and 21 months (Table I). The differences at 18 and 21 months were statistically significant ($P < 0.05$); at 12 and 24 months they were not significant but the numbers of rats were smaller than we usually use. The control average at 12 months was 108 mm Hg, while our usual average for such animals, approximates 100 mm Hg. The differences at 18 and 21 months were in the 15 to 20 mm Hg range which 5 ppm cadmium usually induces under our standard conditions.

The 24-month pressures measured directly by arterial catheterization averaged 129 mm Hg for the four cadmium-treated rats versus 116 mm Hg for the four control rats (Table I). Growth rates and total body weights, which provide a sensitive measure of toxicity from higher doses of cadmium, were the same for cadmium-fed and control animals throughout the entire 24 months of follow-up.

TABLE I. SYSTOLIC BLOOD PRESSURES (mm Hg).[a]

Time (mos)	N	Control	N	5 ppm Cd fed
12	8	108 ± 3	8	117 ± 5 (ns)
18	8	99 ± 4	8	117 ± 7*
21	8	102 ± 5	8	119 ± 5*
24	4	116 ± 6	4	129 ± 8 (ns)

[a] The first three lines cite indirectly measured systolic pressures (mean ± SEM) for eight control and eight cadmium (5 ppm since weaning) rats at 12, 18, and 21 months. The last line cites directly measured systolic pressures (mean ± SEM) for the animals in which electrocardiographic measurements were made at 24 months.
* Significantly different from control, $P < 0.05$.

II. *Electrocardiology.* A significant lengthening of the PR interval, despite a more rapid mean heart rate was evident in the ECGs of the cadmium-fed animals (Table II). This depressed excitability was accompanied by a pronounced increase (30%) in the A-H interval of the HBE. Ventricular depolarization time (QS interval) was prolonged (33%) as well, in the treated animals (Table II). Representative ECG and HBE recordings are shown in Fig. 1. These observations suggest that cadmium may potentially depress the excitability of atrioventricular nodal cells (A-H interval prolongation) and may also interfere with ventricular cell to cell conduction (QS interval increase).

Control ECG intervals in this study were comparable to those reported elsewhere (9, 22). Since the HBE intervals are the first reported from rats to our knowledge, valid comparisons with literature values are not possible.

III. *Tissue cadmium and zinc.* The hearts from control rats had cadmium concentrations that approached the minimum measurable levels; while those from cadmium-fed rats had easily measurable levels (Table III). The average cardiac concentrations of the cadmium-fed group ranged from a minimum of 50 (atrial) to a maximum of 900 (lower septal) times the concentrations found in the comparable control samples. Although the absolute cardiac concentrations were small in comparison with those present in renal and hepatic tissues (with the maximum cardiac concentration being about 3% and 10% of the renal and hepatic concentrations, respectively), it is evident that cadmium accumu-

TABLE II. ELECTROCARDIOLOGY.

			ECG		HBE		
	N	Rate (complexes/min)	PR (sec)	QS (sec)	P-A (msec)	A-H (msec)	H-V (msec)
Control	4	234 ± 23*	0.056 ± 0.002	0.024 ± 0.002	8 ± 1	31 ± 2	19 ± 1
5 ppm Cd	4	294 ± 23	0.073 ± 0.004**	0.032 ± 0.001**	9 ± 1	40 ± 3	22 ± 1

* Mean ± SEM.
Significantly different from control, $P < 0.05$.

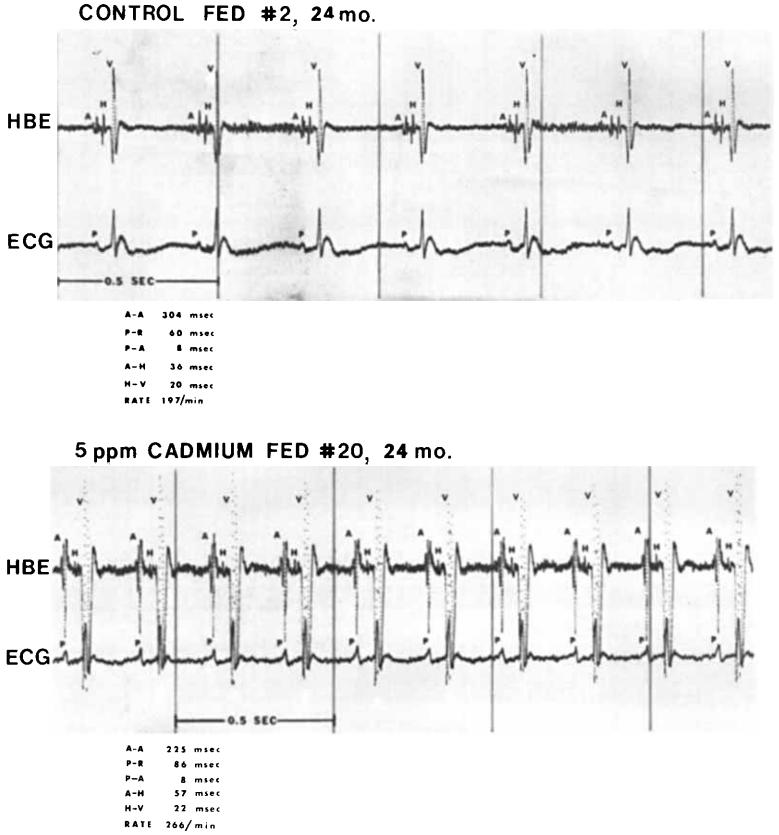

FIG. 1. Representative of ECG and His Bundle electrogram recordings from control and cadmium-fed rats.

lates in the heart. Moreover these data suggest differences in regional uptake within the myocardium.

Zinc was apparently uniformly distributed within the heart and was not affected by cadmium feeding; thus the heart, unlike kidney or liver (11, 15), did not sequester zinc in response to cadmium feeding. Cardiac cadmium-to-zinc ratios were obviously greatly increased by cadmium-feeding.

IV. ^{31}P *NMR.* The integrals of the phosphate spectra for paired heart PCA extracts were analyzed with standard techniques to determine the identity and concentration of compounds present (20, 21) (Table IV). Significant decreases in ATP and total adenine nucleotide contents were detected in hearts from cadmium-fed animals. Control heart ATP and total adenine nucleotide contents were comparable to those reported elsewhere for rat heart (23, 24). Since the sample for each group ($N = 4$) is small, a detailed interpretation of these observations would be inappropriate at this time.

V. *Electron microscopy.* A preliminary survey of all segments of cardiac tissue removed for microscopic examination revealed that ultrastructural alterations resulting from cad-

TABLE III. TISSUE CADMIUM AND ZINC CONCENTRATIONS.[a]

Tissue	Control			5 ppm Cd Fed		
	Cd (μg/g wet wt)	Zn (μg/g wet wt)	Cd/Zn ratio	Cd (μg/g wet wt)	Zn (μg/g wet wt)	Cd/Zn ratio
Heart (animals studied electrocardiographically)						
Atria	0.007 ± .007	10.5 ± 0.6	0.0007	0.363 ± 0.21*	9.3 ± 0.4	0.039
Upper septum	0.004 ± .004	13.7 ± 3.0	0.0003	0.220 ± 0.09*	10.9 ± 0.7	0.020
Lower septum	0.002 ± .002	11.2 ± 0.8	0.0002	1.742 ± 1.61*	9.4 ± 0.6	0.185
Rt. ventricle	0.010 ± .006	12.2 ± 0.6	0.0008	1.233 ± 0.95*	10.3 ± 0.1	0.120
Lt. ventricle	0.008 ± .004	11.2 ± 0.3	0.0007	1.563 ± 1.154*	14.3 ± 4.6	0.109
Heart (animals studied by ^{31}P NMR spectroscopy)						
Apex of lt. ventricle	0.011 ± .004	10.4 ± 1.6	0.0011	0.094 ± 0.026*	9.8 ± 1.0	0.010
Kidney	0.046 ± .008	25.3 ± 1.4	0.0018	45.8 ± 0.65**	32.2 ± 1.0**	1.422
Liver	0.011 ± .003	31.5 ± 2.4	0.0004	16.8 ± 2.3**	36.5 ± 2.1	0.460

[a] Mean concentrations ± standard error of the mean for four control and four cadmium exposed animals in the case of cardiac samples and eight of each in the case of kidney and liver samples.

* Significantly different from control, $P < 0.05$.

** Significantly different from control, $P < 0.01$.

TABLE IV. SELECTED COMPOUNDS DETERMINED BY ^{31}P NMR.

	(μmoles/g Heart wet wt)	
	Control	5 ppm Cd-fed*
Nicotinamide adenine dinucleotide (NAD)	1.3 ± 0.6	1.4 ± 0.1
Inorganic phosphate + phosphocreatine	16.6 ± 2.2	15.4 ± 0.9
ATP	4.4 ± 0.8	2.0 ± 0.7**
ADP	2.4 ± 0.5	1.4 ± 0.4
AMP	2.0 ± 0.4	1.2 ± 0.8
Total adenosine compounds	8.8 ± 0.1	4.6 ± 1.0**
Total heart phosphate	40.0 ± 2.0	32.4 ± 4.0
Protein (mg/g heart)	126.8 ± 1.2	127.2 ± 0.3

* Means of two groups of two pooled hearts ± SEM.

** Significantly different from control, $P < 0.05$.

mium feeding were limited to foci of degeneration within cells of the atrio-ventricular nodal region and apparent increases in electron densities of intercalated disc membranes in septal tissue (Fig. 2). Sections of sino-atrial nodal, atrial and ventricular tissue showed no significant abnormalities relative to control samples. These findings indicate tentatively the presence of ultrastructural changes within atrioventricular nodal and septal tissue exposed to low levels (5 ppm) of cadmium *in vivo*.

Discussion. Previous *in vivo* and *in vitro* investigations (1, 4, 9) have described the depressive action of cadmium on the excitability of the myocardial conduction system. This preliminary study provides additional support for these cadmium-induced changes while extending them to include other electrocardiographic changes and changes in cardiac metabolism and morphology. Long-term (24 months) exposure to low-level dietary cadmium (5 ppm in all drinking water) was found to depress electrical events of the heart characterized by a lengthening of mean PR and QS intervals of the ECG, despite a more rapid but comparable mean heart rate. His bundle electrogram analysis suggests that the prolonged PR interval resulted primarily from an increased A-H interval (30%), indicating impaired conduction through the atrioventricular node, rather than His-Purkinje cell depression. Although still tentative, the concept of selective distribution of trace elements in cardiac tissue (25) would provide a partial explanation for the apparent specificity of cadmium depression for the atrioventricular node of the cardiac conduction system. This hypothesis is further supported by electron microscope evidence showing apparent degenerative cell changes in the atrioventricular node.

The extended QS interval (33%), representative of increased ventricular depolarization time, is consistent with the hypothesis that cadmium also may alter ventricular cell excitability and/or obstruct cell to cell conduction. Septal tissue electron micrographs showed apparent marked increases in mem-

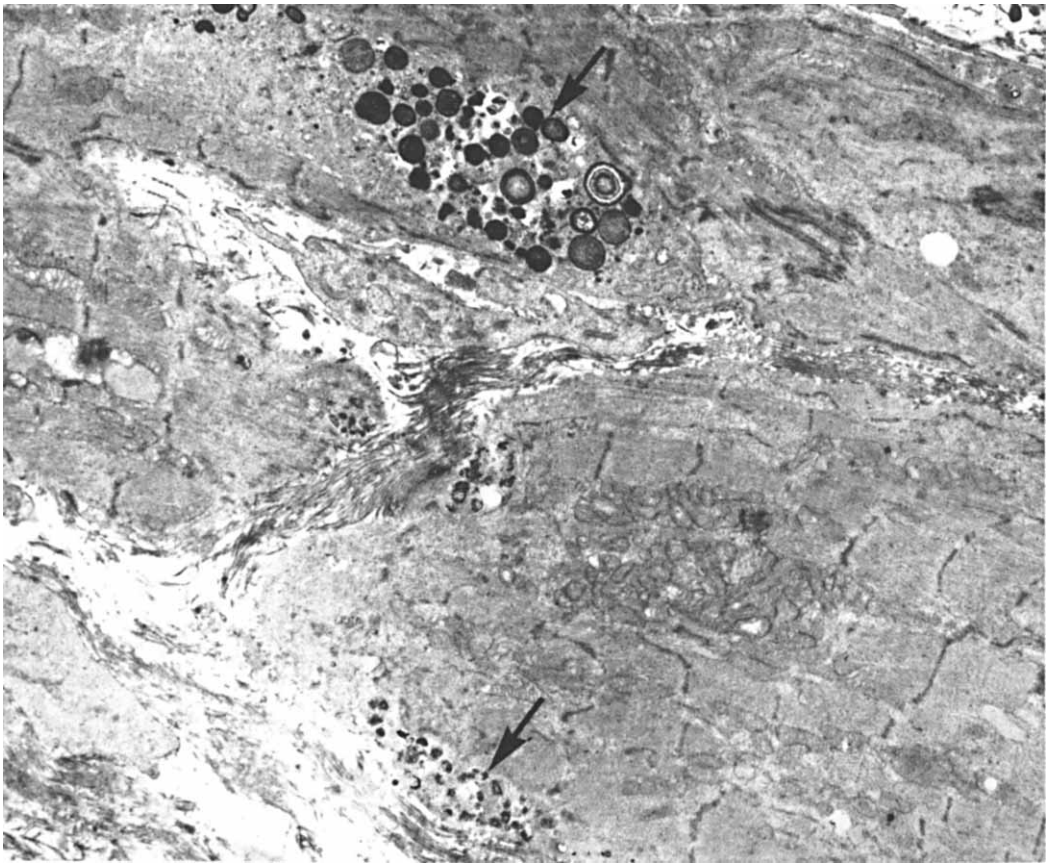

FIG. 2. Cadmium-fed rat, a-v node. Degeneration (arrows) within nodal cells, characterized by an accumulation of dense bodies adjacent to foci of vacuolization Uranyl acetate and lead citrate. Original magnification × 3200.

brane density in the intercalated discs. Since the rapid conductivity of ventricular depolarization is attributed to the intercalated discs, the possibility exists that cadmium may be present in this region bound to membrane structural and/or enzymatic proteins, thereby altering cell to cell conductivity and possibly creating the increases in intercalated disc electron density and mean QS interval of the ECG.

Although the application of ^{31}P NMR spectroscopy to biomedical research has been a recent development, it has provided an analytical method for rapid characterization of the entire phosphate profile of a given tissue. Such analyses of biopsy samples by other investigators have enabled the detection of subtle metabolic disorders (20, 21). ^{31}P NMR spectroscopic analysis of heart tissue from animals treated with dietary cadmium revealed metabolic changes consisting most notably of decreases amounting to 57%, 41% and 43% in ATP, ADP and AMP concentrations, respectively. Speculation regarding the mechanism(s) of the altered high energy phosphate metabolism shown to be associated with cadmium treatment in this study, is premature at this time; however, the functional significance may be related to a reduced excitability associated with a decrease in high energy nucleotide content of myocardial tissue, as reported by Opie (24).

In summary, long term exposure to small concentrations of cadmium is associated with depressed myocardial excitability, decreased high energy phosphate content, and morphological changes. The apparent interconsistency between the electrocardiological, biochemical and morphological findings in this study adds credence to the concept that cadmium exposure, either directly or indirectly, compromises the functional integrity of the

heart. Currently, a more detailed study is in progress which will investigate electrical, mechanical, metabolic and morphological changes in mammalian hearts associated with long term, low level cadmium feeding.

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