Table I—X-Ray Crystallographic Data for Sulfanilamide Polymorphs

Polymorphic Forms	Crystal Systems	Cell Dimensions, Å	Unit Cell Numbers	Monoclinic Angles
α-Form	Monoclinic	$a = 9.042 \pm 0.003$ $b = 9.034 \pm 0.002$ $c = 10.06 \pm 0.02$	4	110°42′
β-Form	Monoclinic	$a = 8.95 \pm 0.02$ $b = 9.06 \pm 0.02$ $c = 9.96 \pm 0.02$	4	110°
γ-Form	Monoclinic	$a = 7.783 \pm 0.006$ $b = 12.944 \pm 0.003$ $c = 7.95 \pm 0.02$	4	106°1′
δ-Form	Orthorhombic	$a = 14.81 \pm 0.02$ $b = 5.65 \pm 0.02$ $c = 18.46 \pm 0.02$	8	—

Table II—Optical Crystallographic Data for Sulfanilamide and Sulfanilamide-d_4 Polymorphs

	Polymorphic Forms	Refractive Indexes α	β	γ
Sulfanilamide polymorphs	α-Form	1.580	1.644	—
	β-Form	1.560	1.680	—
	γ-Form	—	1.676	>1.800
	δ-Form	1.548	1.624	
Sulfanilamide-d_4 polymorphs	α-Form	1.576	1.640	—
	β-Form	1.556	1.672	—
	γ-Form	—	1.664	>1.800
	δ-Form	1.540	1.620	—

transformation. Consequently, the transformation energy is higher for the δ-form as compared with the α- and β-forms.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 18, 1973, from the *College of Pharmacy and the ‡Department of Chemistry, University of Iowa, Iowa City, IA 52242

Accepted for publication September 12, 1973.

Abstracted from a dissertation submitted by H. O. Lin to the Graduate College, University of Iowa, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by funds from the Department of Health, Education, and Welfare, Public Health Service Grant AR01-A 107572.

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Effect of Multiple Doses of Cadmium on Glucose Metabolism in the Rat

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Abstract □ The effect of multiple doses of cadmium on carbohydrate metabolism in rats was studied. The treated groups received two dose levels, 0.25 and 0.50 mg cadmium/kg ip every 2nd day for 20 doses. Plasma glucose, immunoresponsive insulin levels, and body weights were determined before treatment and after 10 and 20 doses of cadmium. After the last dosage, D-glucose-^{14}C (uniformly labeled) was administered to a randomly chosen subgroup and a carbon dioxide radiorespirometry experiment was performed. Statistically significant differences in blood glucose and insulin levels were not detected at any time, indicating no effect of cadmium on the pancreatic secretory activity. There was no effect on body weights. However, cadmium increased the evolution of respiratory $^{14}CO_2$. It is postulated that cadmium affects the rate of glucose metabolism *in vivo* by affecting mechanisms such as glycolysis and the tricarboxylic acid cycle.

Keyphrases □ Cadmium—effect of multiple doses on glucose metabolism, rat □ Glucose metabolism—effect of multiple doses of cadmium, rat □ Glycolysis—possible effect of multiple doses of cadmium on glucose metabolism, rat

In the last 15 years, the role of metals in the structure and function of proteins became a new field for investigation. Unfortunately, there is limited information concerning the nature of the metal-binding sites in metalloproteins. Although different metallic ions may have similar chemical properties, they may

Table I—Effect of Cadmium on Plasma Glucose, Insulin, Insulinogenic Index, and Weight of Rats

Parameter	Replicate	Saline Control			Cadmium Treated			
		Before Treatment	After 10 Doses	After 20 Doses	Dose, mg Cadmium/kg	Before Treatment	After 10 Doses	After 20 Doses
Plasma glucose, mg/100 ml	1	149[a] ±18	154 ±29	182 ±29	0.25	174 ±35	150 ±19	209 ±43
	2	126 ±14	159 ±90	123 ±18		128 ±70	160 ±80	124 ±11
					0.50	128 ±10	160 ±12	129 ±14
Insulin, μunits/ml	1	35.1 ±17.5	36.9 ±17.4	32.4 ±11.5	0.25	33.1 ±19.6	24.2 ±7.6	44.2 ±13.6
	2	37.9 ±9.0	42.6 ±11.0	43.7 ±9.0		52.0 ±9.0	50.5 ±11.0	55.3 ±15.0
					0.50	55.5 ±16.0	47.1 ±16.0	57.6 ±11.0
Insulinogenic index[b]	1	0.24 ±0.13	0.24 ±0.10	0.18 ±0.07	0.25	0.22 ±0.15	0.16 ±0.05	0.21 ±0.09
	2	0.31 ±0.11	0.27 ±0.06	0.36 ±0.09		0.41 ±0.08	0.32 ±0.07	0.45 ±0.12
					0.50	0.43 ±0.11	0.29 ±0.10	0.45 ±0.10
Weight, g	1	215 ±10	257 ±10	271 ±9	0.25	218 ±4	250 ±8	266 ±6
	2	105 ±4	176 ±7	203 ±12		105 ±3	175 ±9	198 ±14
					0.50	104 ±4	165 ±10	195 ±18

[a] Mean of eight animals ± standard deviation. [b] Insulinogenic index = $\frac{\text{insulin, } \mu\text{units/ml}}{\text{blood glucose, mg/100 ml}}$.

Table II—Cadmium Concentrations in Tissues after 20 Intraperitoneal Administrations of 0.25 mg Cadmium/kg Every 2nd Day

Tissue	Saline Control, ppm	Cadmium Treated, ppm
Blood	0.29[a] ± 0.02	2.30 ± 0.24
Kidneys	0.40 ± 0.09	86.82 ± 13.37
Spleen	0.50 ± 0.13	16.24 ± 1.02
Liver	0.57 ± 0.01	120.38 ± 1.30
Pancreas	0.94 ± 0.06	33.46 ± 2.99

[a] Mean of four animals ± standard deviation. Wet basis.

form complexes with different ligands on a metalloprotein. It is not clear to what extent the properties of these complexes can be used to predict the identity of the metal ligands of metalloproteins (1). Cadmium binds more strongly than zinc to metallothionein, and an excess of cadmium can displace zinc (2–4). The parts of a protein with high cysteinyl content are where cadmium and zinc may interact, and this interaction may affect the secondary and tertiary structure of the protein.

In previous studies, investigators (1, 5–8) found that cadmium introduces changes in the three-dimensional structure of enzymes and influences their rate or mechanism of action by activation, inactivation, uncoupling reactions, or other actions. In addition, it was found that cadmium selectively damages pancreatic β-cells (9, 10) and produces changes in blood glucose (5).

Since cadmium may affect the secretory activity of pancreatic β-cells and the rate of reaction of enzymes involved in carbohydrate and cellular energetic metabolism, the following *in vivo* investigation concerned the effect of multiple doses of cadmium on glucose and insulin blood levels and the evolution of respiratory $^{14}CO_2$.

EXPERIMENTAL

Sixteen female rats[1], weighing approximately 200 g, were assigned randomly to two groups, control and treatment, of eight animals each. Animals were housed individually in metal cages. The animals were maintained for 7 days under laboratory conditions prior to experimentation. They were allowed free access to food[2] and tap water.

A dose of 0.25 mg cadmium/kg ip was administered to the treatment group every 2nd day for 20 doses. The cadmium was given as cadmium acetate in water. Two months later, this study was replicated with a different shipment of animals, weighing approximately 100 g. In this replicate, an additional group of eight rats was given twice the previous dose (0.5 mg/kg). Control animals received saline solution instead of cadmium. Plasma glucose and immunoresponsive insulin levels as well as body weights were measured for each animal immediately prior to treatment with cadmium and 24 hr after the 10th and 20th doses.

Twenty-four hours after completion of the last blood sampling, all animals from the first replicate were assigned randomly to two groups, each subdivided into control and treatment animals, to allow for a carbon dioxide respiration experiment and for determination of cadmium concentrations in the pancreas, liver, kidneys, spleen, and blood.

Insulin and Glucose Determination—The immunoresponsive insulin of the plasma was determined quantitatively using a commercially available radioimmunoassay kit[3], which closely resembles the method described by Hales and Randle (11). The method uses an antibody specific to insulin, which is reacted with plasma immunoresponsive insulin and a known amount of ^{125}I-insulin to form an insoluble insulin-antibody complex. This complex is filtered and measured for radioactivity by liquid scintillation spectrometry. The level of radioactivity from ^{125}I-insulin recovered in

[1] Sprague-Dawley descendants, Laboratory Supply Co., Indianapolis, Ind.
[2] Wayne Lab-Blox, Allied Mills, Chicago, Ill.
[3] Amersham/Searle Co., Chicago, Ill.

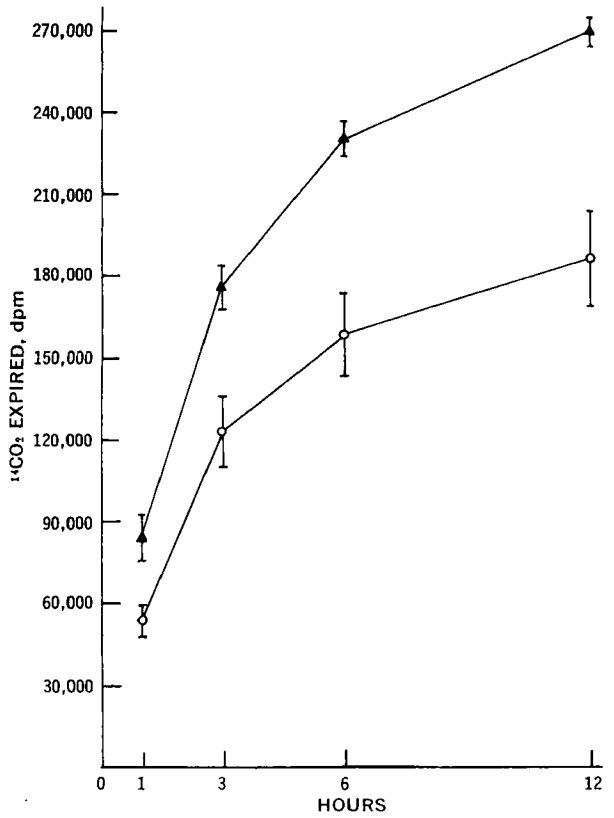

Figure 1—*Amount of $^{14}CO_2$ expired by rats receiving* D-*glucose-U-^{14}C. Key:* ○, *control; and* ▲, *0.25 mg cadmium/kg every 2nd day for 20 doses.*

the complex is related inversely to the concentration of immunoresponsive insulin in the plasma sample.

Plasma glucose was measured with a glucose analyzer containing a digital readout[4]. The principle of this method involves the conversion of glucose to its oxidation products (gluconic acid and hydrogen peroxide) by a glucose oxidase enzyme. The rate of oxygen consumed during the reaction is proportional to the glucose concentration in the sample. The oxygen is detected with an oxygen-sensitive electrode.

Radiorespirometric Study—Eight 15.24-cm, controlled-ventilation, glass metabolism chambers[5] were used for $^{14}CO_2$ collection. Carbon dioxide and water were removed from the incoming air stream (500 ml/min) with a drying tube containing anhydrous calcium sulfate followed by asbestos coated with sodium hydroxide. The chamber exhaust air was dehydrated with anhydrous calcium sulfate, and $^{14}CO_2$ was trapped in 100 ml of a solution of 2-ethoxyethanol and 2-aminoethanol (2:1). Primary and secondary carbon dioxide traps were used, although the efficiency of the primary traps approached 100%.

Prior to the carbon dioxide respiration experiment, the animals were fasted for 24 hr. On the day of testing, each animal was given 3.0 ml po of a 50% (w/v) glucose–water solution containing 4.82 μCi/3.0 ml of D-glucose-U-^{14}C (U = uniformly labeled). Upon completion of dosing, the animals were immediately placed in the metabolism chambers where neither water nor food was provided. Trapping solutions for $^{14}CO_2$ were changed at 1, 3, 6, and 12 hr.

A liquid scintillation counter[6] utilizing internal standardization was used for the determination of ^{14}C activity. The counting efficiencies for the scintillator used[7] ranged from 72 to 75%, the counting error was less than 1%, and the instrument error was minimized by counting each sample twice.

The radiochemical purity of the D-glucose-U-^{14}C was greater than 99%. The purity was determined by paper chromatography, autoradiography (12), and liquid scintillation counting of the spots placed in 15 ml of scintillator[8].

Tissue Digestion—All samples were dissolved by heating with concentrated nitric acid. They were filtered into volumetric flasks and analyzed for cadmium with an atomic absorption spectrophotometer[9].

Table III—Analysis of Variance of Data for Radiorespiration Study

Source	*df*	*MS*	*F*
Cadmium treatment (A)	1	44153.936	218.390[a]
Time (B)	3	71142.130	351.876[b]
AB	3	403.067	1.996[b]
Residual	24	202.180	
Total	31		

[a] At the 0.05 significance level, a significant $F = 4.26$. [b] At the 0.05 significance level, a significant $F = 3.01$.

RESULTS AND DISCUSSION

The effects of multiple doses of cadmium on plasma glucose and insulin levels and body weight are shown in Table I. The insulinogenic index is the ratio of serum immunoresponsive insulin to blood glucose concentration. This index indicates the pancreatic secretory activity. A reduced insulinogenic index implies a decreased pancreatic secretory activity (13). Control animals, which had received repeated doses of saline, were examined at the same time intervals.

The results shown in Table I were analyzed by analysis of covariance. Each replicate and each dose level were analyzed separately. The value before treatment was taken as a covariate for the values after 10 and 20 doses. The significance of the covariate and the difference in treatment effects *versus* control were determined for each case. When the F value of the covariate was lower than 1.0, the analysis was performed on the original cell means; with F values higher than 1.0, the analysis was performed on the adjusted cell means. Since the F values of treatment for $p = 0.05$ with 3 and 27 *df* were significant in all cases, a Newman-Keuls multiple comparison on the adjusted or unadjusted means was performed. The analysis showed that there were no significant differences in glucose, insulin, insulinogenic index, or weight at the $p = 0.05$ level after 10 or 20 doses of cadmium. This was the case for both replicates and for both dose levels.

Analysis of covarince was also run on the combined results from both replicates. Again no significant differences were found.

The cadmium concentrations in the blood, kidneys, spleen, liver, and pancreas were determined, and the results are shown in Table II. The cadmium concentrations in the various tissues of the treated animals were higher than those in controls. The levels varied from 10 times higher for blood to as much as 200 times higher for liver. The relative uptake of the various tissues also was different between the control and treatment groups.

Results from the carbon dioxide radiorespiration study showed that cadmium increased the evolution of respiratory $^{14}CO_2$. The data (Fig. 1) were analyzed by a two-factor analysis of variance (treatment and time effects) to assess the difference between the control and treatment groups. The analysis was done with the square roots of the cumulative amounts (dpm) of $^{14}CO_2$ collected, which provided a response with a variance approximately independent of its mean. The F value for the treatment by time interaction was not significant even at the 10% level (Table III); the main effect difference between control and treatment groups was highly significant over all time periods.

Since cadmium is known to accumulate in the pancreatic islet tissue and repeated administration of cadmium may selectively produce destruction of β-cells (9, 10, 14), a decrease in the insulin secretory activity of the pancreas was expected. The data did not prove this, in spite of the high accumulation of cadmium in the pancreas of treated animals (Table II). Further studies on the effects of multiple cadmium administration on insulin secretion

[4] Beckman, model 2001.
[5] Delmar Scientific Laboratories, Maywood, Ill.
[6] Tri-Carb, Packard Instrument Co., Downers Grove, Ill.
[7] Toluene–2-ethoxyethanol (1:1) and 0.4% 2,5-diphenyloxazole.
[8] Toluene–*p*-dioxane–2-ethoxyethanol (1:3:3), 1% 2,5-diphenyloxazole, and 8% naphthalene.
[9] Perkin-Elmer model 306.

from the perfused pancreas as well as from isolated islets are required to clarify this point.

The significant increase in the evolution of respiratory $^{14}CO_2$ by the animals treated with cadmium indicates that multiple dosages of cadmium alter carbohydrate metabolism. The affected mechanisms may involve glycolysis, the tricarboxylic acid cycle, respiration, or a combination of these. It was reported (15–17) that cadmium ions uncouple oxidative phosphorylation. This uncoupling may be due to the effect of cadmium on the transport of ions across the mitochondrial membrane (16) or to the blocking of some free active site (15). Uncoupling oxidative phosphorylation increases the adenosine diphosphate concentration relative to that of adenosine triphosphate. This causes an increase in the rate of glycolysis and the tricarboxylic acid cycle since it activates certain enzymes such as phosphorylase *a*, adenosine triphosphate:D-fructose 6-phosphate 1-phosphotransferase, adenosine triphosphate:pyruvate phosphokinase, and isocitrate dehydrogenase.

With the increase in the rates of glycolysis and the tricarboxylic acid cycle after cadmium treatment, there may be a concomitant increase in labeled glucose utilization. With the information available, it is not possible to explain further the effect of cadmium on the evolution of carbon dioxide *in vivo* and further work is indicated.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 21, 1973, from the *Departments of Bionucleonics and Statistics, Purdue University, West Lafayette, IN 47907.*

Accepted for publication September 5, 1973.

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Synthesis and Hypotensive Properties of 4-Amino-6,7-dimethoxyisoquinoline

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Abstract □ The compound 4-amino-6,7-dimethoxyisoquinoline hydrochloride, a structural isomer of amiquinsin hydrochloride (4-amino-6,7-dimethoxyquinoline hydrochloride), was synthesized. It was shown to be hypotensive in anesthetized normotensive dogs and produced an antihypertensive effect in unanesthetized renal hypertensive dogs.

Keyphrases □ 4-Amino-6,7-dimethoxyisoquinoline—synthesis and hypotensive properties, structural isomer of amiquinsin hydrochloride □ Amiquinsin hydrochloride—synthesis and hypotensive properties of the structural isomer 4-amino-6,7-dimethoxyisoquinoline □ Antihypertensive agents, potential—synthesis and screening of 4-amino-6,7-dimethoxyisoquinoline, a structural isomer of amiquinsin hydrochloride

Since the hypotensive properties of amiquinsin hydrochloride [1] (4-amino-6,7-dimethoxyquinoline hydrochloride hydrate) (I) were well known (1), there was a desire to prepare the isomeric 4-amino-6,7-dimethoxyisoquinoline hydrochloride (II) for hypotensive screening. This paper discusses the synthesis of II and several related intermediates, as well as the hypotensive properties of II.

The key intermediate, 2,3-dihydro-6,7-dimethoxy-4(1*H*)-isoquinolone (IV), in the synthesis of II was prepared *via* a two-step process (Scheme I). Reductive condensation of veratraldehyde and glycine gave *N*-veratrylglycine (III) which, upon subsequent cyclization in polyphosphoric acid, gave IV [2]. Although the intermediate Schiff-base product of veratraldehyde and glycine was not isolated, it was necessary to heat briefly the reaction solution prior to reduction to avoid formation of *N,N*-diveratrylglycine (VII). The reductive alkylation at room temperature

[1] Norwich Pharmacal Co.

[2] The present synthesis of IV is more direct than the four-step synthesis used in the similar preparation of 2,3-dihydro-6,7-dimethoxy-1-methyl-4(1*H*)-isoquinolone (3).