Effect of Cadmium *In Vivo* on Glucose Catabolism in Normal and Alloxan Diabetic Rats

DIONYSSIS S. ITHAKISSIOS*§, WAYNE V. KESSLER*x, JAMES N. ARVESEN‡, and GORDON S. BORN*

Abstract □ A radiorespirometry experiment was conducted to determine the effect of single and multiple doses of cadmium on the catabolism of D-glucose-^{14}C (uniformly labeled) in normal and alloxan diabetic rats by the collection of $^{14}CO_2$. Cumulative amounts of $^{14}CO_2$ were determined at 1, 3, 6, and 12 hr. The effect of cadmium on glucose catabolism was related to the administered dose as well as to the manner of dosing, single or repeated.

Keyphrases □ Cadmium—effect *in vivo* on glucose catabolism, normal and alloxan diabetic rats, radiorespirometry, single and multiple doses □ Glucose catabolism, *in vivo*—effect of single and multiple doses of cadmium, normal and alloxan diabetic rats, radiorespirometry □ Radiorespirometry—determination of effect of single and multiple doses of cadmium on glucose catabolism, normal and alloxan diabetic rats

An increasing amount of cadmium in the environment is thought to be a risk to the general population. The human hazard usually results after long-term accumulation of relatively small daily increments of cadmium in the microgram range. It has been reported that cadmium alters *in vitro* the activity of certain enzymes (1–5), decreases insulin secretion (6, 7), and produces changes in blood glucose levels (2).

In previous work (8), multiple doses of cadmium did not produce differences in blood glucose or insulin levels, although cadmium did increase the evolution of respiratory $^{14}CO_2$ in rats catabolizing D-glucose-^{14}C (uniformly labeled). It was of interest to investigate further the influence of single and multiple doses of this metal on the rate and extent of evolution of respiratory $^{14}CO_2$ in normal rats. Alloxan diabetic rats were also used to determine if cadmium can disturb the amount of respiratory CO_2 when the amount of insulin present is decreased.

EXPERIMENTAL

Design and Procedures—Forty-two female rats[1] from the same shipment and weighing 195–205 g were used. Four normal and three alloxan diabetic groups, each containing six animals, were established by random assignment. One animal from each group was tested on one of six alternating days. Within each group, the day for testing was established by random assignment. Prior to experimentation, the animals were housed individually in metal cages for 10 days. They were allowed free access to food and tap water but were fasted 1 night before testing to decrease the endogenous glucose level.

Treatments for two normal and two alloxan diabetic groups consisted of either a single dose of 1.0 mg cadmium/kg or a multiple dose of 0.75 mg/kg given for 4 consecutive days, including the day for testing. In addition, a 3.0-mg/kg dose was chosen for one normal group to examine the effect of a single large dose. This dose was not used in the alloxan diabetic group because only seven metabolism chambers were available. The remaining groups served as controls and received saline solution. During the period of multiple dosing, animals receiving single doses were given saline solution.

Table I—Effect of Cadmium on $^{14}CO_2$ Expiration by Rats Metabolizing D-Glucose-^{14}C

Time Interval, hr	Dose, mg Cadmium/kg				
	Normal			Alloxan Diabetic	
	1.0	3.0	4 × 0.75	1.0	4 × 0.75
0–1	NS[a]	NS	NS	NS	NS
0–3	*[b]	NS	**[c]	**	**
0–6	*	NS	**	**	**
0–12	*	NS	**	**	**

[a] Nonsignificant. [b] Significant at the $p = 0.05$ level. [c] Significant at the $p = 0.01$ level.

The cadmium was given intraperitoneally as cadmium acetate dissolved in water. The dose was based on cadmium ion and not on cadmium acetate. The single dose or the last of the multiple doses was followed at once by 3.0 ml *per os* of a 50% (w/v) glucose-water solution containing 5 μCi/3.0 ml of D-glucose-^{14}C (uniformly labeled). Immediately after dosing, the animals were placed in metabolism chambers where neither water nor food was provided. The $^{14}CO_2$ was collected for 12 hr, with the trapping solutions being changed and sampled at 1, 3, 6, and 12 hr. At the end of the collection, the animals were sacrificed and discarded.

Alloxan Diabetic Rats—The diabetic condition was produced by intravenous injection of a 10% aqueous solution of alloxan[2], injected within 10 min after its preparation. The dose administered was 55 mg/kg. Blood samples for glucose determination were obtained by orbital sinus puncture 36 hr after alloxan dosing. Plasma glucose levels were determined enzymatically by a glucose oxidase method[3]. The animals were considered to be diabetic when their plasma glucose level was 350 mg % or greater. All diabetic animals received alloxan 4 days before administration of labeled glucose.

Metabolism Chambers and Radioactivity—Seven chambers were used in the manner previously described (8). Determination of the radiochemical purity of the labeled glucose, which was greater than 99%, and counting of the $^{14}CO_2$ samples were also identical.

RESULTS AND DISCUSSION

The results (Figs. 1 and 2) were analyzed by a two-factor analysis of variance (treatment and time) to assess the differences between the control and treatment groups. The analysis was run separately for the normal and the alloxan diabetic animals and was performed using the square roots of the cumulative amounts, in disintegrations per minute, of $^{14}CO_2$ collected. The square roots yielded a response with a variance approximately independent of the mean. Since the F values for the treatment by time interaction were significant, a Newman-Keuls comparison was done on the cell means (9). At the end of each time interval, the results for the control and the treatments were tested for significant dif-

[1] Sprague-Dawley descendants, Laboratory Supply Co., Indianapolis, Ind.

[2] Alloxan monohydrate (Lot 31C-0220), Sigma Chemical Co., St. Louis, Mo.

[3] ERA model 2001, Beckman glucose analyzer, Fullerton, Calif.

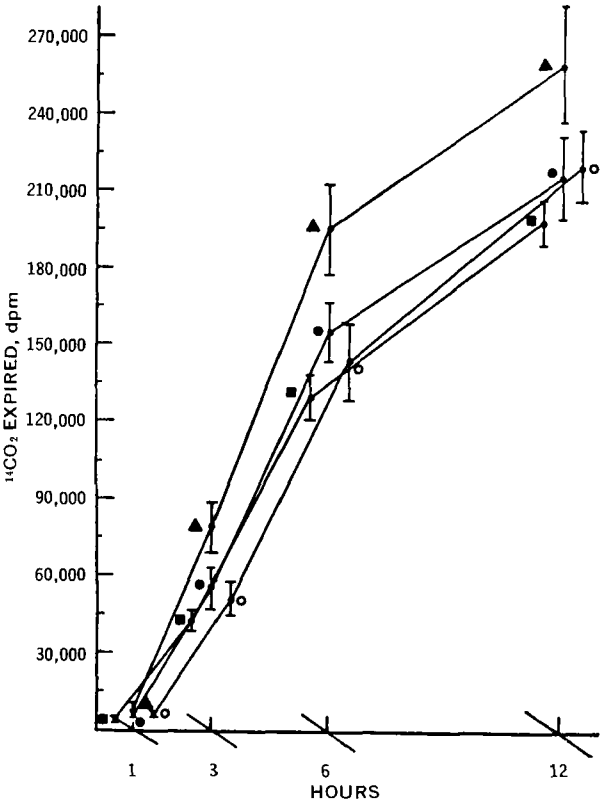

Figure 1—*Cumulative amount of $^{14}CO_2$ expired by normal rats receiving* D-*glucose-^{14}C. Data points are staggered to include error bars which indicate ±1* SD. *Key:* ●, *control;* ■, *1.0 mg/kg;* ○, *3.0 mg/kg; and* ▲, *0.75 mg/kg daily for 4 days.*

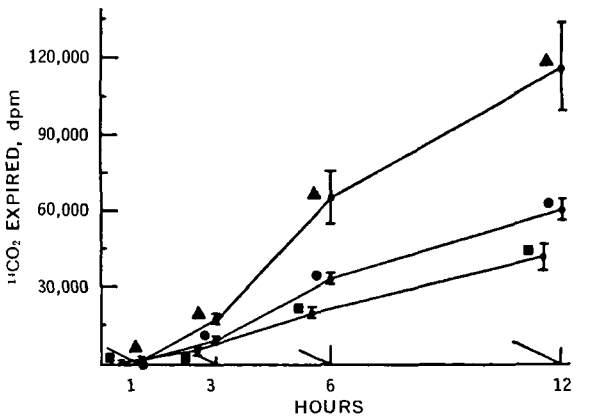

Figure 2—*Cumulative amount of $^{14}CO_2$ expired by alloxan diabetic rats receiving* D-*glucose-^{14}C. Data points are staggered to include error bars which indicate ±1* SD. *Key:* ●, *control;* ■, *1.0 mg/kg; and* ▲, *0.75 mg/kg daily for 4 days.*

ferences at the $p = 0.01$ and $p = 0.05$ levels (Table I). In the first time interval, there were no significant differences in the amounts of $^{14}CO_2$ produced. This was probably related to the availability of labeled glucose at the site of catabolism.

The data for the single doses of cadmium in the normal animals showed that the cadmium effect on glucose catabolism was related to the administered dose. Rats receiving the 1.0-mg/kg dose produced significantly ($p = 0.05$) smaller amounts of $^{14}CO_2$ at the 3-, 6-, and 12-hr intervals, due perhaps to an increase in endogenous glucose levels after administration of cadmium. Mechanisms by which this increase can be explained are: *(a)* cadmium directly stimulates glycogenolysis (2), *(b)* cadmium stimulates release of epinephrine from the adrenals which induces hepatic glycogenolysis and inhibits insulin secretion from pancreatic β-cells (10), and *(c)* cadmium decreases somewhat the hypoglycemic effect of insulin (11). An increase in blood glucose level would decrease the specific activity of the $^{14}CO_2$ because of a decrease in labeled glucose utilization.

With the 3.0-mg/kg dose, there was no significant difference in the amount of $^{14}CO_2$ expired when compared to control at any time interval. Increasing the dosage of cadmium may affect, in addition, a number of enzymes which introduce further changes in glucose catabolism mechanisms and increase the $^{14}CO_2$ evolution rate. Affected mechanisms may involve glycolysis and the tricarboxylic acid cycle.

Increased $^{14}CO_2$ evolution ($p = 0.01$) was found at the 3-, 6-, and 12-hr intervals when 3.0 mg/kg was given in four multiple doses of 0.75 mg/kg. This same effect was seen (8) when 20 doses of 0.25 mg/kg were given over 40 days. The increase in $^{14}CO_2$ evolution with the multiple dose may be due to an uncoupling effect of cadmium on oxidative phosphorylation (12), which increases the rates of glycolysis and the tricarboxylic acid cycle.

From the data in Figs. 1 and 2, it can be seen that the diabetic animals produced decreased amounts of $^{14}CO_2$ in comparison to the normal animals. This was expected because of the higher concentration of glucose in the blood of diabetic animals due to a lack of insulin, with a resulting decrease in labeled glucose utilization. However, except for this overall decrease in labeled glucose utilization in the diabetic animals, cadmium affected evolution of $^{14}CO_2$ in a similar way in both normal and diabetic animals. That is, a single dose of 1.0 mg/kg decreased ($p = 0.01$) $^{14}CO_2$ evolution and the multiple dose increased ($p = 0.01$) $^{14}CO_2$ evolution in both cases. These results indicate that cadmium affects glucose catabolism by a mechanism independent of the presence of insulin.

The results of this study indicate the *in vivo* effect of cadmium on carbohydrate catabolism in relation to the amount of the administered dose and the manner in which the dose is given. Further investigations on the specific pathways and the enzymes involved are needed.

REFERENCES

(1) A. Sporn, I. Dinu, L. Stoenescu, and A. Cirstea, *Nahrung*, **13**, 461(1969).

(2) A. Sporn, I. Dinu, and L. Stoenescu, *Rev. Roum. Biochim.*, **7**, 299(1970).

(3) B. L. Vallee, in "Mineral Metabolism," vol. 2, part B, C. L. Comar and F. Bronner, Eds., Academic, New York, N.Y., 1962, p. 447.

(4) L. Friberg, M. Piscator, and G. Nordberg, "Cadmium in the Environment," Chemical Rubber Co., Cleveland, Ohio, 1971.

(5) B. L. Vallee and W. E. C. Wacker, in "Metalloproteins," 2nd ed., vol. 5, H. Neurath, Ed., Academic, New York, N.Y., 1970, p. 142.

(6) L. Barbieri, R. Colombi, and G. Straneo, *Folia Med. (Naples)*, **44**,1120(1961).

(7) N. Havu, *Acta Endocrinol. Suppl.*, **139**, 1(1969).

(8) D. S. Ithakissios, W. V. Kessler, J. N. Arvesen, and G. S. Born, *J. Pharm. Sci.*, **63**, 146(1974).

(9) B. J. Winer, "Statistical Principles in Experimental Design," McGraw-Hill, New York, N.Y., 1971, p. 215.

(10) D. T. Hart, M.S. thesis, Purdue University, West Lafayette, Ind., 1972.

(11) A. O. Voinar, *Mikroelem. Zhizni Rast. Zhivotn., Tr. Konf. M.*, **1950**, 580(1952); through *Chem. Abstr.*, **49**, 468(1955).

(12) G. P. Brierley, *J. Biol. Chem.*, **242**, 1115(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 20, 1973, from the *Departments of *Bionucleonics and ‡Statistics, Purdue University, West Lafayette, IN 47907*

Accepted for publication February 22, 1974.

§ Fellow, Purdue Research Foundation. Present address: Nuclear Products Division, 3M Center, St. Paul, MN 55101

ˣ To whom inquiries should be directed.