Effect of Cadmium on Palmitic Acid Metabolism by Rat Liver Homogenate

NAGY A. FARID *, GORDON S. BORN *x, WAYNE V. KESSLER *, and VIRGIL L. ANDERSON ‡

Abstract □ An *in vitro* study was conducted to determine the effect of cadmium on the metabolism of sodium palmitate-1-^{14}C by rat liver homogenate through the collection of $^{14}CO_2$. Concentrations of cadmium ion ranged from 8.9 to 115.7 μM. The results indicated a significant inhibitory effect of cadmium on palmitate metabolism. Increased cadmium concentrations caused a corresponding decrease in $^{14}CO_2$ production in a linear relationship.

Keyphrases □ Cadmium—effect on palmitic acid metabolism by rat liver homogenate, respirometry □ Palmitic acid—effect of cadmium on metabolism by rat liver homogenate, respirometry □ Metabolism, fatty acid—effect of cadmium on palmitic acid metabolism by rat liver homogenate

Many studies (1) on the toxicity and distribution of cadmium indicate that it accumulates mainly in the liver and kidneys regardless of the route of administration. It is not considered to be an essential element in humans or animals (2), but its influence on liver function has been demonstrated in both (3–8). In the liver, a large portion of the cadmium can be found in the homogenate supernate (9, 10) and it has been found in the mitochondria (10). The literature does not contain information about the effects of cadmium on fatty acid metabolism. Since the liver is one organ where cadmium accumulates to a large extent and the liver is known to oxidize about 50% of the free fatty acids in the body at rest (11), the effect of cadmium on the metabolism of sodium palmitate by rat liver homogenate was studied.

EXPERIMENTAL

The experimental design was a randomized complete block (12) with four replicates and eight cadmium concentrations plus a control in each. The liver of one rat was used in each replicate.

Compounds—Sodium palmitate-1-^{14}C[1] had a specific activity of 27.8 mCi/mmole. TLC of the free acid on silica gel G plates with two solvent systems showed one spot. The solvent systems were ethyl acetate–benzene–acetic acid (69:29:2) and petroleum ether (bp 60–80°)–ether–acetic acid (80:4:2), giving R_f values of 0.74 and 0.31, respectively. Of the total activity on the plate, 98% was detected in the palmitic acid spot. Unlabeled sodium palmitate was added to make the specific activity about 2 μCi/mg. Bovine serum albumin[2], fraction V, fatty acid poor, was used to solubilize the sodium palmitate. A fresh sodium palmitate–bovine serum albumin solution was prepared on the day of each replicate. The buffer used was calcium-free Krebs–Ringer phosphate buffer, pH 7.4. Calcium was omitted since it was found to decrease the solubility of the fatty acid (13).

Animals—Sprague–Dawley descendent male albino rats weighing 150–210 g[3] were used. Prior to use, each was fasted for 19 hr but had free access to water. All were sacrificed at about 10:45 am by decapitation on the day of use. The liver was removed and perfused with 15 ml of ice-cold buffer to remove blood. About 5 g was cut from different lobes and homogenized with 4 ml of buffer. The homogenate was centrifuged for 10 min at 2500 rpm to remove unground tissue.

System and Incubation—A Warburg respirometer with a shaking rate of 82 shakes/min was used. The temperature of the water bath was kept at 37°. The reaction flasks had two side arms, only one of which was used, and a center well. A 4 × 4.5-cm folded filter paper moistened with an agent[4] to trap the evolving $^{14}CO_2$ was placed in the center well. The total volume of fluid in each flask was 3 ml and consisted of 2.5 ml of buffer, 0.1 ml of cadmium acetate solution (or water), 0.2 ml of sodium palmitate–bovine serum albumin solution in the buffer in the side arm, and 0.2 ml of the liver homogenate supernate.

There were two flasks for each of the eight concentrations of cadmium and two that contained water for controls. The concentration of cadmium ion in the medium was 8.9, 17.8, 44.5, 53.4, 71.2, 89.0, 106.8, or 115.7 μM. The concentration of bovine serum albumin ranged from 40.6 to 43.5 μM based on a molecular weight of 69,000 (14) and that of sodium palmitate ranged from 0.100 to 0.110 mM (0.16–0.18 μCi/flask).

The flasks were attached to the manometers and placed in the water bath for 45 min, with the system opened to the air and shaking. After this incubation period, the systems were closed and the palmitate–bovine serum albumin solution was dropped into the main compartment from the side arm. Four hours after the addition of the palmitate–bovine serum albumin, the flasks were removed and placed in an ice bath.

Counting and Calculations—From each flask, a 0.2-ml sample was counted in a liquid scintillation spectrometer. The entire filter paper containing the $^{14}CO_2$ was also counted. Samples and background were counted three times for 10 min each. The counting data were converted to disintegrations per minute with correction for counter efficiency. The effect of cadmium on palmitate metabolism was determined using the following two equations:

$$\frac{A}{15B + A} \times 100 = N_f \text{ or } N_c \qquad \text{(Eq. 1)}$$

$$\frac{N_f}{N_c} \times 100 = Y \qquad \text{(Eq. 2)}$$

where A was the disintegrations per minute of $^{14}CO_2$ for a given flask, B was the disintegrations per minute of the 0.2-ml sample from a given flask, N_f was the percentage of total activity in the $^{14}CO_2$ for a given flask that contained cadmium, and N_c was the

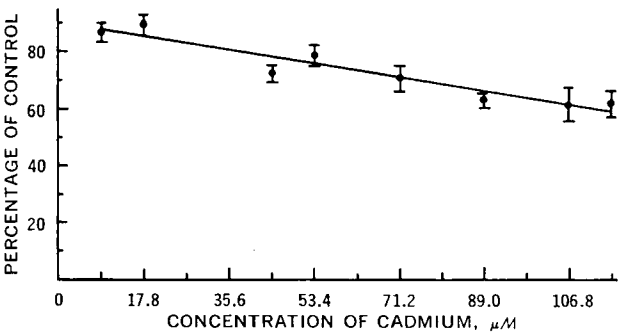

Figure 1—*Effect of cadmium on carbon dioxide production from palmitate by rat liver homogenate. Error bars represent the standard error of the mean.*

[1] International Chemical and Nuclear Corp., Irvine, Calif.
[2] Calbiochem, San Diego, Calif.
[3] Laboratory Supply Co., Indianapolis, Ind.

[4] Hydroxide of Hyamine 10-X, a 1 M solution in methanol, Packard Instrument Co., Downers Grove, Ill.

Table I—Effect of Cadmium on $^{14}CO_2$ Production

Cadmium Concentration in Flask, μM	Percentage of Control				
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average ± SE
8.9	95.24	91.25	72.81	95.83	86.41 ± 3.36
	88.57	86.25	71.05	90.28	
17.8	97.14	77.50	—[a]	97.22	89.18 ± 3.62
	91.43	78.75	—[a]	93.06	
44.5	76.19	80.00	69.30	83.33	72.39 ± 3.03
	—[a]	62.50	63.16	72.22	
53.4	87.62	71.25	62.28	83.33	78.52 ± 3.62
	86.67	73.75	—[a]	84.72	
71.2	77.14	63.75	67.54	95.83	70.65 ± 4.58
	78.10	68.75	54.39	59.72	
89.0	70.48	55.00	57.02	65.28	62.95 ± 2.62
	75.24	60.00	55.26	65.28	
106.8	80.95	62.50	47.37	77.78	61.56 ± 6.09
	—[a]	—[a]	53.51	47.22	
115.7	59.05	55.00	52.63	72.22	61.78 ± 4.59
	—[a]	—[a]	52.63	79.17	

[a] Rejected results. See text for explanation.

average percentage of total activity in the $^{14}CO_2$ for the two control flasks of each replicate; N_c was the 100% reference level for comparison, and Y was the result from a given flask relative to the control and was termed the percentage of control.

RESULTS AND DISCUSSION

The effect of cadmium on $^{14}CO_2$ production from labeled sodium palmitate is shown in Table I. For eight of the 64 flasks, the results were rejected because it was known that the filter papers were contaminated with the palmitate–bovine serum albumin solution and their activities did not represent only the collected $^{14}CO_2$. This problem was anticipated prior to the initiation of the experiments; the experimental design chosen allowed for the missing data.

An unequal subclass numbers least-squares analysis of variance (12) was run to determine differences between treatments and replicates. The difference between treatments was highly significant ($p = 0.005$), indicating that cadmium does affect carbon dioxide production from palmitate. A difference ($p = 0.005$) between replicates was probably due to day-to-day variability that was not entirely removed by expressing the results as the percentage of control. The treatment by replicate interaction was not significant even at the conservative probability level of $p = 0.25$ (15). Therefore, the values for the different doses could be pooled for calculation of the means shown in Table I.

A regression analysis was run on the means. The means rather than the individual values were used to average over the day-to-day variability and to allow for a better test on dose effects. The departures from a linear trend were not significant ($p = 0.25$), so the trend was considered linear. The equation for the straight line shown in Fig. 1 is:

$$\bar{Y} = 90.20 - 0.269X \qquad \text{(Eq. 3)}$$

where X is the concentration of cadmium ion in the flask. This equation is valid only for the range in cadmium-ion concentrations studied. It is not valid for concentrations less than 8.9 μM. The estimated linear correlation coefficient is 0.96, and the 95% confidence interval for the true correlation coefficient is $0.77 < \rho < 0.99$.

The number of concentrations studied was limited by the number of reaction flasks available on the Warburg respirometer. The highest concentration studied was dependent on the limited solubility of cadmium ion in the buffer, and the lowest concentration was chosen to give a substantial inhibitory effect. A preliminary study showed that the effect was slight and highly variable at concentrations lower than those used in this experiment. At low concentrations the inhibitory effect probably is not linear. Determining the relationship with certainty at this low range was not important enough to warrant running the large number of replicates required.

In this study, $^{14}CO_2$ was measured while the other oxidation products, the ketone bodies, were not. Ketone body formation from palmitic acid by rat liver homogenate has been reported to be strongly inhibited by arsenite (13), and the resemblance between cadmium and arsenite ions in their inhibitory actions has been cited (16). It was also reported that the production of the ketone bodies ceased 1 hr after the start of incubation (17). Their concentration in the medium then started to decrease gradually over the remaining 3 hr, while that of the $^{14}CO_2$ rose over this time. The net effect of not measuring the amounts of ketone bodies in this experiment was that the effect of cadmium on the metabolism of palmitate might have been slightly underestimated.

This *in vitro* study showed that increased cadmium concentration in the medium caused a corresponding decrease in the production of $^{14}CO_2$ resulting from the metabolic oxidation of sodium palmitate by rat liver homogenate. The inhibition was about 60% of the control at the highest cadmium concentration. This suggests that cadmium inhibits enzymes involved in the transformation of palmitic acid to acetyl coenzyme A and/or enzymes involved in the metabolism of acetyl coenzyme A to carbon dioxide.

REFERENCES

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

(2) H. A. Schroeder, A. P. Nason, I. H. Tipton, and J. J. Balassa, *J. Chronic Dis.*, **20**, 179(1967).

(3) L. Friberg, *Acta Med. Scand.*, **138**, 240(1950).

(4) L. H. Cotter and B. H. Cotter, *Arch. Ind. Hyg. Occup. Med.*, **3**, 495(1951).

(5) R. deRosa, *Boll. Soc. Ital. Biol. Sper.*, **30**, 68(1954).

(6) P. Andreuzzi and C. P. Odescalchi, *ibid.*, **34**, 1376(1958).

(7) P. Andreuzzi and C. P. Odescalchi, *Lav. Um.*, **11**, 164(1959).

(8) B. Axelsson and M. Piscator, *Arch. Environ. Health*, **12**, 360(1966).

(9) G. F. Nordberg, M. Piscator, and B. Lind, *Acta Pharmacol. Toxicol.*, **29**, 456(1971).

(10) Z. A. Shaikh and O. J. Lucis, *Arch. Environ. Health*, **24**, 419(1972).

(11) I. B. Fritz, *Physiol. Rev.*, **41**, 52(1961).

(12) B. Ostle, "Statistics in Research. Basic Concepts and Techniques for Research Workers," 2nd ed., Iowa State University Press, Ames, Iowa, 1963.

(13) J. A. Ontko, *Biochim. Biophys. Acta*, **137**, 1(1967).

(14) D. S. Goodman, *J. Amer. Chem. Soc.*, **80**, 3892(1958).

(15) H. Bozivich, T. A. Bancroft, and H. O. Hartley, *Ann. Math. Statist.*, **27**, 1017(1956).

(16) D. R. Sanadi, M. Langley, and F. White, *J. Biol. Chem.*, **234**, 183(1959).

(17) J. A. Ontko, *ibid.*, **247**, 1788 (1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 4, 1974, from the *Bionucleonics and ‡Statistics Departments, Purdue University, West Lafayette, IN 47907

Accepted for publication April 29, 1974.

[x] To whom inquiries should be directed.

COMMUNICATIONS

Particle-Size Distributions of Milled Granulations and Powders

Keyphrases □ Particle size—distributions in milled granulations and powders, theory and equations □ Granulations, milled—particle-size distributions, theory and equations □ Powders—particle-size distributions, theory and equations

To the Editor:

Steiner *et al.* (1) recently demonstrated that milling of monodisperse granulations gives rise to log-normally distributed particle sizes. Log-normal distributions are not uncommon for solid particles; crystallization often leads to log-normal distributions that are truncated from below and above, a fact that has been explained (2) on theoretical grounds. However, no explanation has been put forth on why milling produces a log-normal distribution. Such an explanation may be sought in the following simplfied theoretical model.

Let it be assumed that a sample of N particles of size x_0 is milled and that a fraction, α, of the particles is "halved" on each impact. It follows that there will be $(N)(1 - \alpha)$ particles left of the original size after one impact and $(N)(1 - \alpha)^2$ left after two impacts. After the second impact there will be $(N)(4)(\alpha)(1 - \alpha)$ of size $x_0/2$ and $(N)(4)(\alpha^2)$ of size $x_0/(2^2)$. The total number of particles after the second impact is $(N)(1 + \alpha)^2$. The number of particles of size x_0, $x_0/2$, $x_0/(2^2)$, and $x_0/(2^3)$ after zero, one, two, three, and m impacts is listed in Table I. It is seen from the bottom line of the table that the total number of particles after m impacts is $(N)[(1 + \alpha)^m]$. After m impacts the possible particle sizes are $x_0/(2^p)$, where p is an integer between zero and m. The number of particles of size $x = x_0/(2^p)$ is seen from the last column of Table I to be:

$$N\begin{bmatrix} m \\ p \end{bmatrix}[(2\alpha)^p][(1 - \alpha)^{m-p}] \qquad \text{(Eq. 1)}$$

i.e., since the number of particles is $(N)[(1 + \alpha)^m]$, the fraction having a particle size of $x = x_0/(2^p)$ is given by:

$$Pr[(x_0/2^p)] = [(1 + \alpha)^{-m}]\begin{bmatrix} m \\ p \end{bmatrix}[(2\alpha)^p][(1 - \alpha)^{m-p}] \qquad \text{(Eq. 2)}$$

The right-hand side of Eq. 2 is a normalized binomial expression which, for large values of m, approaches a normal distribution. The *sizes* of the particles, however, are log-linear; for instance, $\log(2^{p+1}) - \log(2^p) = \log 2$, regardless of the value of p. Hence, for large m, the distribution described by Eq. 2 approaches a log-normal equation.

The same type of argument holds for any situation where the particle breaks into a number of equally sized fractions. The model is, of course, greatly simplified since it assumes that an impact will always produce the same (whether two, three, or more) fractional particles, and it does not account for removal of fines. However, the model describes the main feature of milling which accounts for the log-normally distributed end-product.

(1) G. Steiner, M. Patel, and J. T. Carstensen, *J. Pharm. Sci.*, 63, 1395(1974).

(2) R. R. Irani and C. F. Callis, "Particle Size: Measurement,

Table I—Number of Particles Remaining after Number of Indicated Impacts[a]

Size	Impacts				
	0	1	2	3	m
x_0	N	$(N)(1 - \alpha)$	$(N)[(1 - \alpha)^2]$	$(N)[(1 - \alpha)^3]$	$(N)[(1 - \alpha)^m]$
$x_0/2$		$(N)(2\alpha)$	$(N)(2)(2\alpha)(1 - \alpha)^b$	$(N)(2)(2\alpha)[(1 - \alpha)^2]$	$(N)\begin{bmatrix} m \\ 1 \end{bmatrix}(2\alpha)[(1 - \alpha)^{m-1}]$
$x_0/4$			$(N)(4)(\alpha^2)$	$(N)(3)[(2\alpha)^2](1 - \alpha)$	$(N)\begin{bmatrix} m \\ 2 \end{bmatrix}[(2\alpha)^2][(1 - \alpha)^{m-2}]$
$x_0/8$				$(N)[(2\alpha)^3]$	$(N)\begin{bmatrix} m \\ 3 \end{bmatrix}[(2\alpha)^3][(1 - \alpha)^{m-3}]$
Total	N	$(N)[(1 + \alpha)]$	$(N)[(1 + \alpha)^2]$	$(N)[(1 + \alpha)^3]$	$(N)[(1 + \alpha)^m]$

[a] Initial number is N. [b] The general procedure is to note that the amount of size $x_0/2$ disappearing is $N\alpha 2\alpha$; $N2\alpha(1 - \alpha)$ remains. The amount of $x_0/2$ produced from size x_0 is twice $N\alpha(1 - \alpha)$, so the amount of size $x_0/2$ produced is $(N)(2\alpha)(1 - \alpha)$. The total is $(2)(N)[\alpha(1 - \alpha)]$.