Variability of Cadmium-109 Uptake in Rats as Affected by Route of Administration and Manner of Expressing Results

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In studies on the uptake of cadmium in certain rat tissues in our laboratory, there was a high variability in the measured cadmium content. This variability was seen even among the same type of tissues from different animals. Other investigations in our laboratory indicate that high variability is common in certain types of tracer work.

It was thought that the route of administration may affect the variability. Also, the manner in which the results are expressed, either uptake for the total organ or uptake per gram, may be important. Consequently an experiment was designed to test the effect of the route of administration, both intraperitoneal and intravenous, and the manner of expressing the results. Although cadmium was used, the findings may apply to other tracer work of a similar type.

EXPERIMENTAL

The experiment was replicated twice at times about 2 months apart. Each replicate was run with a different group of animals.

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Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 3 © 1974 by Springer-Verlag New York Inc.

For each replicate, 24 female rats[1] weighing 195 to 205 g. were assigned randomly to two groups, one containing 10 animals for intraperitoneal injection and one containing 14 animals for intravenous injection into the tail vein. The number of animals in the group for intravenous injection was greater to allow for elimination in case of a bad injection; this was not necessary. Animals in each group were then assigned randomly to two subgroups to allow for injections on 2 consecutive days since 24 animals could not be conveniently handled on a single day.

Prior to experimentation the animals were housed individually in metal cages under laboratory conditions for 1 week. They were allowed free access to food[2] and tap water.

To ensure uniformity of dosage, animals with a small range in weight were used. The dose consisted of exactly 0.25 ml. of a solution containing about 15 μCi. of ^{109}Cd and 0.0625 mg. of cadmium ion as cadmium acetate in water. The ^{109}Cd was found to be radionuclidically pure.

On day 1 of the experiment, the order of all injections was randomly assigned and all were completed within about 0.5 hr. All animals were sacrificed by decapitation with a small guillotine 12 hr. later. Sacrificing and removal of organs were completed within 1.5 hr. The liver, kidney, and spleen were removed and washed quickly with cold water to remove surface blood. This

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[1] Sprague Dawley descendants, Laboratory Supply Co., Indianapolis, IN 46241.

[2] Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL 60606.

entire procedure was repeated on day 2. A uniform time interval between dosing and sacrificing was not considered necessary since both the literature and our preliminary studies indicated that the amount of cadmium after a single dose in major organs does not change appreciably between 8 and 24 hr. after its administration.

Each organ was weighed and placed in a small polyethylene bottle. The 0.088-MeV gamma ray of ^{109}Cd was counted in a large well-type sodium iodide scintillation detector. The counting error for all samples was less than 1%. Count rates above 500,000 c.p.m. were corrected for a small amount of coincidence loss.

RESULTS AND DISCUSSION

The activity of the ^{109}Cd is expressed as counts per minute for the whole organ and as counts per minute per gram of organ. Means are given in Table 1. The differences between the means for the two replicates resulted from the use of different injection solutions.

An analysis of variance was run to assess the differences between the route of administration, intraperitoneal versus intravenous, for the different organs. A separate analysis was run for each way of expressing the results, per whole organ and per gram of organ. The model for the experimental design was a nested factorial.

The results of the analysis of variance showed that there was a significant difference ($P = .01$) between replicates. This was expected because of the use of different injection solutions. There was a significant difference ($P = .01$) between days within

TABLE 1

Cadmium content of organs

Day	C.p.m. per whole organ				C.p.m. per gram of organ			
	Replicate 1		Replicate 2		Replicate 1		Replicate 2	
	IP	IV	IP	IV	IP	IV	IP	IV
				Liver				
1	1,018,400[a] ± 26,800	931,700 ± 22,000	651,400 ± 26,800	721,600 ± 18,000	137,200 ± 14,200	134,800 ± 9,400	100,300 ± 8,400	117,600 ± 6,600
2	979,400 ± 24,300	956,300 ± 22,500	804,800 ± 25,000	807,100 ± 11,100	135,300 ± 11,900	132,900 ± 10,100	136,500 ± 8,700	120,400 ± 6,600
				Kidney				
1	38,620 ± 4699	32,495 ± 4242	21,252 ± 2134	22,633 ± 1683	23,465 ± 2652	20,509 ± 2997	14,453 ± 1887	14,620 ± 622
2	31,927 ± 2946	32,594 ± 3539	24,908 ± 1822	26,093 ± 919	19,908 ± 2930	19,837 ± 1607	17,255 ± 1603	16,919 ± 586
				Spleen				
1	2821 ± 628	2181 ± 237	2672 ± 780	2049 ± 218	5664 ± 1049	4702 ± 399	5796 ± 1456	4441 ± 487
2	2968 ± 890	2138 ± 302	3049 ± 374	1827 ± 126	6362 ± 1830	4408 ± 475	7134 ± 936	4140 ± 269

[a]Mean of five animals for intraperitoneal (seven for intravenous) injection ± standard deviation. Values for liver have been rounded off since individual counts were corrected for coincidence loss.

replicates which was not important in the statistical analysis of this study. The only significant difference ($P = .05$) between treatments, intraperitoneal versus intravenous, was found in the spleen using total weight of organ. The other treatment difference for the spleen, using the activity per gram of organ, was significant only at the $P = .10$ level. All other treatment differences were nonsignificant. It is believed that the lack of significant differences for the two routes of administration in liver and kidney resulted from the rapid absorption of cadmium after intraperitoneal injection (PERRY and ERLANGER 1971). The reason for the one significant difference for the spleen is not known. It cannot be explained by differences in blood levels of cadmium since these were measured and were essentially the same for both routes of administration.

To study variabilities, means and coefficients of variation were calculated and are shown in Table 2. The analysis that follows was done after the parameters of the nested factorial model were estimated. Thus, combining means and variances to get the estimates of the coefficients of variation was possible. Results are given for both replicates and their combined values. In addition, since the intraperitoneal versus intravenous treatment differences for liver and kidney were nonsignificant, means and coefficients of variation for the combined data were calculated. There are no combined values for spleen because the treatment difference was significant.

The coefficient of variation for liver counts recorded on a total weight basis is much less than on a per gram basis. The comparison is 2.6% versus 7.5% for the combined data. Using the

TABLE 2

Means and coefficients of variation

	Liver			Kidney			Spleen		
	Repl. 1	Repl. 2	Both	Repl. 1	Repl. 2	Both	Repl. 1	Repl. 2	Both
				Per whole organ					
IP	998.9[a] ± 2.6%	728.1 ± 3.6%	863.5 ± 3.0%	35.27 ± 11.1%	23.08 ± 8.6%	29.18 ± 10.7%	2.90 ± 26.6%	2.86 ± 21.3%	2.88 ± 24.3%
IV	944.0 ± 2.4%	764.4 ± 2.0%	854.2 ± 2.2%	32.54 ± 12.0%	24.36 ± 5.6%	28.45 ± 10.3%	2.16 ± 13.0%	1.94 ± 9.3%	2.05 ± 11.7%
Combined	966.9[b] ± 2.4%	749.3 ± 2.7%	858.1 ± 2.6%	33.68 ± 11.6%	23.83 ± 7.1%	28.75 ± 10.4%			
				Per gram of organ					
IP	136.2 ± 9.6%	118.4 ± 7.3%	127.3 ± 8.7%	21.69 ± 12.9%	15.85 ± 11.0%	18.77 ± 12.4%	6.01 ± 24.1%	6.47 ± 18.9%	6.29 ± 21.6%
IV	133.8 ± 7.2%	119.0 ± 5.5%	126.4 ± 6.6%	20.17 ± 11.9%	15.77 ± 3.8%	17.97 ± 9.7%	4.56 ± 9.9%	4.29 ± 9.1%	4.42 ± 9.5%
Combined	134.8 ± 8.5%	118.8 ± 6.5%	126.8 ± 7.5%	20.80 ± 12.5%	15.80 ± 7.6%	18.30 ± 10.9%			

[a] Means have been divided by 1000.

[b] Combined means were calculated using the five animals for intraperitoneal and seven animals for intravenous injection; they therefore do not equal the average of the IP and IV means.

jackknife technique (ARVESEN 1972), this observed difference was found to be significant at the P = .01 level. No significant differences were found for kidney or spleen. This result implies that under the experimental conditions, and contrary to what one might predict, cadmium uptake is not a function of liver size. This pattern of uptake may result when an organ takes up a high percentage of the dose, as was the case for liver. The uptake may then be a constant and nearly independent of organ size.

The spleen count data gave a much higher coefficient of variation for intraperitoneal injection than for intravenous injection, on both a total organ basis and a per gram of organ basis (P = .01). Although the differences for liver and kidney were not significant, there appears to be a trend toward more variability for the intraperitoneal injection. The reason for the difference for the spleen is not apparent.

CONCLUSIONS

Under the conditions of this experiment, variability in the cadmium content of liver and kidney, as measured by the coefficient of variation, was the same for both intraperitoneal and intravenous injection. For spleen, intravenous injection gave less variability.

For liver, where cadmium uptake was large, variability was less when uptake for the whole organ rather than per gram of organ was used. For kidney and spleen, there was no difference in variability for the two ways of expressing the results.

ACKNOWLEDGMENT

This research was supported in part by the Purdue Research

Foundation.

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