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Apparent Ornithine Decarboxylase Activity, Measured by $^{14}CO_2$ Trapping, after Frozen Storage of Rat Tissue and Rat Tissue Supernatants

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Ornithine decarboxylase (ODC) activity of rat tissues was measured by the standard $^{14}CO_2$ trapping method after frozen storage (−60 or −70°C) of the tissues or their 105,000*g* supernatants. True ODC activity was determined by two methods: (a) addition of the inhibitors α-difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC, or aminooxyacetate (AOA), an inhibitor that blocks the decarboxylation of ornithine by mitochondrial enzymes; and (b) chromatographic analysis of the reaction products. In the frozen supernatants of liver and spleen, ODC activity changed only slightly after 1 day but increased 29 and 14%, respectively, by 30 days; activity in kidney supernatant decreased 17% after 1 day and remained near that level at 30 days. Kidney and spleen ODC activity was inhibited 90–100% by DFMO, but apparent liver ODC activity was inhibited only 60–75%. In the supernatant prepared from tissue stored frozen for 1 day, apparent ODC activity in liver increased 500% over that activity in the freshly prepared supernatant; at 23 days, apparent activity increased 755% for liver and 121% for kidney. After 23 days, DFMO did not inhibit apparent ODC activity in supernatants from frozen liver and inhibited ODC in frozen kidney by only 49%. With AOA, the ODC activities of the fresh and frozen supernatants were similar, indicating that the large increase in apparent ODC activity in frozen tissue was due to artifacts from the metabolism of ornithine via the mitochondrial pathway. HPLC analysis of the reaction products resulting from the incubation of uniformly labeled [^{14}C]ornithine with the fresh and frozen preparations indicated no increase in putrescine with the frozen preparation. Thus frozen storage of the 105,000*g* supernatants of these tissues for short periods produces little change in ODC activity, but freezing and thawing whole tissue, at least in regard to rat liver and kidney, produces large artifactual increases, as measured by the commonly accepted $^{14}CO_2$ method.

KEY WORDS: ornithine decarboxylase; difluoromethylornithine; frozen storage; enzyme preparation.

Polyamines are organic cations present in all types of living cells, ranging from bacteria through mammals; they play important regulatory roles in cell growth (1). One area of polyamine research that has received a great deal of interest is ornithine decarboxylase (ODC),[2] the enzyme which catalyzes the first reaction in the biosynthetic pathway of polyamines to yield the diamine putrescine.

The most widely accepted technique for assay of ODC has been the procedure originated by Russell and Snyder (2), in which carboxyl-labeled radioactive ornithine is used as a substrate and the evolved $^{14}CO_2$ is trapped as an indicator of ODC activity. The method of preparation of the tissue extracts used for the assay varies considerably among researchers. Rozhin *et al.* (3) centrifuged the homogenates and froze the supernatants at −80°C until assay. Others (4–7) froze whole tissue; at the time of assay, the tissue was homogenized and centrifuged, and the supernatant was removed and analyzed. Similar procedures have been used for tumors (8,9).

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[2] Abbreviations used: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; AOA, aminooxyacetate; DTT, DL-dithiothreitol; PLP, pyridoxal 5-phosphate; PMSF, phenyl methyl sulfonyl fluoride; GABA, 4-aminobutyric acid.

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Lima and Shiu (10) made a suspension of human breast cancer cells, stored the suspension at −70°C, and, on the day of assay, sonicated the suspensions, centrifuged the cell lysates, and measured ODC activity in the supernatants.

Since preliminary evidence indicated that the use of freezing and thawing produced tissue preparations with unusually high ODC activities, we evaluated the effect of different freezing regimens on the measurement of apparent ODC activity, determined by $^{14}CO_2$ counting, in several rat tissues. We also investigated the effect of storing tissue supernatant preparations under ultra-cold freezing conditions on ODC activity. Concentration of EDTA and both heating and dilution of preparation were also examined for their roles in the measurement of ODC. True ODC activity was evaluated by two methods: (a) by the use of α-difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC, and aminooxyacetate (AOA), an inhibitor that blocks the action of a mitochondrial aminotransferase and thereby prevents release of $^{14}CO_2$ from [1-^{14}C]ornithine by a secondary pathway; and (b) by chromatographic measurement of putrescine formed by the reaction. The results of representative experiments are presented.

MATERIALS AND METHODS

Animals. Male Osborne–Mendel (FDA strain) rats, 3–4 months old (365–475 g), were used and were housed individually in stainless steel cages. Room temperature (22–25°C), relative humidity (approximately 50%), and lighting (12 h light, 12 h dark) were automatically controlled. Diets (Purina Chow pellets) and tap water were provided *ad libitum.*

Materials. Sucrose, enzyme grade, was purchased from Schwarz/Mann, (Orangeburg, NY). DL-Dithiothreitol (DTT), DL-ornithine, pyridoxal 5-phosphate (PLP), AOA, pargyline, aminoguanidine, and phenyl methyl sulfonyl fluoride (PMSF) were obtained from Sigma Chemical Co. (St. Louis, MO). DL-[1-^{14}C]Ornithine (47.2 mCi/mmol, used only in the initial two experiments), L-[1-^{14}C]ornithine (54.3 mCi/mmol), and L-[^{14}C (U)]ornithine (291.0 mCi/mmol) (used in experiments to characterize products of metabolic reaction) were purchased from New England Nuclear (Boston, MA). DFMO and γ-acetylenic GABA were obtained from Merrell Dow Research Institute (Cincinnati, OH). All other chemicals were of the highest commercial grade available.

Tissue preparation. Rats were decapitated and exsanguinated. Tissues were prepared for enzyme assay in one of two ways. In the first procedure, the kidneys, spleens, and random portions of the livers from seven rats were quickly removed, pooled according to organ, and placed on ice. A 25% homogenate of each pool was prepared and centrifuged as described below, and the resulting supernatants were divided into four portions. Three of the portions from each organ were immediately frozen in liquid N_2 and stored at −60°C. The remaining portion from each organ was used for measuring ODC activity that same day (0 day) as described below. On Days 1, 7, and 30 the frozen portions were thawed and analyzed for ODC activity. In the second procedure, a whole liver was quickly removed, placed on ice, and divided into three portions. Two of the three pieces were frozen in liquid N_2 and stored at −60°C. The unfrozen portion was assayed for enzyme activity that day (0 day), and the frozen portions were thawed and assayed on Days 1 and 23. In addition, whole kidneys from two rats were removed and sliced longitudinally into approximate halves which were distributed into two separate pools. The pools were treated as above for whole liver except that ODC activity was determined only at 0 and 23 days.

A third experiment was conducted to test the effect of various freezing conditions on rat liver and kidney. A single liver was divided into approximately five equal portions, and the portions were distributed into five ice-chilled beakers. One piece was frozen in liquid N_2 (approximately −196°C), a second piece was frozen with dry ice (approximately

−78.5°C), and the third and fourth pieces were stored at −70 and −20°C, respectively. Kidneys from five rats were sectioned as above and distributed into four separate and approximately equal pools. The pools were treated as above for the liver portions with the exception of freezing at −70°C. Storage of both liver and kidney tissues at −70°C was for 7 days. Unfrozen portions of liver and kidney were assayed fresh at 0 day.

To determine whether the effect of freezing tissue with liquid N_2 on ODC activity might be due to an increase of nonenzymatic activity or to the release of metal ions catalyzing a nonspecific decarboxylation reaction, the liver and kidneys from six rats were prepared as described in the previous experiment. In this case distribution of sectioned tissue for each of the two organs was among four pools. Two pools for each of the two organs were homogenized in the standard fashion (see next section) by using 0.1 mM EDTA in the homogenization buffer for one and 1.0 mM EDTA in the homogenization buffer for the other. A portion of the tissue supernatants from the 0.1 mM EDTA preparation was boiled for 5 min before enzyme analysis. The remaining two pools of tissue for each of the organs were frozen in liquid N_2, stored for 1 week at −70°C, and analyzed for ODC activity as described for the fresh tissue in this experiment.

To determine whether dilution might reduce the spurious activity, a fivefold dilution was made of aliquots of both the original fresh kidney homogenate and the homogenate made from kidney frozen in liquid N_2 and stored at −70°C for 1 week. A final experiment, using pooled liver lobes from four rats, was carried out to test the effect of two additional metabolic inhibitors on possible spurious (non-ODC) activity generated as a result of the freeze–thaw process. γ-Acetylenic GABA at a final concentration of 0.1 mM was used (6) to minimize generation of $^{14}CO_2$ by inhibiting decarboxylation of glutamate (11) possibly formed from ornithine (12,13), and a mixture of pargyline (10 μM) and aminoguanidine (50 μM) was employed separately to reduce accumulation of H_2O_2 reportedly capable of inducing nonenzymatic decarboxylation (14). For this experiment the tissue was homogenized in buffer containing 1.26 μM PMSF, a protease inhibitor (15). In the same experiment the effect of preincubating (15 min at 37°C) the fresh and frozen-thawed tissue supernatants in the presence and absence of all the inhibitors tested was determined. All preparations were analyzed for ODC activity.

ODC assay. The assay for ODC was a modification of the methods of Ono *et al.* (16) and Danzin *et al.* (17). Briefly, tissues were homogenized in 3 vol of 30 mM sodium phosphate buffer (pH 7.1) containing 0.25 mM sucrose, 0.1 mM EDTA, 0.1 mM PLP, and 5.0 mM DTT. Except where indicated, the homogenization procedure was carried out by using a motor-driven Teflon-lined pestle and a glass vessel. The homogenate was centrifuged at 105,000*g* for 60 min at 2°C and the supernatant was used for the assay. The assay medium contained 400 μl of tissue supernatant, 400 μl of incubating buffer (30 mM sodium phosphate, pH 7.1, 0.1 mM PLP, and 6.3 mM DTT), and 200 μl of substrate (10.35 mM DL-ornithine and 1.0 μCi DL-[1-^{14}C]ornithine or L-[1-^{14}C]ornithine). In experiments in which the reaction products were chromatographed, the assay medium contained 200 μl of kidney tissue supernatant, 200 μl of incubating buffer, and 100 μl of substrate (41.4 mM DL-ornithine and 1.5 μCi L-[^{14}C(U)]ornithine) to facilitate detection of the reaction products. In some cases DFMO or AOA was added in assay buffer (as were the aforementioned inhibitors) to give final concentrations of 5 and 0.01 mM or 0.02 mM, respectively. Reactions were carried out in 25-ml Erlenmeyer flasks equipped with rubber stoppers supporting polypropylene center wells (Kontes Scientific Glassware, Vineland, NJ). The flasks were incubated at 37°C for 60 min, and the reaction was terminated by placing the flasks in ice water and adding to each 1.0 ml of either 2 M citric acid or 0.67 N HCl. NCS (Amersham Corp., Arlington Heights, IL) was added (0.2 ml) to each center

well and the flasks were incubated for an additional 60 min at 25°C to collect the liberated $^{14}CO_2$. Center wells were removed and placed in vials containing either 15 ml of toluene-based Permafluor I (98% 2,5-diphenyloxazole, 2% dimethyl 1,4-bis(5-phenyloxazol-2-yl)benzene; Packard Instrument Co., Inc., Downers Grove, IL) or OCS (Amersham Corp.). Triplicate determinations were performed and blanks containing homogenizing buffer in place of the supernatant were included. Protein content of the enzyme preparation was determined by either the biuret method (18) or a modified Lowry procedure (19). Results were expressed as picomoles CO_2 per hour per milligram protein. Preliminary analysis indicated activity to be approximately proportional to protein concentration and linear with time under conditions of the assay.

Chromatographic analysis. [^{14}C]Putrescine was assayed by HPLC according to the method of Seiler and Knödgen (20). The HPLC column was a Beckman (Fullerton, CA) ODS-IP (4.6 × 250 mm, 5-μm particle size). Radioactivity was monitored using a Flo-one β-detector (Radiomatic Instrument Co., Tampa, FL).

Statistical evaluation. The difference between means was analyzed statistically by Student's t test. A P value of 0.05 or less was accepted as an indication of statistical significance.

RESULTS AND DISCUSSION

Effect of Inhibitors

DFMO is an enzyme-activated irreversible inhibitor of ODC (21). Table 1 shows activity of ODC in liver, kidney, and spleen of the fresh and frozen stored supernatants with and without DFMO. Compared with values at 0 time, ODC activity for liver and spleen changed only slightly after 1 day but increased 25 and 14%, respectively, by 30 days; activity for kidney decreased 17% after 1 day and remained near that level at the end of 30 days. DFMO inhibited kidney and spleen ODC activity by 90–100%, while liver activity was inhibited only 60–75%.

Table 2 shows ODC activity in supernatants prepared from frozen whole tissue. After 1 day, apparent activity in liver increased 500%; at 23 days this apparent activity increased 755% for liver and 121% for kidney. In similarly conducted experiments with intact liver tissue stored frozen from 1 to 4 weeks, the percentage increase in apparent activity ranged from 114 to 11,496, respectively (data not shown). After 23 days, DFMO did not inhibit activity in liver and inhibited ODC in kidney by only 49%.

Summarized data in Table 3 demonstrate the dependency of changes in ODC activity of liver and kidney on freezing conditions. Increases were seen at all freezing temperatures, with the greatest increase seen with liquid N_2 for both tissues and the least increase with dry ice for liver and at −20°C for kidney.

Earlier studies (22) demonstrated that 0.01 mM AOA depresses mitochondrial ornithine-2-oxo acid aminotransferase activity without seriously affecting the measurement of ODC. It is apparent that ODC activities determined in the presence of this concentration of AOA increased very little after freezing at all tested temperatures, while all activities measured in the presence of DFMO, the specific inhibitor of ODC, increased, accounting to a large extent for the increases in apparent ODC activity in the absence of the inhibitors. However, since the combination of inhibitors prevented all activity in the fresh preparation but not in the frozen–thawed preparations, some evidence is presented for the appearance or increase in the latter preparations of a nonenzymatic mode of decarboxylation. This interpretation, however, may be erroneous as indicated by the results obtained for boiled preparations in Table 4. Boiled supernatant preparations of both liver and kidney with and without DFMO showed no increase in apparent ODC activity as a result of freezing the whole tissue in liquid N_2. Incidentally, the incomplete elimination of apparent ODC activity after the fresh liver supernatant was boiled (unlike the results for kidney) was an effect observed repeatedly in this laboratory and probably reflects the generation during

TABLE 1

EFFECT OF FROZEN STORAGE (−60°C) ON ODC ACTIVITY OF 105,000*g* TISSUE SUPERNATANTS[a]

Tissue	Time (days)			
	0 (Fresh)	1	7	30
Liver				
−DFMO	45.7 ± 0.6	42.4 ± 1.0[b]	59.5 ± 0.3[b]	57.2 ± 2.1[b]
+DFMO	10.9 ± 0.9	10.6 ± 0.2	22.3 ± 1.8	23.0 ± 1.6
Kidney				
−DFMO	582 ± 5.5	481 ± 8.0[b]	450 ± 40.0[b]	474 ± 8.6[b]
+DFMO	22.5 ± 0.5	19.2 ± 1.4	40.1 ± 4.0	45.7 ± 5.0
Spleen				
−DFMO	29.2 ± 0.7	32.6 ± 0.4[b]	63.1 ± 3.4[b]	33.3 ± 1.1[b]
+DFMO	0.0 ± 0.0	0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] Values are given as picomoles CO_2 per hour per milligram protein and are means ± SE of three replicates.
[b] $P \leq 0.05$ compared with 0 time (−DFMO).

boiling of a chemical species that acts as a weak catalyst for the decarboxylation reaction. These results reaffirm the admonition of Slotkin and Bartolome (23) and demonstrate the inadvisability of using boiled preparations of tissues as "blanks" for estimation of ODC activity (24).

The inability of 1.0 mM EDTA to appreciably diminish the apparent increase in ODC activity following freezing in liquid N_2, especially in liver, argues against a metal ion-catalyzed reaction being responsible for this phenomenon. In the case of kidney, the increase in activity in the presence of DFMO (non-ODC decarboxylation) was somewhat diminished by the higher concentration of EDTA, but the increase was still appreciable and statistically significant.

Another observation that can be made regarding the data in Table 4 is that DFMO consistently reduced the activity of the boiled preparation. This is not terribly surprising since we likewise repeatedly observed that DFMO decreased to a small extent the nonenzymatic decarboxylation in the "no-tissue" blanks also referred to by Slotkin and Bartolome (23).

The diluted kidney preparation does appear to prevent an increase in nonspecific activity under frozen (liquid N_2) conditions—in the presence of DFMO the activity was 0.0 ± 0.0 in fresh or frozen tissue. However, in the absence of DFMO, ODC activity in the fresh preparation apparently decreased 18% compared to the activity of the frozen preparation (2733 ± 25.5 vs 3330 ± 11.0). Although this procedure appeared to dilute out some "factor(s)" responsible for nonspecific activity, such an approach for measurement of ODC in tissues with inherently low levels

TABLE 2

EFFECT OF FROZEN STORAGE (−60°C) ON APPARENT ODC ACTIVITY OF WHOLE TISSUE[a]

Tissue	Time (days)		
	0 (Fresh)	1	23
Liver			
−DFMO	86.3 ± 11.6	518 ± 13.3[b]	737 ± 50.0[b]
+DFMO	12.4 ± 0.5[c]	529 ± 3.8[d]	840 ± 26.2[d]
Kidney			
−DFMO	792 ± 12.8	n.d.[e]	1,750 ± 19.6[b]
+DFMO	36.6 ± 1.1[c]	n.d.	885 ± 47.6[d]

[a] Values are given as picomoles CO_2 per hour per milligram protein and are means ± SE of three replicates.
[b] $P \leq 0.05$ compared with 0 time (−DFMO).
[c] $P \leq 0.05$ compared with −DFMO (0 time).
[d] $P \leq 0.05$ compared with 0 time (+DFMO).
[e] n.d., Not determined.

TABLE 3

EFFECT OF FREEZING TEMPERATURES ON APPARENT ODC ACTIVITY OF LIVER AND KIDNEY AFTER STORAGE AT −70°C FOR 7 DAYS[a]

Tissue	Fresh	−20°C[b]	−70°C[b]	−78°C[b] (dry ice)	−195°C[b] (liquid N_2)
Liver					
−Inhibitor	173 ± 1.2	474 ± 3.5	431 ± 8.3	337 ± 3.5	737 ± 39.9
+DFMO	30 ± 2.7	412 ± 3.4	354 ± 2.6	225 ± 5.2	658 ± 19.0
+AOA	115 ± 2.0	187 ± 2.0	168 ± 2.0	183 ± 6.1	170 ± 10.4
+DFMO & AOA	0 ± 0.0	68 ± 2.9	53 ± 6.7	46 ± 5.5	77 ± 11.9
Kidney					
−Inhibitor	2987 ± 40.4	3775 ± 8.7	n.d.[c]	4155 ± 27.9	5780 ± 59.3
+DFMO	218 ± 3.5	1273 ± 23.3	n.d.	1417 ± 13.6	1873 ± 19.5
+AOA	2572 ± 48.6	2079 ± 29.1	n.d.	2197 ± 17.3	2895 ± 30.6
+DFMO & AOA	0 ± 0.0	202 ± 9.9	n.d.	274 ± 0.5	371 ± 21.1

[a] Values are given as picomoles CO_2 per hour per milligram protein and are means ± SE of three replicates.
[b] For every value in this column, $P \leq 0.05$ compared with fresh (nonfrozen preparation).
[c] n.d., Not determined.

of enzyme (e.g., liver and pancreas) would be impractical under typical assay conditions.

Brief preincubation (prior to addition of labeled substrate) lowers to a small degree the activity of the fresh preparation but reduces strikingly the activity of the frozen–thawed tissue preparation (Table 5). Since there was only a very small effect of preincubation in the case of the AOA-treated samples relative to the nontreated or DFMO-treated samples, we can infer that the preponderance of activity lost during the preincubation period was probably of the nonspecific or mitochondrial type. Further evidence that the huge increase in apparent ODC activity following freezing and thawing was of a nonspecific nature was seen in the negative effect of the two antioxidants, aminoguanidine and pargyline. The initial absence of effect of γ-acetylenic GABA, an enzyme-activated irreversible inhibitor of glutamic acid decarboxylase (11), on the dramatic induction of $^{14}CO_2$ production from the labeled ornithine by the frozen–thawed preparation of liver would tend to eliminate decarboxylation of ^{14}C-glutamate as a significant source of this increased activity. Preincubation, however, did slightly increase the inhibitory action of this compound as was also the case for the antioxidants. It can be inferred from these results that much of the increase in $^{14}CO_2$ production in tissue preparations may be, as discussed by Murphy and Brosnan (22), the result of the conversion

TABLE 4

EFFECT OF EDTA CONCENTRATION AND BOILING ON APPARENT ODC ACTIVITY OF FRESH AND FROZEN (LIQUID N_2) LIVER AND KIDNEY AFTER STORAGE AT −70°C FOR 7 DAYS[a]

Tissue	Boiled	Fresh	Frozen (liquid N_2)
Liver			
0.1 mM EDTA	−	131 ± 1.5	547 ± 17.2[b]
+DFMO	−	75 ± 0.6	541 ± 10.8[b]
0.1 mM EDTA	+	312 ± 4.4	234 ± 13.9[b]
+ DFMO	+	233 ± 11.2	214 ± 1.0
1.0 mM EDTA	−	99 ± 0.7	362 ± 7.9[b]
+DFMO	−	24 ± 0.7	406 ± 36.0[b]
Kidney			
0.1 mM EDTA	−	1517 ± 56.0	1778 ± 14.8[b]
+DFMO	−	214 ± 4.9	507 ± 3.0[b]
0.1 mM EDTA	+	245 ± 5.7	188 ± 0.3[b]
+DFMO	+	188 ± 1.8	160 ± 21.7
1.0 mM EDTA	−	1552 ± 4.2	1338 ± 5.5[b]
+DFMO	−	188 ± 3.8	334 ± 3.4[b]

[a] Values are given as picomoles CO_2 per hour per milligram protein and are means ± SE of three replicates.
[b] $P \leq 0.05$ compared with fresh (nonfrozen preparation).

TABLE 5

EFFECT OF PREINCUBATION OF INHIBITORS ON APPARENT ODC ACTIVITY WITH TISSUE SUPERNATANT FROM FRESH AND FROZEN (LIQUID N_2) LIVER AFTER STORAGE AT −70°C FOR 7 DAYS[a]

Treatment	Preincubated (37°C, 15 min)	Fresh	Frozen
−Inhibitor	−	119 ± 6.5	2384 ± 32.0
	+	86 ± 0.6[b]	714 ± 11.8[b]
+DFMO	−	47 ± 2.0[c]	2231 ± 23.2[c]
	+	23 ± 0.2[b,c]	506 ± 33.0[b,c]
+0.01 mM AOA	−	74 ± 2.7[c]	432 ± 9.3[c]
	+	64 ± 0.2[b,c]	303 ± 18.5[b,c]
+0.02 mM AOA	−	63 ± 0.7[c]	267 ± 5.3[c]
	+	54 ± 0.9[b,c]	207 ± 3.6[b,c]
+Aminoguanidine & Pargyline	−	123 ± 7.1	2344 ± 22.8
	+	78 ± 0.3[b,c]	610 ± 16.9[b,c]
+γ-Acetylenic GABA	−	134 ± 2.3	2324 ± 30.4
	+	77 ± 0.7[b,c]	548 ± 3.5[b,c]

[a] Values are given as picomoles CO_2 per hour per milligram protein and are means ± SE of three or four replicates.
[b] $P \leq 0.05$ compared with no preincubation.
[c] $P \leq 0.05$ compared with no treatment (−inhibitor).

of the L-[1-^{14}C]ornithine to glutamate via ornithine-2-oxo acid aminotransferase with the subsequent production of $^{14}CO_2$ formed by the sequential action of 1-pyrroline-5-carboxylate dehydrogenase, either glutamate dehydrogenase or aspartate aminotransferase, and 2-oxogluterate dehydrogenase. Of course only isolation and quantitation of the radiolabeled products can establish this as the pathway.

Chromatographic Analysis

To obtain additional evidence that the sizable increase in enzyme activity from the frozen preparation was not due to ODC, a direct method (20) was used for the detection of ODC, based upon HPLC detection and quantitation of the [^{14}C]putrescine produced. Putrescine is the product formed from the decarboxylation of ornithine by ODC. If, indeed, the enzyme activity increased greatly, a concomitant increase in product would be expected.

Table 6 presents a comparison of apparent ODC activity from the commonly accepted $^{14}CO_2$ trapping method with the direct measurement of [^{14}C]putrescine from the same reaction flasks. Relatively good agreement is obtained in the measurement of ODC activity of fresh tissue by both methods. When $^{14}CO_2$ is measured in fresh and frozen kidney tissue without DFMO, there appears to be an increase of 19% in ODC activity in the frozen tissue. The measurement of [^{14}C]putrescine in the media from the same flasks not only fails to reflect this increase in apparent ODC activity but also demonstrates a 20% loss of putrescine. That this activity as measured by $^{14}CO_2$ generation is ostensible rather than actual is confirmed by the results showing an 800% increase in activity of the frozen tissue in the presence of DFMO relative to that of the fresh tissue also incubated with DFMO. These results confirm preliminary analyses carried out using an ion exchange paper chromatographic technique (25) (data not shown).

Other investigators (26–28) have also measured ODC activity based on the detection of putrescine instead of the more commonly used method of trapping $^{14}CO_2$. One group (29,30) acknowledged the release of nonspecific $^{14}CO_2$ in the determination of plant

TABLE 6

COMPARISON OF APPARENT ODC ACTIVITY FROM $^{14}CO_2$ WITH HPLC ANALYTICAL RESULTS FOR [^{14}C]PUTRESCINE FROM FRESH AND FROZEN KIDNEY[a]

	pmol CO_2/h/mg protein[b]		pmol putrescine/h/mg protein[b]	
Treatment	Fresh	Frozen	Fresh	Frozen
−DFMO	2665 ± 16.0	3178 ± 37.5[c]	3013 ± 237.7	2400 ± 145.1[d]
+DFMO	150 ± 2.3	1355 ± 60.5[c]	104 ± 104	294 ± 52.0

[a] Seven days at −70°C.
[b] Values are means + SE of two or three replicates.
[c] $P \leq 0.05$ compared with fresh (nonfrozen preparation).
[d] $P \leq 0.1$ compared with fresh (nonfrozen preparation).

ODC and suggested verification of the standard $^{14}CO_2$ trapping method by assessing labeled putrescine as demonstrated above.

DFMO is a valuable tool for validating the ODC assay. In Table 2, subtraction of the 23-day activity of the DFMO kidney preparation from the 23-day activity of the same preparation without DFMO leaves approximately the same amount of ODC activity present in the fresh (0 day) preparation. Without the use of DFMO, the assumption would be made that the kidney stored frozen for 23 days contained 2.3 times the activity that was measured in the freshly made preparation. Recently, researchers (23,31) have begun correcting ODC values by subtracting values for blanks that contain the appropriate supernatant incubated with DFMO. However, in tissues such as liver that have inherently low baseline ODC values (32), ODC measurements with DFMO of frozen–thawed preparations would at best be imprecise because of the relatively high levels of nonspecific activity apparently present.

Many cell culture workers (33–40) lyse cells by freeze–thawing up to three times before centrifugation and ODC determination. Any effect this technique might have on the release of nonspecific decarboxylases and their subsequent measurement by the $^{14}CO_2$ trapping method has not been investigated.

Because the mitochondria contain a number of decarboxylases and transaminases, any cell-disrupting or homogenizing technique that produces severe damage to mitochondrial membranes should be carefully evaluated for use in enzyme preparation. Our laboratory, in developing methods for measurement of ODC in different tissues, found that use of the Polytron (Brinkmann Instruments, Westbury, NY) in homogenizing rat liver for longer than 10 s at maximum speed also resulted in abnormally high values for ODC activity. However, this did not appear to be true for rat kidney; in fact, this method of homogenization was used in the third experiment described above.

Thus it is important, when cell-disrupting or homogenizing techniques are used to prepare tissue for ODC assays, to confirm the specificity of the assay by means such as those described here. In agreement it is also recommended that a simple and prudent method for assaying tissue preparations for ODC under such conditions include 0.01 mM AOA in the incubation media, possibly with a preincubation step. This method is especially useful in assaying materials of low activity such as liver preparations in which DFMO may inhibit only a small portion of the total activity, leading to difficulties in statistical interpretation of the data.

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