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# EVALUATION OF RADIORESPIROMETRY FOR THE DETERMINATION OF MONOAMINE OXIDASE ACTIVITY *IN VIVO* UTILIZING [$^{11}$C]OCTYLAMINE AS A SUBSTRATE*

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**Abstract**—The inhibition of MAO *in vivo* by pargyline in mice has been studied to evaluate the usefulness of *n*-octylamine · HCl[1-$^{11}$C], a substrate of MAO that is rapidly metabolized to $^{11}CO_2$, as a probe of MAO activity *in vivo*. In pargyline-pretreated mice, the rate of decline in tissue levels of [$^{11}$C]*n*-octylamine after intravenous administration was decreased to an extent that varied with the particular tissue and the time after pargyline pretreatment. Likewise, pargyline-treated mice showed a reduced excretion of $^{11}CO_2$ after [$^{11}$C]*n*-octylamine administration but not after octanoic acid[1-$^{11}$C] or octanol[1-$^{11}$C] injection. The duration of inhibition of $^{11}CO_2$ excretion after pargyline treatment paralleled reasonably well the inhibition of MAO measured directly in intestine and liver, but the inhibition of MAO in the lung, kidney and brain persisted longer. Direct measurement of mitochondrial MAO activity using [$^{14}$C]octylamine showed the following: intestine > liver > lung > brain ≫ kidney. At 94 hr after pargyline administration, $^{11}CO_2$ excretion was close to the control value yet the MAO activity of brain, lung and kidney was less than half of the control value. Thus, the rate-determining step in the overall rate of $^{11}CO_2$ excretion appears not to be the MAO-catalyzed step but the reactions occurring after deamination.

Previous studies [1–4] have suggested the potential utility of suitably labeled aliphatic amines as monoamine oxidase (MAO) [monoamine: $O_2$ oxidoreductase (deaminating); EC 1.4.3.4] substrates *in vivo*. Unlike the biogenic amines, whose metabolites are usually excreted via the kidney, aliphatic amines isotopically labeled in the α-carbon position undergo initial metabolism by MAO to the corresponding aldehyde, then conversion to the labeled carboxylic acid via aldehyde dehydrogenase and, ultimately, oxidation to labeled $CO_2$ via the various β-oxidation reactions [5].

Carbon-11 is a short-lived ($t_{1/2}$ = 20.4 min) isotope of carbon which decays by positron emission, resulting in the production of two 511-keV annihilation photons. The distribution of radioactivity *in vivo* after the injection of a carbon-11-labeled tracer can be externally visualized and the relative change in the tissue radioactivity determined as these photons traverse the body barrier. Previous work [2] had shown that *n*-octylamine · HCl[1-$^{11}$C] ([$^{11}$C]OA) was a suitable substrate *in vivo* for MAO in mice showing both a high tissue uptake and a rapid metabolism to $^{11}CO_2$. Subsequent studies in rabbits [3], dogs and humans [4] have demonstrated, non-invasively, the dynamic process *in vivo* of amine uptake and metabolism by lung and other tissues utilizing [$^{11}$C]octylamine. However, the correlation between the exhalation of labeled $CO_2$ and the MAO activity of the intact organism has not been adequately characterized. We have studied the MAO initiated metabolism of *n*-octylamine · HCl[1-$^{11}$C] to $^{11}CO_2$ in some detail to determine the extent to which $^{11}CO_2$ excretion can be correlated with MAO activity in several major organs of the mouse after a single dose of the irreversible MAO inhibitor, pargyline.

## MATERIALS AND METHODS

*n*-Octylamine · HCl[1-$^{11}$C] was synthesized as detailed previously [2], dissolved in 0.9% saline and injected intravenously into a lateral tail vein of 8-week-old male Swiss albino mice (BNL strain) which had been pretreated by the intraperitoneal injection of 10 or 32 mg/kg of pargyline or saline. The expiration of $^{11}CO_2$ was monitored [2] for 20 min, the mice were sacrificed by cervical fracture, and the lungs, liver, brain, kidneys and small intestine were removed, blotted to minimize adhering blood, weighed and counted for radioactivity in an automated NaI well-counter and the data corrected for isotopic decay [2].

Portions of the above organs were extracted with 5–10 vol. methanol–chloroform (2:1) in a ground-glass homogenizer, and the insoluble material was filtered onto glass wool. The soluble fraction (extraction efficiencies were 61–99 per cent of the activity in the intact tissue) was evaporated to dryness under reduced pressure and the residue dissolved in 2–3 ml of 0.05 M phosphate buffer, pH 6.0, and applied to a 1 × 3 cm BioRex 70 column ($Na^+$ form). The deaminated metabolites were separated from the unchanged amine by washing with 75 ml water, and

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aliquots of this fraction containing the deaminated metabolites were counted for radioactivity [2]. The amine fraction remaining on the resin was also counted for radioactivity. Overall recoveries after the initial extraction through the separation procedures were 80–97 per cent.

Mitochondria were prepared from lung, liver, brain, kidney and small intestine. Organs were minced finely and homogenized in 3–5 vol. of 0.25 M sucrose–0.1 M potassium phosphate buffer, pH 7.4, at 0° in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 600 *g* twice, the pellets were discarded, and the supernatant was centrifuged at 12,100 *g* at 4° for 10 min. The mitochondrial pellet was resuspended in sucrose–phosphate buffer and re-centrifuged as above. The pellet was finally suspended in 0.05 M phosphate buffer, pH 7.4, and stored frozen at −20°. MAO activity was stable for several weeks using this procedure.

MAO activity was determined by an adaptation of an earlier method [6] by incubating an aliquot of the mitochondrial fraction from each tissue (120–310 μg protein) with 86.7 μM *n*-octylamine · HCl[1-$^{14}$C] (10 mCi/m-mole, synthesized in our laboratory) in a total volume of 300 μl of 0.1 M potassium phosphate buffer at 37° for 25 min. Reactions were linear with regard to protein concentration and time under these conditions. Reactions were stopped by the addition of 200 μl of 0.25 M zinc sulfate followed by 200 μl of 0.25 M barium hydroxide solution. Distilled water (1 ml) was added, and the mixture was agitated well on a vortex shaker and centrifuged at 1000 *g* for 5 min to remove the precipitate. A 0.5-ml aliquot of the supernatant was applied to a 1 × 2 cm column of Bio-Rex 70 ($Na^+$ form) equilibrated with 0.4 M potassium phosphate buffer, pH 6.0. The deaminated metabolites were washed from the resin with 2.5 ml of distilled water and counted in 10 ml Aquasol (New England Nuclear, Boston, MA) in a liquid scintillation spectrometer. Protein was determined [7] using bovine serum albumin as a standard.

## RESULTS

After the injection of [$^{11}$C]octylamine, the clearance of this compound from the blood and the distribution to the various organs in the mouse are quite rapid [2]. The lung and kidney typically contained the highest concentration of activity/g tissue (Fig. 1) and accounted for 7.12 ± 0.18 and 11.79 ± 0.47 per cent/organ, respectively, of the total injected dose at 1 min.

The liver contained 8.07 ± 0.55 per cent/organ and the brain 1.98 ± 0.15 per cent/organ of the injected dose although the radioactivity/g tissue in these two tissues was similar (~5 per cent/g of tissue) (data not shown). Pretreatment with pargyline (32 mg/kg) resulted either in a decreased net clearance of the total radioactivity as found with the lung (and heart) or a net accumulation of radioactivity with time as shown by the kidney (and liver, brain, spleen and muscle). The total radioactivity in these organs from animals treated 1 hr previously with 32 mg/kg of pargyline at 1, 5 and 15 min after the administration of [$^{11}$C]octylamine was often significantly higher than

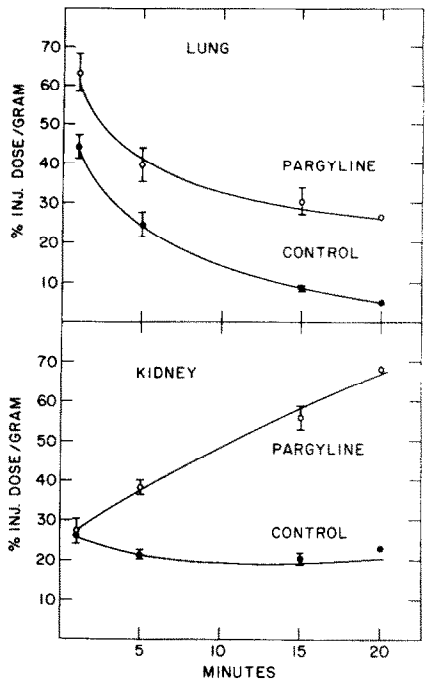


Fig. 1. Clearance of radioactivity in mouse lung and kidney after the i.v. injection of [$^{11}$C]octylamine in control (●——●) or pargyline treated (32 mg/kg) (○——○) animals. Each point represents the mean ± S.D. of two to eight animals.

control in several organs particularly at 5 and 15 min post injection (Fig. 2).

Pretreatment of animals 1 hr previously with 32 mg/kg of pargyline inhibited the excretion of $^{11}CO_2$ from [$^{11}$C]octylamine quite markedly with 8.8 per cent of the injected radioactivity being excreted as $^{11}CO_2$ in drug-treated animals compared to 48.1 per cent excreted in 20 min by control animals (Table 1). Since some recent reports [8, 9] have shown that high doses (>50 mg/kg) or pargyline inhibit aldehyde dehydrogenase, we explored this possibility to insure that the decrease in $^{11}CO_2$ excretion was not the result of inhibition of enzymes other than MAO (Table 1). Pargyline only inhibited the excretion of $^{11}CO_2$ with [$^{11}$C]octylamine as the substrate and did

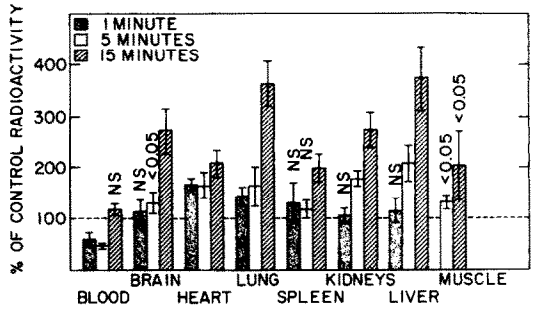


Fig. 2. Distribution of total radioactivity after the i.v. injection of [$^{11}$C]octylamine expressed at per cent of control in animals pretreated with pargyline (32 mg/kg) 1 hr previously. Each point represents the mean ± S.D. of three (experimental) and five (control) mice and is significant (student's *t*-test) at $P < 0.01$ except where noted.

Table 1. The effect of pargyline on the cumulative excretion of $^{11}CO_2$ from octylamine, octanoic acid and octanol*

| Substrate | (n) | (mg/kg) Pargyline | Cumulative $^{11}CO_2$ as % Injected Dose, minutes 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|---|
| $^{11}$C-octylamine | 5 | 0 | 19.8 ± 0.4 | 33.1 ± 0.5 | 41.8 ± 0.5 | 48.1 ± 1.1 |
| | 4 | 32 | 1.3 ± 0.6 | 3.4 ± 0.9 | 5.4 ± 1.1 | 8.8 ± 1.1 |
| $^{11}$C-octanoic acid | 6 | 0 | 30.6 ± 0.9 | 43.5 ± 1.3 | 51.2 ± 1.0 | 56.5 ± 1.2 |
| | 4 | 32 | 26.6 ± 1.4 | 40.7 ± 1.3 | 51.4 ± 2.5 | 55.4 ± 1.2 |
| $^{11}$C-octanol | 6 | 0 | 24.6 ± 1.2 | 38.5 ± 1.4 | 47.7 ± 1.4 | 53.9 ± 1.3 |
| | 5 | 32 | 29.3 ± 0.9 | 42.0 ± 0.9 | 50.7 ± 1.1 | 56.5 ± 1.2 |

* Groups of mice were pre-treated with saline or 32 mg/kg pargyline 1 hr prior to the injection of $^{11}$C-octylamine, $^{11}$C-octanoic acid or $^{11}$C-octanol and the cumulative $^{11}CO_2$ excretion measured for 20 minutes.

Values for octylamine are highly significant ($p < 0.001$) and are not significant for the other substrates except 5 minute octanoic acid ($p < 0.05$).

not significantly inhibit the 20-min $^{11}CO_2$ excretion from [$^{11}$C]octanoic acid or [$^{11}$C]octanol ([$^{11}$C]octanoic acid was slightly slower for the first 5 min but was at the control level by 10 min). Since [$^{11}$C]octanol shares [$^{11}$C]octanal as a common intermediate with [$^{11}$C]octylamine, we conclude that, if aldehyde dehydrogenase was inhibited, it could not be demonstrated by a decreased $^{11}CO_2$ excretion.

Thus, the $\beta$-oxidation enzymes also appear not to be greatly influenced by pargyline using either [$^{11}$C]octanol or [$^{11}$C]octanoic acid as model substrates. To further insure that the observed effects were not due to a possible non-specific action of pargyline, we utilized a lower dose (10 mg/kg) in all the further experiments, below levels which have been found to cause non-specific responses.

The return of the ability to oxidize [$^{11}$C]octylamine to $^{11}CO_2$ is quite rapid after the single administration of 10 mg/kg of pargyline (Fig. 3). This return is characterized by a phase of rapid recovery between 1 and 22 hr followed by a slower rate of return of cumulative $^{11}CO_2$ excretion toward control values

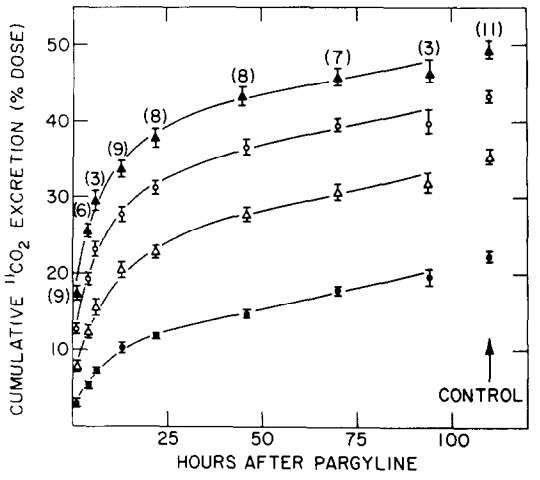


Fig. 3. Cumulative $^{11}CO_2$ excretion expressed as per cent of injected dose excreted at 5 (●——●), 10 (△——△), 15 (○——○), and 20 (▲——▲) min after the i.v. injection of [$^{11}$C]octylamine at 1–94 hr after pargyline (10 mg/kg) pretreatment. Control values are plotted at the far right for comparison. The number of animals used for each point is in parentheses.

seen at later times. By 94 hr after the administration of pargyline, the cumulative $^{11}CO_2$ excretion was 88.4, 89.5, 92.4 and 94.4 per cent of control values at 5, 10, 15 and 20 min respectively.

The retention of radioactivity by several organs in MAO-inhibited animals after the injection of [$^{11}$C]octylamine can be attributed to the accumulation of the unchanged amine and/or a decreased rate of metabolism based on the analysis of the distribution of the total radioactivity in the organs studied between amine and deaminated metabolite fractions (Fig. 4). Considerably greater activity as the unchanged amine was present at 20 min in all the organs studied at 1 and 4 hr after pargyline pretreatment. The retention of the unchanged amine decreased rapidly with increasing time after pargyline administration and varied with the different tissues studied. The per cent per organ as unchanged amine in the liver returned to control levels the most rapidly, in ~13 hr, the intestine and kidney by 46 hr. The lung and the brain showed the slowest rate of return toward the control value.

The decrease in the tissue radioactivity present as unchanged amine, as the MAO in these organs recovered from the effects of pargyline, correlates in a general pattern with the studies of MAO activity in these organs (Fig. 5). The two organs showing the highest retention of the unchanged amine compared

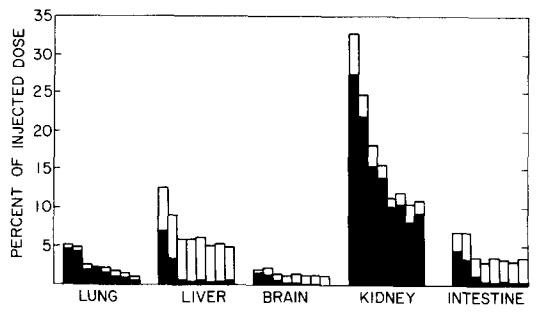


Fig. 4. Effect of pargyline (10 mg/kg) on the distribution of total radioactivity per organ (unshaded) and [$^{11}$C]*n*-octylamine activity (shaded) in mouse tissues 20 min post-injection. Bars for each tissue are arranged from left to right representing 1, 4, 13.5, 22, 46, 70 and 94 hr after pargyline and control animals, respectively, and are the average of duplicate determinations.

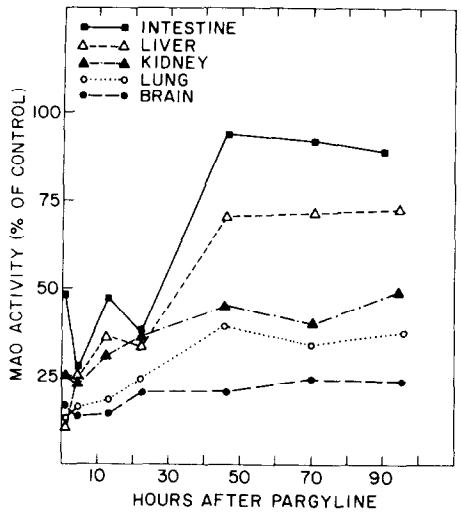


Fig. 5. MAO activity (for [$^{14}$C]octylamine) of major mouse organs at 1–94 hr after pretreatment with pargyline (10 mg/kg). Points are the average of two determinations. Per cent of control MAO activity represents:

$$100 \cdot \frac{\text{nmoles product/mg of mitochondrial protein (pargyline-treated)}}{\text{nmoles product/mg of mitochondrial protein (saline controls)}}$$

The control MAO activity for each tissue is listed in Table 2.

to controls, the brain and lung, also had the slowest return of MAO activity measured in mitochondrial preparations with [$^{14}$C]octylamine as the substrate. The two organs with the greatest rate of MAO recovery, the liver and intestine, likewise showed the fastest return to control for tissue amine levels at 20 min after the injection of [$^{11}$C]octylamine. This correlation was not true for the kidney in which the amount of radioactivity as unchanged amine at 20 min returned to control levels by 46 hr while MAO activity in this organ was still strongly inhibited (< 50 per cent of control).

These findings may in part reflect the marked differences in the rate of deamination of [$^{14}$C]*n*-octylamine by the tissues studied (Table 2). The activity expressed as nmoles product/mg of mitochondrial protein/hr shows that the liver and intestine have the highest activity for this substrate. The lung also has considerable activity, about twice that of brain. The

Table 2. Mitochondrial MAO activity of mouse tissues for octylamine

| Tissue | nmole Product/mg protein/hour | % Highest Activity* |
|---|---|---|
| Lung | 19.8 | 67.3 |
| Liver | 28.8 | 98.0 |
| Brain | 7.8 | 26.5 |
| Kidney | 1.8 | 6.1 |
| Intestine | 29.4 | 100.0 |

Values are the average of two determinations.

* % highest activity is listed for the sake of intercomparison between tissues and was determined by setting the intestinal MAO specific activity to 100% and expressing the activity of the other tissues as a proportion of this tissue.

kidney showed the lowest MAO activity (about 6 per cent that of liver and intestine) of the organs studied for this substrate, and thus deamination may play only a minor role in the clearance of octylamine from this organ. The per cent of the total radioactivity present in the kidney as the unchanged amine at 20 min after the injection of [$^{11}$C]OA was independent of the time after pargyline administration and always greater than 83 per cent of the total. Thus, the relatively slow rate of oxidation of octylamine by kidney may account for the high levels of unchanged amine at 20 min in control animals.

## DISCUSSION

Monoamine oxidase activity has been extensively studied with regard to age [10, 11], drug treatment [12, 13], species [14], disease states [15, 16] and turnover rate [17–21]. Most of these studies, however, have relied upon the use of techniques *in vitro* employing tissue homogenates. We have sought to develop a method to measure MAO activity *in vivo* employing aliphatic amines labeled in the α-position with carbon-11.

We have shown previously that aliphatic amines, particularly *n*-octylamine, labeled with carbon-11, an isotope which decays by the emission of a positron which upon annihilation results in the production of gamma radiation which can be detected externally, are of potential value for the non-invasive study of amine uptake by the lung in animals and in man [2–4]. The use of such tracers is being pursued with a view toward the understanding of basic physiological processes and how they are perturbed by disease or injury. The goal of such studies is the application of radiopharmaceuticals such as [$^{11}$C]*n*-octylamine to disease diagnosis in man.

Although the short half-life of carbon-11 (20.4 min) and the need for a cyclotron limit the use of this isotope, the labeling of many organic molecules of biomedical importance has been achieved [22–24] and the desirability of carbon-11 for studies *in vivo*, particularly in humans, is great. The short half-life results in very low radiation doses to the patient and the pharmacodynamics of labeled substrates can be monitored externally with a gamma camera. Furthermore, the exceedingly high specific activities which can be achieved (2000 Ci/m-mole) greatly exceed those possible with carbon-14 and permit studies to be carried out at true tracer levels.

Even at the near carrier-free level, octylamine has shown dramatic differences both in the tissue distribution and/or clearance of activity from various mouse organs and the rate of ultimate metabolism to $^{11}CO_2$ after low (10 mg/kg) doses of the MAO inhibitor, pargyline. Because of the relative ease by which $^{11}CO_2$ excretion (or $^{14}CO_2$) can be measured, we have investigated this parameter to determine how well it reflects the MAO activity of several mouse organs. The recovery in the rate of $^{11}CO_2$ excretion after pargyline appears to be independent of lung, brain and kidney MAO recovery and is qualitatively similar to MAO activity in liver and intestine, organs to which an appreciable amount of radioactivity initially distributes. These organs also showed the highest MAO activity for octylamine of the tissues studied and re-

covered from the effects of pargyline much faster than the kidney, brain and the lung. This latter finding is based both on the biochemical measurement of MAO activity in these tissues and on the analysis of the radioactivity present as unchanged amine after intravenous administration (Fig. 4). However, these two parameters did not correlate well for the kidney. At 46 hr after the injection of pargyline, the amount of radioactivity present as amine was at control levels, yet the MAO activity in this organ in the same animals was less than 50 per cent of the control value. Although the kidney shows a high affinity for octylamine, its ability to deaminate this substrate is very low. Another study [25] failed to detect any deamination of a similar aliphatic MAO substrate, *iso*-amylamine, in preparations of mouse kidney. Thus, the high levels of octylamine present in the kidney, particularly at short times after pargyline treatment, may reflect only the affinity of this organ for the higher levels of unchanged amine circulating in the blood during MAO inhibition [2]. These higher blood levels of octylamine are most likely the result of decreased deamination by the liver and intestine. The lung probably also contributes to the whole body metabolism of this substrate, particularly in some other species such as the rabbit, dog and human [3, 4] in which 60–70 per cent of the injected octylamine is removed from the circulation during the first pass by the lung, and within 1 min, 95 per cent of the blood activity in humans is due to deaminated metabolites.

It is interesting to note the differences in the MAO turnover in the various organs of the mouse after irreversible inhibition. A recent report [26] has also demonstrated a much slower rate of return of mouse brain MAO relative to the liver, requiring more than 21 days to achieve control values while liver activity was at the control level by 7 days. The present study has confirmed and extended these earlier results. The lung was similar to the brain in the relatively slow return of MAO activity and the kidney also showed a relatively slow return of MAO activity. We have observed that the recovery rate of animals 10 weeks old at the start of the experiment was slower than that of the 8-week-old animals although 10-week control animals (injected only with saline) showed no difference from 8-week-old controls in the cumulative $^{11}CO_2$ excreted (unpublished observations).

Perhaps the most important observation is that at times (70–94 hr) when the $^{11}CO_2$ excretion is very close to control values, the MAO activity of at least the brain, lung and kidney is less than half of control and, the lung and brain also show significantly higher tissue levels of amine. Thus, although these organs may normally contribute in part to the whole body metabolism of this substrate in control animals, during periods when lung and brain MAO activity is strongly inhibited, the liver and intestine may have significantly recovered, contributing at almost control levels to the total $^{11}CO_2$ excreted. That is, the MAO activity of the partially recovered liver and intestine may be sufficient to metabolize [$^{11}$C]octylamine such that the overall rate of $^{11}CO_2$ excreted is almost at control levels. This suggests that the rate-determining step in the excretion of $^{11}CO_2$ from [$^{11}$C]octylamine is not dependent upon MAO activity but on the subsequent metabolic steps. This may in part be due to the dilution of the labeled metabolites with endogenous pools. Other explanations are also possible and it is difficult to assign the rate of $^{11}CO_2$ excretion to any single process. The overall rate represents the sum of several coupled and competing processes which may be altered dramatically by drug treatment. Nonetheless, it is interesting to note the seeming specificity of pargyline for MAO inhibition in view of the finding that the $^{11}CO_2$ from [$^{11}$C]octanol and [$^{11}$C]octanoic acid was not significantly affected by this drug. We are continuing in our attempts to devise methods by which it is possible to measure MAO activity *in vivo* by utilizing $^{11}$C-labeled serotonin, norepinephrine and phenylethylamine as well as octylamine and the gamma camera. It is possible to dynamically and non-invasively measure the clearance of radioactivity from various organs *in vivo* by using such techniques [3, 4], and this approach may provide a more precise technique than $^{11}CO_2$ excretion for determining MAO activity *in vivo*.

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