# Ornithine Metabolism in Normal Subjects and Patients with Cancer

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**The metabolism of L-(1-$^{14}$C)ornithine monohydrochloride was monitored in patients with histologically proven cancer and in normal volunteers. Following i.v. injection of 8 μCi C-14 ornithine (160 nmoles), the decarboxylation of ornithine—yielding $^{14}CO_2$—was monitored for a 2.5-hr period using the ionization chamber and vibrating-reed electrometer of Tolbert, as modified by Davidson and Schwabe. Twelve normal subjects exhaled 7.3–15.7% of the administered C-14 (mean 12.6%, s.d. 3.11%). In ten patients tested before initiation of therapy, recovery ranged from 18.2–32.1% (mean 23.02%, s.d. 4.52%). A t-test indicates a confidence level of >99.5% that a significant difference exists between the two means. Re-testing of two normal volunteers showed little or no change in ornithine metabolism over a 2–5-mo period. Results from testing three cancer patients before and after therapy correlate well with clinical evidence of the presence of tumor burden.**

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Ornithine is an ubiquitous amino acid, whose metabolism appears to be closely associated with the growth process in mammalian systems, but the role of ornithine in growth has been only partly elucidated (*1–3*). Growth stimuli produce a rapid rise in ornithine decarboxylase (ODC) activity—which has been shown to be associated with the early formation of RNA polymerase I—and a significant increase in the polyamines, putrescine, spermidine, and spermine (*4*). Decarboxylation, in this instance, most likely represents a diversion of ornithine from its role in the urea cycle and from its deamination and oxidative metabolism in the Krebs cycle (*2, 4*). In mammals, increased polyamine excretion has been linked to the increased growth of malignant tissues, and the use of urinary polyamine levels as a possible marker of malignancy has been explored by Russell (*4*) and others (*5–7*).

Utilizing this background information, we previously reported the results obtained by the use of carboxyl-labeled C-14 ornithine in normal and tumor-bearing rats (*8*). The amount of exhaled radioactive $CO_2$ was plotted as a function of time. Significant increases in exhaled $^{14}CO_2$ were found in tumor-bearing animals, compared with controls. On the basis of these findings, a very similar procedure has been applied to human subjects and the results are reported here.

## MATERIALS AND METHODS

L-(1-$^{14}$C)ornithine, specific activity 49.2 mCi/mmol, was purchased as a sterile, pyrogen-free solution.* The monitoring apparatus used a 14.8-l ionization chamber with appropriate pumps, an expired-air hood, and a dryer, as initially described by Tolbert (*9, 10*) and later modified by Davidson and Schwabe (*11*). The apparatus is designed to monitor $^{14}CO_2$ in expired air continuously from the subject by creating a gentle flow from the plastic hood through the dryer and into the ionization chamber, where the activity is measured.

The criteria for the selection of normal volunteers were that they were in good health at the time, not taking any medications, and had fasted for at least 8 hr before being tested. Patients volunteering as test subjects had histologically proven neoplasia and had also fasted for at least

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**TABLE 1. PERCENTAGE OF INJECTED ORNITHINE METABOLIZED IN 2.5 HR**

| Category | Age | Sex | % $^{14}CO_2$ Recovered | Mean* | s.d. |
|---|---|---|---|---|---|
| Normal | 37 | M | 8.1 | | |
| Normal | 61 | M | 7.3 | | |
| Normal | 19 | F | 7.5 | | |
| Normal | 23 | M | 14.0 | | |
| Normal | 19 | M | 13.8 | 12.6% | ±3.11% |
| Normal | 39 | F | 14.5 | | |
| Normal | 48 | M | 13.7 | | |
| Normal | 30 | F | 15.0 | (N = 12) | |
| Normal | 54 | F | 15.7 | | |
| Normal | 46 | F | 14.2 | | |
| Normal | 38 | M | 15.0 | | |
| Normal | 59 | F | 12.0 | | |
| Cancer | 38 | F | 18.9† | | |
| Cancer | 31 | F | 18.2† | | |
| Cancer | 60 | F | 21.7 | | |
| Cancer | 40 | F | 20.9† | 23.02% | ±4.52% |
| Cancer | 41 | F | 32.1 | | |
| Cancer | 59 | M | 20.9 | (N = 10)* | |
| Cancer | 63 | F | 28.8 | | |
| Cancer | 58 | F | 25.3 | | |
| Cancer | 28 | F | 23.7 | | |
| Cancer | 59 | F | 19.7 | | |

Diagnoses include: Metastatic breast CA; epidermoid CA of oropharynx; reticulum sarcoma of femur; squamous-cell CA of larynx; infiltrated ductal breast CA; squamous-cell CA of lung.

* t-Test indicates a confidence level of >99.5% that a significant difference exists between the means of the two groups.

† Metastatic breast cancer patients undergoing chemotherapy.

8 hr. None had other illnesses and the estimated tumor burdens ranged from 4–70 g. After obtaining informed consent, data were acquired by measuring expired $^{14}CO_2$ in the 2.5-hr period following the intravenous injection of 8.0 $\mu$Ci (160 nmoles) of radiolabeled ornithine.

The percentage of the administered dose metabolized was calculated for each subject as follows.

1. Total activity recovered per test period: $A = 10\,RT$, where R = machine constant ($1.42 \times 10^{-4}$ $\mu$Ci/min) and T = sum of 10 min readings minus background after i.v. administration of 8 $\mu$Ci; therefore $A = 1.42 \times 10^{-3}\,T$ $\mu$Ci.

2. % administered dose recovered: $X = 100\,A/8 = 0.0178T\%$.

## RESULTS AND DISCUSSION

The results are shown in Table 1. Twelve normal subjects exhaled $CO_2$ containing 7.3–15.7% of the administered dose (mean 12.6% ± 3.11% s.d.) in 2.5 hr. Ten patients with neoplasia, ranging from 18.2–32.1%, yielded a mean of 23.03% ± 4.52% s.d. Application of the t-test indicates a confidence level of more than 99.5% that a significant difference (10.42%) exists between the two means.

One volunteer, a 21-year-old woman who was originally thought to be normal, had a reading of 33.1%, the highest of any test subject. After careful history and re-examination, we found no evidence of disease but concluded that she had been 7–9 days pregnant at the time of the study. The pregnancy terminated in a spontaneous abortion a few days later. Repeat tests at 1 and 3 mo were 24 and 18%, respectively.

Table 2 gives preliminary results on the reproducibility of the test in two normal individuals, and of the effect of radiation therapy on three cancer patients. Both of the normals had repeat values very close to the original. The two cancer patients with a good response to treatment, on the other hand, showed a marked drop in $^{14}CO_2$ production, bringing them into the normal range. The third patient, whose tumor failed to respond to radiation, had only a minimal drop in $^{14}CO_2$ production, which correlates well with the clinical evaluation of her lack of response to therapy.

All of the cancer patients tested were ambulatory, in good health apart from the neoplasia, in no distress, and showed minimal changes in eating habits, weight, amount of sleep, and the like during their treatment. All were attempting to maintain their normal manner of

**TABLE 2. EFFECTS OF TIME AND THERAPY ON THE AMOUNT OF ORNITHINE METABOLIZED**

| Category | Age | Sex | % $^{14}CO_2$ Recovered | | Remarks |
|---|---|---|---|---|---|
| | | | 1st test | 2nd test | |
| Normal | 61 | M | 7.3% | 6.9% | 5 mos. apart |
| Normal | 46 | F | 14.2% | 15.5% | 2 mos. apart |
| Cancer* | 60 | F | 21.7% | 16.3% | No apparent tumor burden |
| Cancer | 41 | F | 32.1% | 14.0% | No apparent tumor burden |
| Cancer | 58 | F | 25.3% | 23.4% | Therapy minimally effective |

* All cancer patients were tested before, and 1 wk after, radiation therapy courses.

living. None of the cancer patients who were re-tested presented any severe side effects of their courses of radiation therapy.

This preliminary study demonstrates a highly significant difference in ornithine metabolism in a group of cancer patients as compared with a group of normal volunteers. The results are similar to those seen in a previously reported animal study (*8*). The biochemical explanation for this phenomenon remains to be elucidated. It is felt that the altered utilization of ornithine may lead to the development of a method for detecting the presence and quantity of malignant tissue within the body.

Our observations suggest that in normal individuals ornithine metabolism by ornithine decarboxylase or other processes proceeds quite slowly, possibly indicating slow utilization of a relatively large pool. In the patient with neoplasia, however, the higher utilization suggests that more ornithine is acted upon by ODC or other enzymes, and/or that the pool is smaller. In either case, the results suggest that altered L-(1-$^{14}$C)ornithine utilization may be a sensitive test for malignancy arising in several different cell types.

It can be anticipated that some patients with nonmalignant proliferative disease may also yield increased levels of $^{14}CO_2$, but it is likely that they can be differentiated on a clinical basis. This would also be true of pregnancy. Ongoing studies will examine differences in ornithine utilization in normal subjects of both sexes and in various age groups, in patients with neoplasia before and after therapy, and in individuals with nonmalignant disorders.

FOOTNOTE

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