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DIMINISHED OXIDATION OF ^{14}C-UL-L-ASPARAGINE TO $^{14}CO_2$ IN MICE AND HUMANS WITH TUMORS: A POSSIBLE MEANS FOR ASSESSING EFFICACY OF THERAPY?

Tuhin K. Chaudhuri* and H. S. Winchell

Donner Laboratory, University of California, Berkeley, California

Certain transplantable tumors in animals and some forms of neoplasm in man appear to respond to therapy with L-asparaginase (*1,2*). These tumors appear to have a diminished ability to synthesize L-asparagine, and they require L-asparagine synthesized by normal cells for the growth of the tumor cells. If this is the case, animals with L-asparagine-dependent tumors should demonstrate abnormalities in the metabolism of this amino acid. In the work presented here we tried to show these abnormalities by measuring $^{14}CO_2$ excretion in the breath subsequent to the i.v. administration of ^{14}C-UL-L-asparagine to mice and humans with malignant tumors. In mice a contrast study was also made with ^{14}C-UL-L-aspartic acid.

MATERIALS AND METHODS

The ^{14}C-UL-L-asparagine was obtained from Amersham/Searle (218 mCi/m*M*). The ^{14}C-UL-L-aspartic acid was obtained from the same source (208 mCi/m*M*). Male A-Heston mice weighing between 15 and 17 gm and a transplantable anaplastic carcinoma (#15091A) were obtained from Jackson Laboratories, Bar Harbor, Maine. Each mouse received 3.3 μCi of ^{14}C-UL-L-asparagine or 5–10 μCi of ^{14}C-UL-aspartic acid immediately before being placed in a device which continuously measured the excretion of $^{14}CO_2$ in the breath. This device was similar to those described previously (*3,4*).

Five to six days after subcutaneous implantation of the anaplastic carcinoma and 1–3 hr after i.v. administration of ^{14}C-labeled asparagine or aspartic acid, the mice were sacrificed. The tumor at the site of the subcutaneous implantation was excised as were the liver, spleen, kidney and a portion of muscle. These tissues were weighed, and a small aliquot was digested in 2 ml of NCS solution (Nuclear-Chicago) and suspended in 15 ml of liquid scintillation solution containing the following proportion of ingredients: PPO 6.5 gm, POPOP 0.65 gm, naphthalene 104 gm, ethanol 300 ml, toluene 500 ml and dioxane 500 ml. The samples were counted in a Mark I Nuclear-Chicago liquid scintillation counter. To correct for quenching, each sample was recounted

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For reprints contact: Tuhin K. Chaudhuri, Nuclear Medicine Div., University of Iowa, Iowa City, Iowa 52240.

* Present address: See above.

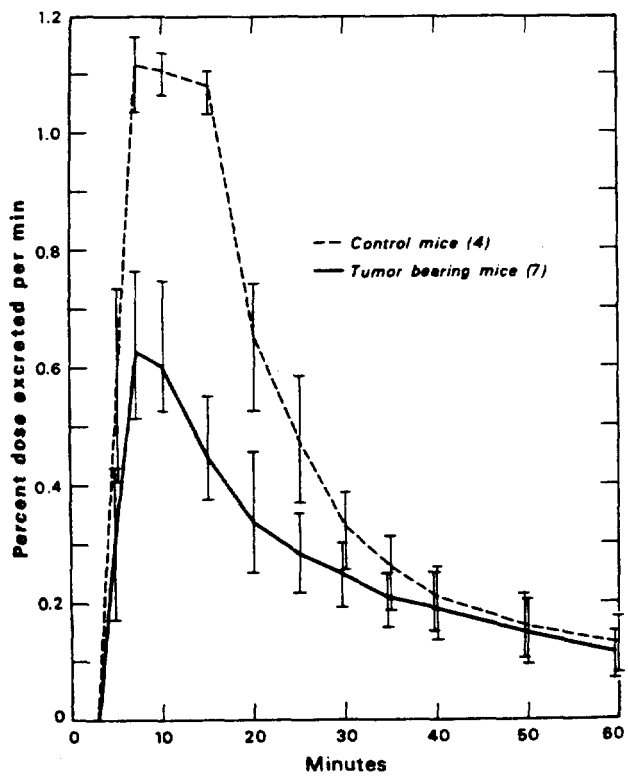

FIG. 1. Graphical presentation of rate of ^{14}C-excretion in breath of four control mice and seven mice with anaplastic carcinoma following intravenous administration of ^{14}C-UL-L-asparagine. Percent of administered dose excreted per minute is plotted on ordinate and time after administration of labeled asparagine is plotted on abscissa. Curve is drawn through average value obtained for each group of mice. Range of highest and lowest values obtained in individual mice at any given point is shown by ends of line drawn above and below mean value.

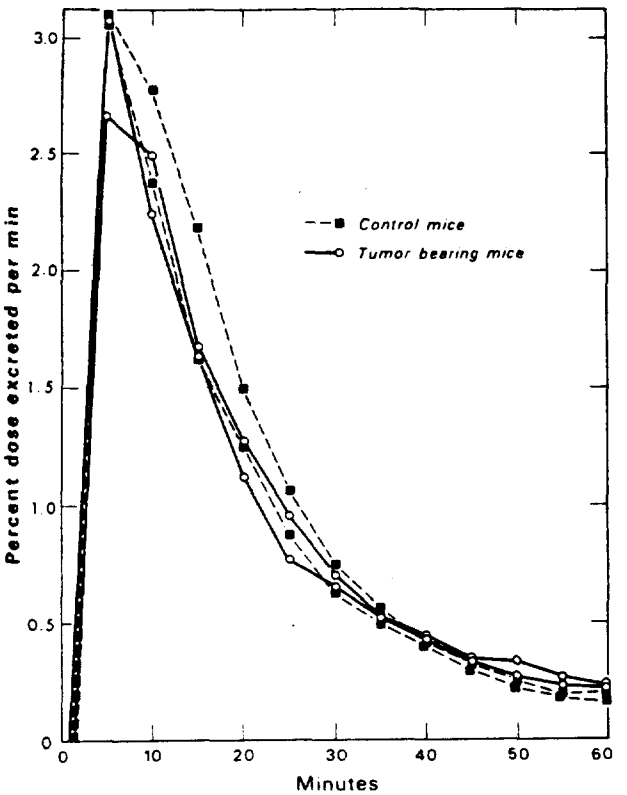

FIG. 2. ^{14}C-excretion following i.v. administration of ^{14}C-UL-L-aspartic acid to two control mice and two mice with same type of anaplastic carcinoma. Ordinate and abscissa are same as in Fig. 1.

after a known amount of ^{14}C-toluene (New England Nuclear Corp.) was added. Concentration of activity and total activity in the tissue were calculated in a routine fashion.

Two human subjects with malignant neoplasia were studied before and after institution of specific therapy. Each patient received 5–10 μCi of ^{14}C-UL-L-asparagine at the beginning of the study. Total CO_2 excretion and oxygen consumption as well as the rate of $^{14}CO_2$ excretion in the breath was measured using a device described previously (*5*).

RESULTS

Figure 1 presents graphically the rate of excretion of $^{14}CO_2$ in the breath in four control mice and in seven mice with anaplastic carcinoma subsequent to the intravenous administration of ^{14}C-UL-L-asparagine. Percentage of the administered dose excreted per minute is plotted on the ordinate and time after administration of the labeled asparagine is plotted on the abscissa. The curve is drawn through the mean value obtained for each group at the given time. The ends of the lines through each point define the range of highest and lowest values in individual mice. It is clear from these curves that the rate of excretion of $^{14}CO_2$ in the breath subsequent to the administration of ^{14}C-UL-L-asparagine to control animals was much greater than that seen in similar animals with tumor. The mice with tumor were similar in age and weight to those in the control series but had received a subcutaneous injection of transplantable carcinoma cells in the groin 5–6 days before the study. At the time of the study the average size of the solid tumor mass varied between 1 and 2 cm in dia. The average of the administered ^{14}C-L-asparagine excreted as $^{14}CO_2$ in the breath during the first hour after its administration in the control mice (four animals) was 24.9% ± 3.2% s.d. while that in the tumor-bearing group (seven animals) was 15.4% ± 2.2% s.d. (Table 1).

Figure 2 shows the results of similar measurements made in two control mice and two mice with tumors after the intravenous administration of ^{14}C-UL-L-aspartic acid. The average of the administered ^{14}C-L-aspartic acid excreted as $^{14}CO_2$ in the breath during the first hour after its administration was 65–70% for both the control and tumor-bearing mice. This is three times faster than that of asparagine in control mice. It is clear that the mice bearing tumors

TABLE 1. PERCENT ADMINISTERED ^{14}C EXCRETED IN BREATH IN 1 HR

	Control (4)*	Tumor bearing (7)*
Mice	24.9 ± 3.2 (s.d.)	15.4 ± 2.2 (s.d.)
Patients	After treatment	Before treatment
[redacted]	4.9	2.7
	1.5	1.0

* Number of animals in each group.

TABLE 2. ^{14}C CONCENTRATION IN NORMAL AND TUMOR TISSUE OF MICE AFTER I.V. ADMINISTRATION OF ^{14}C-UL-L-ASPARAGINE

	Control		Tumor bearing	
	% dose/gm	% dose/organ	% dose/gm	% dose/organ
Liver	7.5	6.2	6.4	6.7
Spleen	8.7	1.3	8.0	1.3
Kidneys	7.0	3.0	5.9	2.0
Muscle	1.1	—	1.1	—
Tumor	—	—	2.7	6.8

Each figure represents average values obtained in 2–5 mice sacrificed 1–3 hr after administration of ^{14}C-UL-L-asparagine. Concentration is expressed as percent of administered dose/gm wet tissue weight. The percent dose/organ is obtained by multiplying concentration by organ weight. The percent dose/organ for the kidney is given for both kidneys.

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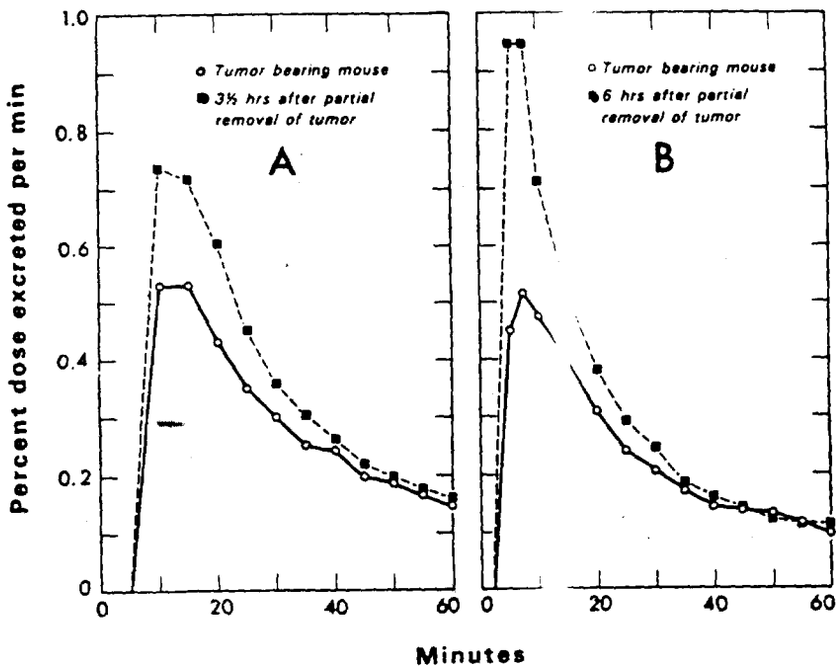

FIG. 3. A shows ^{14}C-excretion following i.v. administration of ^{14}C-UL-L-asparagine to tumor-bearing mouse before and at 3½ hr after partial removal of tumor. B is for similar mouse before and 6 hr after partial removal of tumor. Ordinate and abscissa are same as in Fig. 1.

excrete $^{14}CO_2$ in the breath after the administration of the labeled aspartic acid in a similar fashion to that seen in the control animals.

Figure 3 shows the rate of excretion of $^{14}CO_2$ in the breath after the administration of ^{14}C-UL-L-asparagine to animals both before and after partial removal of the subcutaneous tumor growth. Figure 3A gives the data obtained in a mouse studied before and 3½ hr after incomplete surgical removal of the tumor, while Fig. 3B gives data for another animal studied before and 6 hr after incomplete removal of the subcutaneous portion of the tumor. In each case the tumor had spread from the site of initial subcutaneous implantation in the groin to involve the rectus sheath, and at the time of surgery only the major subcutaneous portion of the tumor could be removed. Within 3½ hr after incomplete removal of the tumor there was an increase in the rate of $^{14}CO_2$ excretion following the administration of labeled L-asparagine. This increased rate of $^{14}CO_2$ excretion was even more pronounced 6 hr after incomplete removal of the tumor in a similar animal.

Table 2 gives the results of experiments attempting to measure the incorporation of ^{14}C from ^{14}C-UL-L-asparagine into tumors and normal tissue. It can be seen that the concentration of ^{14}C label in tumor, while greater than that in muscle, was generally less than the ^{14}C concentration in liver, spleen and kidney.

Figure 4 shows results obtained in two humans with malignant tumors studied before and after specific therapy for their disease was started. Patient [redacted] is a [redacted]year-old C.F. who presented with pancytopenia, weight loss and fever of unknown origin. Bone marrow biop showed replacement of the bone marrow by shee of lymphoblasts. Similar cells were found infiltrati a cervical lymph node. The patient could either b classified as having aleukemic lymphoblastic leuk ia or lymphoblastic lymphosarcoma with primary one marrow involvement. The $^{14}CO_2$ excretion f owing administration of labeled L-asparagine was studied on May 10, 1969 before any specific thera y was given and again on Aug. 7, 1969 after app oximately 2½ months of therapy with steroids an 6-mercaptopurine. At the time of the second study e patient was felt to have only a partial remissio from her disease. In the upper portion of Fig. 4 can be seen that there is a clear increase in the te of $^{14}CO_2$ excretion following the administratio of L-asparagine at the time of the second study (% of administered dose excreted within the firs hour before therapy compared with 4.9% after erapy—Table 1).

[redacted] was a [redacted]year- d W.F. with plasmoblastic multiple myeloma with widespread skeletal involvement, nodular infiltratio of the kidneys and stomach and hepatomegaly with probable hepatic infiltration by tumor. Between th time of the first study on May 20, 1969 and th second study on Aug. 14, 1969, the patient rece ved radiotherapy to skeletal lesions and systemic therapy with cytoxan, vincrystine and prednisone. At the time of the second study, there was only modest clinical evidence o response to therapy.

The results of the initial study on May 20, 196 before the initiation of specific therapy, and that o

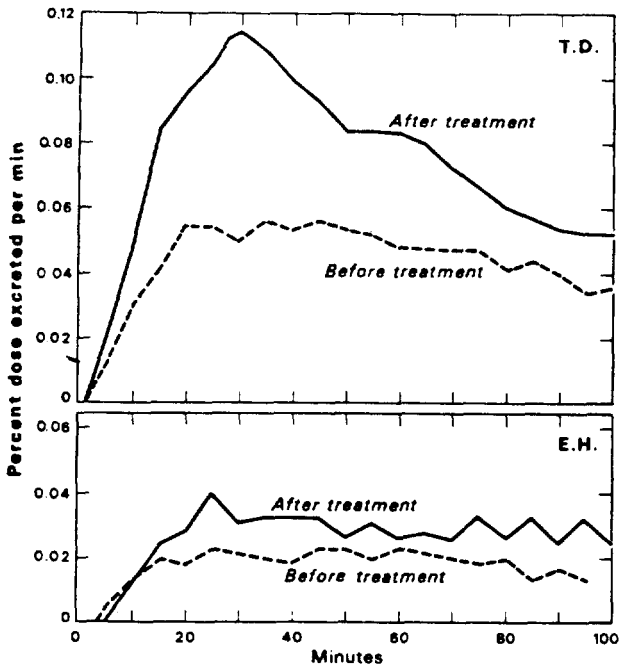

FIG. 4. ^{14}C-excretion following administration of ^{14}C-UL-L-asparagine to patients before and after therapy of their tumors. Ordinate is expressed as percentage of administered dose excreted per minute and abscissa is time in minutes after administration of ^{14}C-labeled asparagine. Upper portion of figure shows data obtained in patient TD before and after therapy of her lymphoblastic leukemia and lower portion of figure shows data obtained in EH before and after treatment of her plasmoblastic myeloma.

Aug. 14, 1969 after therapy, are shown in the lower portion of Fig. 4. It is again clear that there was an increase in the rate of $^{14}CO_2$ excretion in the breath on the second study after the administration of therapy and induction of partial remission (1.0% of administered dose excreted within the first hour before therapy compared with 1.5% after therapy—Table 1).

DISCUSSION

These results can be explained by considering the tumor as a large asparagine "sink" which rapidly removes asparagine from the circulation and incorporates it into materials required by the tumor. This would effectively diminish the pool of labeled circulating asparagine which could be metabolized to CO_2 by normal tissue. Alternatively, the results presented in this paper may be due to an effect of the tumor on the metabolism of asparagine by normal tissues such as the liver. It has long been appreciated that systemic effects such as cachexia and asthenia associated with many malignant tumors are frequently disproportionate to the amount of tumor mass present in the body, and it is often difficult to explain such systemic effects on the basis of actual tumor invasion of organs and tissues. If indeed our results obtained with metabolism of asparagine represent abscopal effects of the tumor on the metabolism of normal cells, then perhaps these results can be used as a model for explaining such systemic manifestations of malignancy. In such a model the malignant cells would somehow "instruct" normal tissue to provide the tumor with needed metabolites, resulting in a diversion of the metabolism of the normal cells to the requirements of the tumor rather than its own requirements.

The fraction of administered ^{14}C-UL-L-asparagine rapidly oxidized to $^{14}CO_2$ was much greater in mice (15–25% in 1 hr) than men (1–5% in 1 hr). In part this may be due to administration of nontracer, pharmacologic amounts of L-asparagine when 3.3 μCi of ^{14}C material was introduced (0.015 μM) to the small, mouse L-asparagine pool compared with the *relatively* small amount of L-asparagine (0.023 μM) when 5 μCi of ^{14}C material was administered to the relatively large human L-asparagine pool. Additionally, this result may reflect relatively greater anabolic utilization of exogenous L-asparagine by human cells than by mouse cells.

Tumor-induced alterations in systemic metabolisms of specific substrates are probably not limited to L-asparagine. In preliminary experiments similar to those presented in this paper we found results with L-serine similar to those obtained with L-asparagine. The method of studying $^{14}CO_2$ excretion from ^{14}C-labeled substrates administered before and after therapy to patients with tumors lends itself to study of tumor effects on systemic metabolism of a large variety of materials. We are presently engaged in such studies.

The results obtained in mice were clearly related to the presence or absence of tumor. However, the results in humans could reflect the effect of antitumor therapy on normal tissue as well as the effect of such therapy on the tumor itself. The results described in the present communication are preliminary and further studies will be required to confirm these findings and elucidate their meaning. If $^{14}CO_2$ production from ^{14}C-UL-L-asparagine or other substrates correlates with metabolic activity of tumors, then such measurements may be useful in assessing therapeutic efficacy. Furthermore, markedly abnormal $^{14}CO_2$ production from ^{14}C-UL-L-asparagine might indicate sensitivity of the given tumor to the enzyme L-asparaginase and may be useful in choosing patients for such therapy.

SUMMARY

$^{14}CO_2$ excretion in the breath was measured after i.v. administration of ^{14}C-UL-L-asparagine to control mice, to mice with transplanted anaplastic carcinoma and to two humans with plasmoblastic

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myeloma and lymphoblastic leukemia, respectively. Excretion was measured before and after specific therapy. Tumor-bearing mice excreted approximately 40% less $^{14}CO_2$ than control animals. On the other hand, excretion of $^{14}CO_2$ after administration of ^{14}C-UL-L-aspartic acid was identical in normal and tumor-bearing mice. After partial extirpation of the tumor, the $^{14}CO_2$ excretion rate following administration of ^{14}C-UL-L-asparagine increased toward normal values. Concentration of ^{14}C from labeled asparagine in the mouse tumor was generally greater than in muscle but less than that in liver, spleen and kidney. The two patients showed increased $^{14}CO_2$ excretion following ^{14}C-asparagine administration after therapy compared with before therapy. $^{14}CO_2$ measurements similar to those described may be useful in assessing efficacy of therapy of tumors. Studies with ^{14}C substrates other than L-asparagine may prove useful in delineating systemic metabolic abnormalities associated with various neoplastic processes.

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