
Nitrogen-13 Glutamate Uptake and Perfusion in Walker 256 Carcinosarcoma Before and After Single-Dose Irradiation

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Nitrogen-13 (^{13}N) glutamate uptake was recorded in 18 anesthetized rats, both before and at least once after intervention. Each investigation was immediately followed by imaging of blood flow distribution using [^{11}C]butanol. All animals had Walker 256 carcinosarcoma implants in one hind leg. Tumors were locally irradiated with a dose of 800 rad in 14 rats; in four rats, the vasoactive substance 5-hydroxytryptamine (5-HT) was administered. Prior to interventions, the [^{13}N]glutamate tumor-to-muscle uptake showed a linear correlation with blood flow close to identity ($y = 0.117 + 0.915x$, $r = 0.97$). After irradiation, a discordant pattern was observed: blood flow tended to increase, while [^{13}N]glutamate tumor-to-muscle uptake dropped from 4.30 ± 0.66 (s.e.m.) to 3.06 ± 0.36 ($p < 0.005$) during 30 min and attained 4.04 ± 0.67 2 days later. If [^{13}N]glutamate tumor-to-muscle uptake was related to that of [^{11}C]butanol in each individual animal, this index dropped from 0.93 ± 0.03 (s.e.m.) to 0.62 ± 0.04 ($p < 0.001$) 30 min after irradiation and attained 0.90 ± 0.09 after 2 days. In animals treated with 5-HT, [^{13}N]glutamate and [^{11}C]butanol showed a parallel drop from 6.60 ± 0.84 to 2.10 ± 0.60 ($p < 0.05$) and from 6.8 ± 0.78 to 2.08 ± 0.74 ($p < 0.05$), respectively. Thus, single-dose irradiation causes [^{13}N]glutamate uptake to be uncoupled with respect to flow, while [^{13}N]glutamate uptake in untreated tumors is flow-limited and responds together with flow on vasomotion.

J Nucl Med 27:1604-1610, 1986

There is increasing interest in methods for in vivo assessment of metabolic activity in tumors concerning the individual control of therapeutic regimes in cancer patients. Particular attention has been focused on Nitrogen-13 (^{13}N) L-glutamate, since amino acids are known to be involved in membrane and enzymatic alterations in malignancy (1-3) and its stereospecific synthesis with positron emitters was described a decade ago (4). Clinical and experimental studies using [^{13}N]L-glutamate have shown a relationship between the amount of radionuclide uptake by the tumor on one side and the malignant activity or the response to antineoplastic therapy on the other (5,6,7,8).

In order to evaluate the mechanisms which cause an increased [^{13}N]L-glutamate uptake by malignant tissue, previous work was dedicated to elucidate the role of tumor perfusion (9). In different lines of tumor trans-

plants as well as in human neoplasms, blood-flow-limited radionuclide uptake was found, i.e., high tumor-to-muscle ^{13}N uptake was observed when the tumor-to-muscle perfusion ratio attained the same high value, and low perfusion values coincided with low [^{13}N]L-glutamate uptake. This inter-relationship, however, was only established for nontreated tumors. In order to better understand the value of [^{13}N]L-glutamate scintigraphy in therapy control, it appeared necessary to monitor perfusion changes and [^{13}N]L-glutamate uptake changes in the same individual before and after therapeutic intervention. The present study reports on the effect of a single-dose irradiation on these parameters.

MATERIAL AND METHODS

Radionuclide Production and Syntheses

1. The ^{13}N activity was produced by the ^{16}O (p, α) ^{13}N reaction according to the method described by Vaalburg and co-workers (10). Typical yields (15 min irradiation at 10 μA) were ~ 100 mCi $^{13}\text{NH}_3$. The conversion of $^{13}\text{NH}_3$ and α -ketoglutarate into [^{13}N]L-glu-

Received Dec. 3, 1985; revision accepted April 4, 1986

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tamate was first described by Gelbard et al. (4). Details of our procedure have been reported previously (9). The specific activity obtained was 590 mCi/ μ mole glutamate.

2. No-carrier-added 1- ^{11}C -n-butanol (9,11) was employed as a freely diffusible tracer with appreciable tissue retention and equally good solubility in lipid and aqueous components. The precursor $^{11}\text{CO}_2$ was produced through the $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$ reaction by irradiation of pure nitrogen gas with 14.0 MeV protons. With a beam current of 15–20 μA and an irradiation time of 30–40 min, 700–900 mCi $^{11}\text{CO}_2$ was produced. The synthesis of 1- ^{11}C -n-butanol was based on the carboxylation of n-propylmagnesium chloride with $^{11}\text{CO}_2$ and subsequent reduction of the resulting free (1- ^{11}C) butyric acid with LiAlH_4 dissolved in anhydrous ether. Detailed information on the synthesis (12) as well as the methodological description and validation of the use of this compound for blood-flow estimation has been published elsewhere (11).

Animal Experiments

Tumor cell suspensions of the Walker 256 carcinoma were injected i.m. into the hind leg of 18 Sprague-Dawley rats, 5–8 days prior to use. When first investigated, the legs bearing these inocula attained diameters of 16–28 mm (diameters of contralateral legs: 11–12 mm). Fourteen rats were irradiated while four had injections of 8.0 mg/kg 5-hydroxytryptamine (5-HT), an agent which is able to reduce the blood flow of the Walker carcinoma significantly without important effects on the systemic circulation (13). In each animal, both radiotracers [^{13}N]L-glutamate and [^{11}C]butanol were injected before and after the intervention. The musculature of the healthy leg, contralateral to the tumor, served as reference tissue.

For measurements and interventions the rats were anesthetized with Inactin[®] (60–80 mg/kg of a 5% solution) and Pancuronium[†] (1 mg/kg) injected i.p. A mixture of O_2 and N_2O (1:3) together with 0.3–0.5 vol% halothane was delivered to the trachea through a polyethylene tube. The output was 56–64 ml/min at a pumping rate of 70–80/min.

For local irradiation, the tumors were fixed at a position 75 cm from a cobalt-60 source[‡] equipped with a tungsten collimator to limit the irradiation field. The dose rate at this distance was about 110 rad/min, the exposure period was chosen 7.5 min. In the nonirradiated control rats, 5-HT creatinine sulfate[§] in 0.5 ml isotonic saline was injected i.p. at a dose of 4.0 mg/kg, immediately after the first and 5–10 min prior to the second radionuclide measurement.

Radionuclide Studies

One millicurie [^{13}N]L-glutamate and 3–5 mCi [^{11}C]butanol were successively injected by tail vein in 0.5 ml saline solution with a delay of 5–10 min. Imaging was

performed with a single-crystal gamma camera equipped with a pinhole collimator with additional shielding against the 511 keV positron annihilation photons. Therefore, a 5 cm thick lead plate was positioned around the pin-hole. Data acquisition began with the injection of the tracer using a framing rate of 1/10 sec for 5 min.

The impulses from the tumor activity were divided by those from a segment of the contralateral leg (containing muscle). Tumor and control area were graphically defined in the scintigrams by equal size regions of interest (ROIs). The tumor-to-muscle quotient was plotted and used as a parameter of [^{13}N]L-glutamate uptake and, in the case of [^{11}C]butanol; of flow. Apart from constant values, only decreasing slopes were observed for both radioagents. In cases where tumor-to-muscle ratios varied during the observation period, the initial value was taken. To determine this value, the 90–300 sec slope postinjection was fitted (11). This procedure allowed the elimination of random errors due to low initial values of the denominator.

The processing of quotients, instead of dealing with absolute uptake data, eliminates variations of the arterial input function and renders arterial blood sampling superfluous. Since sampling techniques may produce a distortion of the slope of the arterial input curve (14), resulting in additional experimental errors, it can be assumed that the abandonment of absolute uptake data in μCi per gram tissue is of advantage in view of our questions posed.

In all instances, whether [^{13}N]glutamate or [^{11}C]butanol uptake was determined, identical ROIs were used for both measurements and before and after irradiation or pharmacological intervention. Residual activity was considered as follows. The activity before injection was extrapolated and subtracted from the recording and errors were minimized by the above-mentioned sequence of doses used. Furthermore, the decay-corrected background activity showed only minor minute-to-minute changes.

RESULTS

Prior to interventions the [^{13}N]L-glutamate tumor-to-muscle uptake was proportional to the tumor-to-muscle perfusion. The uptake values for both radioagents [^{13}N]glutamate and [^{11}C]butanol (Fig. 1) showed a linear correlation represented by $y = 0.117 + 0.915x$ with $r = 0.97$, a relationship which approximates the line of identity. The average [^{13}N]glutamate tumor-to-muscle uptake of the total animal population was 4.82 ± 2.47 (s.d., $N = 18$), the average value for [^{11}C]butanol was 5.14 ± 2.63 .

Fourteen animals of this group were irradiated. The values of the radionuclide tumor-to-muscle uptake before and after irradiation are listed in Table 1. On

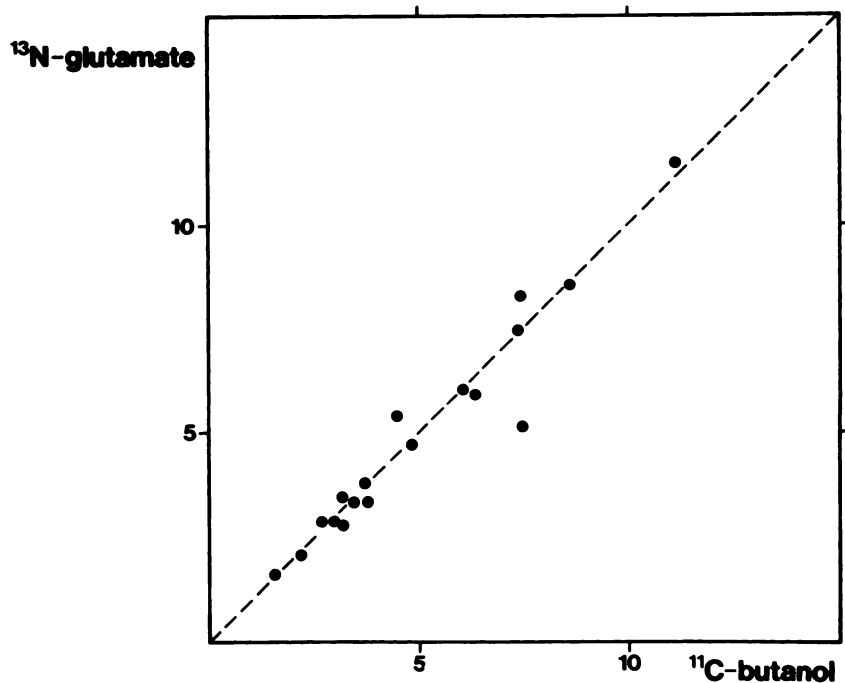


FIGURE 1
Correlation between [¹³N]glutamate uptake and blood flow as determined by [¹¹C]butanol uptake in untreated Walker carcinomas. $r = 0.97$

average, [¹³N]glutamate uptake decreased from 4.30 ± 0.66 (s.e.m.) to 3.06 ± 0.36 ($p < 0.005$), 30 min postirradiation. An example is given in Fig. 2. Two days postirradiation [¹³N]glutamate uptake recovered and attained 4.04 ± 0.67 .

Regarding the behavior of perfusion, [¹¹C]butanol

uptake changed in the same irradiated rats insignificantly from 4.69 ± 0.75 to 5.52 ± 0.89 (30 min; $p > 0.05$) and to 5.4 ± 1.28 (48 hr postirradiation) (Table 1; mean values). Its time-course is clearly discordant to that of [¹³N]glutamate uptake (Fig. 3).

Four rats received 4 mg/kg 5-HT. In these animals,

TABLE 1
Radionuclide Tumor-to-Muscle Uptake Before and After Irradiation

Diameter (T/M)	Before irradiation			30 min after irradiation			Diameter (T/M)	2 days after irradiation		
	[¹³ N]glu	[¹¹ C]but	¹³ N/ ¹¹ C	[¹³ N]glu	[¹¹ C]but	¹³ N/ ¹¹ C		[¹³ N]glu	[¹¹ C]but	¹³ N/ ¹¹ C
28/12	3.3	3.5	0.93	2.3	3.4	0.69	30/12	1.8	1.9	0.94
26/12	2.9	2.8	1.05	2.9	3.7	0.79	34/12	2.2	2.27	0.96
21/11	3.1	2.9	1.07	2.3	2.6	0.88	30/11	3.6	12.3	0.29
19/11	1.6	1.7	0.91	1.4	1.8	0.77	23/11	1.0	1.0	1.00
17/11	3.4	3.2	1.05	2.2	7.2	0.31	16/11	4.0	3.8	1.05
20/11	3.8	3.8	1.00	2.5	3.0	0.83	19/11	6.7	5.9	1.12
16/12	2.8	3.2	0.88	2.3	3.9	0.59	-	-	-	-
18/12	2.1	2.2	0.95	1.4	2.5	0.56	-	-	-	-
22/11	11.0	11.3	0.97	5.6	14.0	0.40	24/11	6.5	8.5	0.76
23/11	5.1	7.5	0.68	3.6	5.5	0.65	-	-	-	-
21/11	4.5	5.4	0.83	4.0	7.2	0.57	30/11	7.0	11.5	0.61
23/11	7.4	8.2	0.90	5.8	9.2	0.63	-	-	-	-
20/11	6.0	6.1	0.98	3.5	7.4	0.47	29/11	4.0	3.2	1.25
21/11	3.3	3.8	0.87	3.0	5.9	0.51	27/11	3.7	3.6	1.03
Mean value	4.3	4.7	0.93	3.1 [†]	5.5 [‡]	0.62 [§]		4.0	5.4	0.90
Standard deviation	2.5	2.8	0.10	1.4	3.3	0.17		2.1	4.0	0.28
Standard error of the mean	0.66	0.75	0.03	0.36	0.89	0.04		0.67	1.28	0.09

[†] Tumor-bearing thigh/contralateral thigh (mm).

[‡] $p < 0.005$ Compared with value before treatment (Student's t-test for paired values).

[§] N.S. Compared with value before treatment (Student's t-test for paired values).

[§] $p < 0.001$ Compared with value before treatment (Student's t-test for paired values).

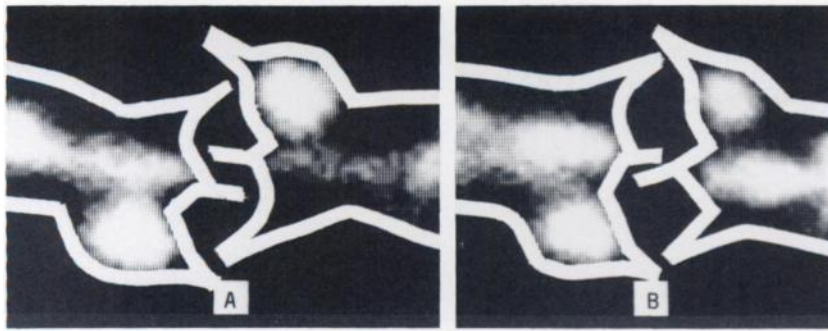


FIGURE 2
 $[^{13}\text{N}]$ glutamate uptake before (A) and 30 min after (B) irradiation. Each scintigram shows two animals: outlines of animals show part of trunks, left legs with tumors (spots of high activity) and right legs. Because of high activity contrast, right legs are not visualized with scaling used. Note decrease in tumor activity from A to B when compared with trunk

$[^{13}\text{N}]$ glutamate uptake dropped significantly from 6.60 ± 0.84 to 2.10 ± 0.60 ($p < 0.05$), while $[^{14}\text{C}]$ butanol uptake fell from 6.8 ± 0.78 to 2.08 ± 0.74 ($p < 0.05$). 5-HT induced parallel changes in both perfusion and $[^{13}\text{N}]$ glutamate, in contrast to irradiation.

When tumor-to-muscle uptake of both radioagents are related to each other, untreated animals showed ratios of 0.93 ± 0.03 (s.e.m.). This ratio dropped to 0.62 ± 0.04 ($p < 0.001$) 30 min postirradiation, and increased 2 days later to 0.90 ± 0.09 (Fig. 3). No significant change of this ratio was observed in animals treated

with 5-HT, while both nominator and denominator showed a dramatic decrease (Fig. 4).

DISCUSSION

Previous work suggested flow-limited uptake of $[^{13}\text{N}]$ glutamate by untreated tumors, since the tumor-to-muscle uptake ratio equaled that of tumor-to-muscle perfusion, in animals as well as in humans (9). Accordingly, similar tumor-to-muscle uptake of $[^{13}\text{N}]$ gluta-

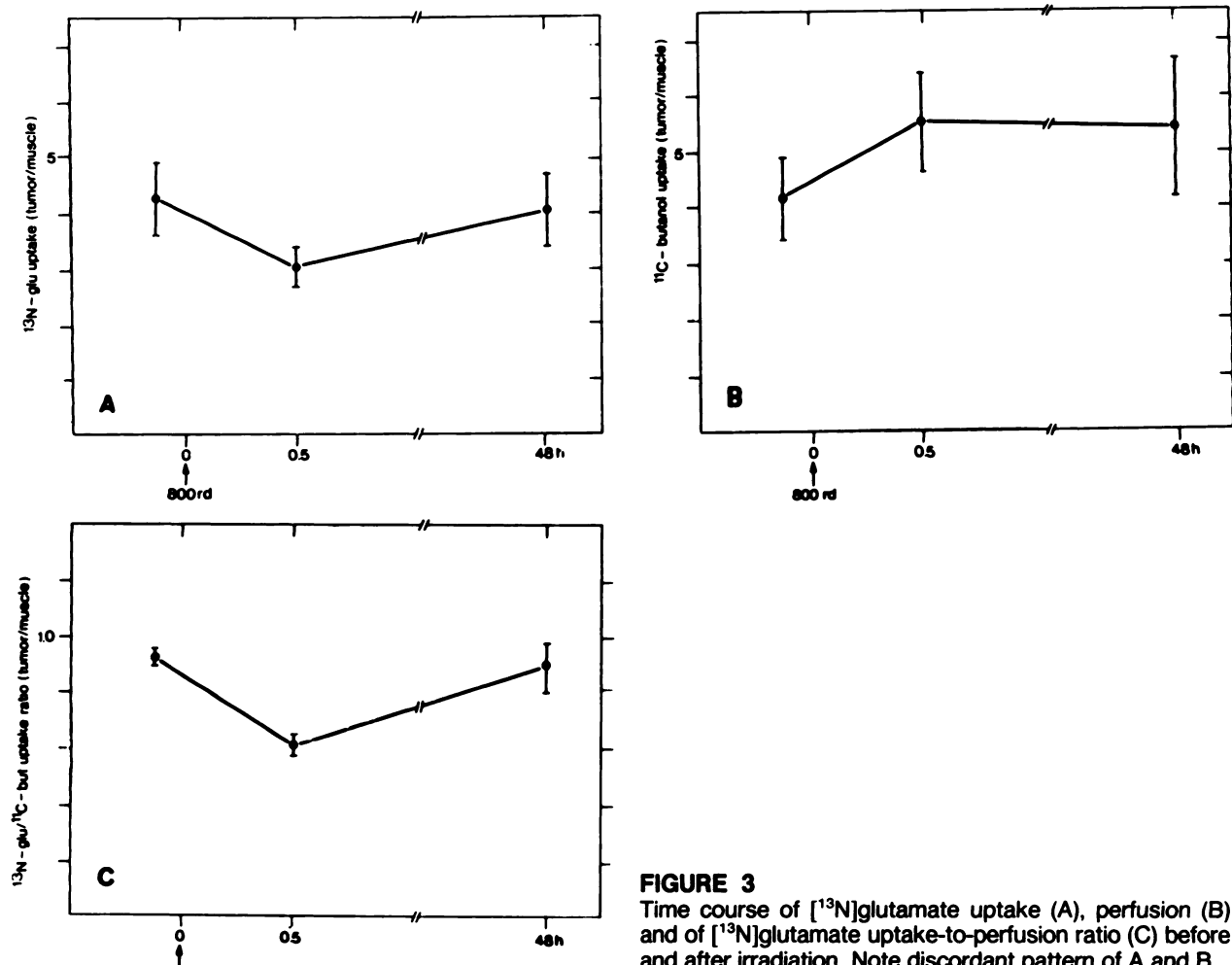


FIGURE 3
 Time course of $[^{13}\text{N}]$ glutamate uptake (A), perfusion (B) and of $[^{13}\text{N}]$ glutamate uptake-to-perfusion ratio (C) before and after irradiation. Note discordant pattern of A and B

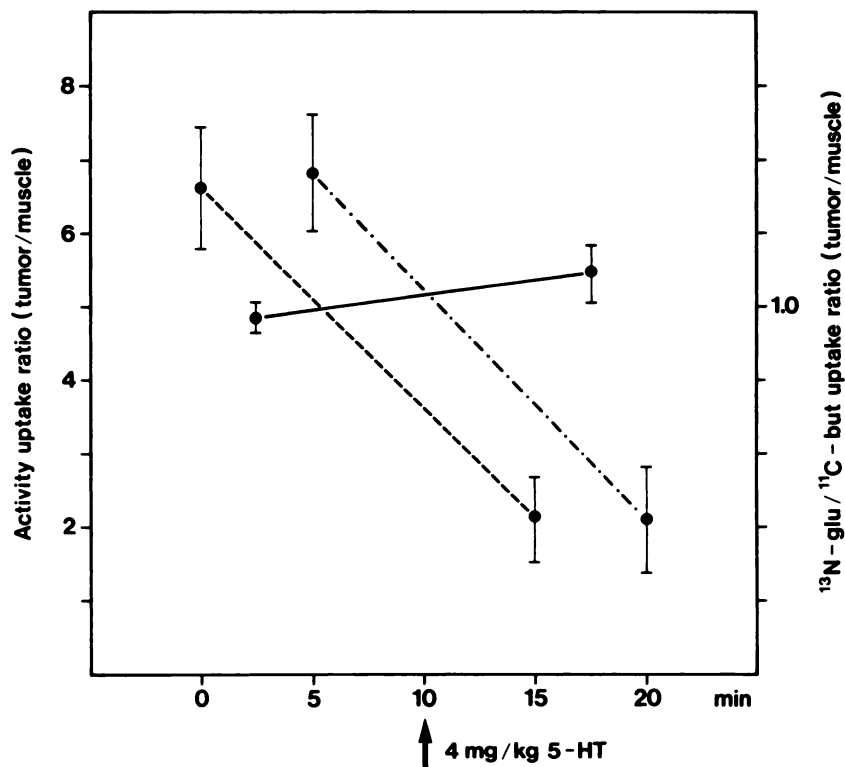


FIGURE 4
Time course of [¹³N]glutamate uptake, perfusion and of [¹³N]glutamate uptake-to-perfusion ratio before and after administration of vasoactive substance (5-HT). Note concordant pattern of substrate uptake and perfusion. (—) [¹³N]glu/[¹¹C]but uptake ratio (tumor/muscle); (---) [¹³N]glu uptake (tumor/muscle); (-.-.) [¹¹C]but uptake (tumor/muscle)

mate and [¹¹C]butanol was found in this study before interventions. In contrast to previous investigations, [¹¹C]butanol was employed for the first time systematically to assess perfusion together with substrate uptake. The use of this substance is certainly an advantage over the use of microspheres, the nobel-gas washout technique, or the use of thallium-201. The principal reasons are briefly summarized as follows.

1. No active transport is required for the tracer to be distributed in the intracellular space.
2. The uptake is not dependent on the vascular microarchitecture.
3. The tissue volume under investigation as well as the primary data (count rate within a given tissue volume) correspond exactly with those of the labeled-substrate study.

Physical and biological parameters influencing the tumor-to-muscle count rates of [¹³N]glutamate and [¹¹C]butanol were identical except that [¹³N]glutamate tissue uptake is dependent on active carrier systems and metabolism (1), while [¹¹C]butanol is freely diffusible. In flow-limited uptake, this difference in the uptake mechanism of both substances does not become relevant. Accordingly, the pretreatment relationship between [¹³N]glutamate and [¹¹C]butanol uptake was highly consistent and showed only a small variability.

Regarding the perfusion alone, marked differences between individual tumors were found. Similar variations in the same tumor model were reported based on different washout techniques (13,15,16). The same great variation in perfusion was obtained after irradiation.

Even the individual changes did not show any uniform pattern. This is in agreement with the results of various investigators showing partly increasing, partly decreasing flow response after x-ray irradiation, even when all experimental conditions were kept constant (17-19).

Data which could be directly compared with our results, however, are not available in the literature. At least daily intervals were commonly used to establish time sequences of irradiation effects. No measurements have been made as early as 30 min postirradiation. Furthermore, most investigations dealt with the change in blood volume as a parameter for vascular effects instead of dealing with flow changes. Despite the principal methodological differences, some conclusions on blood flow can be drawn from the reported data. Single low-dose irradiations tend to increase tumor blood flow temporarily (20-23,24,17). After a few days, flow tends to decrease again (20,21,25,26). A similar pattern was found in human tumors in which blood flow increased during the first week and decreased during the second week of radiotherapy (27).

Data on the uptake of substrates following irradiation are not available in the literature. The dramatic decrease of [¹³N]glutamate uptake by the tumor as early as 30 min postirradiation underscores the adequacy of the widely accepted hypothesis which attributes the effect of ionizing radiation on tumor to both direct action on the cells and indirect action mediated by the tumor's blood vessels and stroma (18).

Regarding the great individual variability not only of

perfusion changes but also of changes in [¹³N]glutamate uptake, it appears noteworthy that the perfusion-related [¹³N]glutamate uptake values behave more consistently, particularly following interventions. The ratio between [¹³N]glutamate and [¹¹C]butanol tumor uptake—both related to muscle—approximated unity before treatment. Thirty minutes postirradiation this ratio decreased to 0.62 on average. Since this ratio expresses the discordance of the distribution of [¹³N]glutamate and the freely diffusible [¹¹C]butanol and since the capillary walls do not limit the distribution of amino acids (1), it is concluded that the transport into the cellular compartment becomes the limiting factor for [¹³N]glutamate uptake. Thus, the ratios mentioned indicate that low-dose irradiation causes a temporary conversion from flow-limited to transport-limited substrate uptake, or that the primary radiation effect decreases the ability of the cell to utilize the substrates supplied by the blood stream. This can be due to damage to the active transport systems involved in the intracellular transport of amino acids (1) and/or to a decrease in the intermediary metabolism of the cell. The most important functions of glutamate in the intermediary metabolism are to provide the NH₂ group for transamination reactions and the carbon skeleton for the citrate cycle (28,29). A relatively small amount is incorporated in protein synthesis. It is understood that glutamate utilization decreases with a decrease in metabolic rates of the pathways mentioned. In this case, the net glutamate transport into the cell and, thus, [¹³N]glutamate retention may be metabolically inhibited.

The adequacy of the [¹³N]glutamate/[¹¹C]butanol index to express changes of uptake kinetics is underlined by the results of the control experiments in which 5-HT was administered. This substrate revealed a highly selective vasoactive effect on different tumor lines (13). Both interventions, irradiation, and administration of 5-HT, produced a decrease of [¹³N]glutamate uptake by the tumor. In contrast to the irradiation effect, however, 5-HT did not change the [¹³N]glutamate/[¹¹C]butanol index.

In conclusion, radiation therapy causes [¹³N]glutamate uptake to be uncoupled with respect to flow. In low-dose irradiation, damage to the cellular integrity occurs already when microcirculation is not yet impaired. Thus, [¹³N]glutamate appears to be a more sensitive indicator of the response of tumor tissue to radiotherapy than a mere flow tracer. Furthermore, the double-tracer method described offers a means for therapy research to monitor cellular and vascular effects separately.

FOOTNOTES

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ACKNOWLEDGMENT

The authors thank L. Gerlach for expert technical assistance.

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