

methionine or leucine is more suitable for hyperglycemic patients.

## ACKNOWLEDGMENTS

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# Intrathecal 5-[<sup>125</sup>I]Iodo-2'-Deoxyuridine in a Rat Model of Leptomeningeal Metastases

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The antitumor effect of 5-[<sup>125</sup>I]iodo-2'-deoxyuridine (<sup>125</sup>IUdR) was examined in a rat model of leptomeningeal metastases. In this model, 50% of rats develop paralysis of hind limbs in 9.20 ± 0.02 days and die in 12.1 ± 2.1 days after intrathecal (i.t.) implantation of 5 × 10<sup>5</sup> 9L rat gliosarcoma cells. **Methods:** Three days after implantation of 9L gliosarcoma cells, <sup>125</sup>IUdR was administered intrathecally to rats as: (a) a single injection (500 μCi/rat), (b) five daily injections (100 μCi/day) or (c) a continuous 5-day infusion (0.5 μl/hr, total of 500 μCi), and the animals were monitored for the onset of paralysis. Control groups received physiologic saline. For biodistribution studies, rats received a bolus injection of <sup>125</sup>IUdR (10 μCi) 5 days after tumor-cell implantation and were killed 1, 8, 24, and 48 hr later. Tissues and organs, including the spinal cord, were isolated and their radioactive content determined. The results were expressed as percent injected dose per gram of wet tissue. Histological sections of the spinal cord were also prepared and used for autoradiographic detection of DNA-incorporated <sup>125</sup>IUdR. **Results:**

Treatment with i.t. administered <sup>125</sup>IUdR (500 μCi/rat) significantly (p ≤ 0.005) prolonged the median time of paralysis to 11.2 ± 0.1, 12.3 ± 0.1 and 15.2 ± 0.4 days for the single-dose, five daily injections and continuous infusion groups, respectively. Radioactivity cleared rapidly from all tissues except the thyroid and tumor cells growing within the spinal cord. Autoradiography demonstrated that normal cells in the tumor-bearing spinal cord were void of radioactivity. **Conclusion:** The results suggest that a selective antitumor effect could be achieved in treating leptomeningeal metastases with i.t. administered <sup>125</sup>IUdR.

**Key Words:** leptomeningeal metastases; intrathecal tumor; iodine-125-IUdR; gliosarcoma

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Leptomeningeal metastases are a serious complication of cancer characterized by neurologic dysfunction at multiple levels of the neuraxis. This disease develops in 5%-8% of patients with solid tumors, in 5%-29% of patients with non-Hodgkin's lymphoma and in 11%-70% of patients with leukemia (1,2). The prognosis of patients who develop leptomeningeal

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geal metastases is poor. Without therapy, the median survival is 4–6 wk. With current treatment regimens, the median survival is 3–6 mo (1–3).

Iodine-125 is a prolific emitter of low-energy (<1 keV) electrons (~20 electrons per decay) that dissipate their energy typically within nanometer distances from the decay site (4,5). Consequently, the biologic toxicity of this Auger electron emitter resembles that of high-LET radiations (exponential decrease in survival of mammalian cells) when  $^{125}\text{I}$  decays in close proximity to DNA (5,6–8). The thymidine analog 5-iodo-2'-deoxyuridine radiolabeled with  $^{125}\text{I}$  is readily incorporated into DNA of proliferating cells during DNA synthesis. The DNA-incorporated  $^{125}\text{IUdR}$  is retained in the cells and their progeny and has been shown to be extremely radiotoxic to cells (5–7). Intravenously administered  $^{125}\text{IUdR}$ , however, is unlikely to be useful as an antitumor agent because of its nonspecific uptake by all proliferating cells (9,10) and its rapid dehalogenation ( $T_{1/2} = 5\text{--}7$  min) in the liver (9,11,12). On the other hand, locoregional administration of IUdR radiolabeled with the Auger-electron-emitting radionuclide  $^{125}\text{I}$  or  $^{123}\text{I}$  has been shown to be therapeutically effective in mice with intraperitoneal ovarian tumors (13,14) and in rats with solid brain tumors (15). In this investigation, we demonstrate the therapeutic effectiveness of intrathecally administered  $^{125}\text{IUdR}$  in a rat model of leptomeningeal metastases.

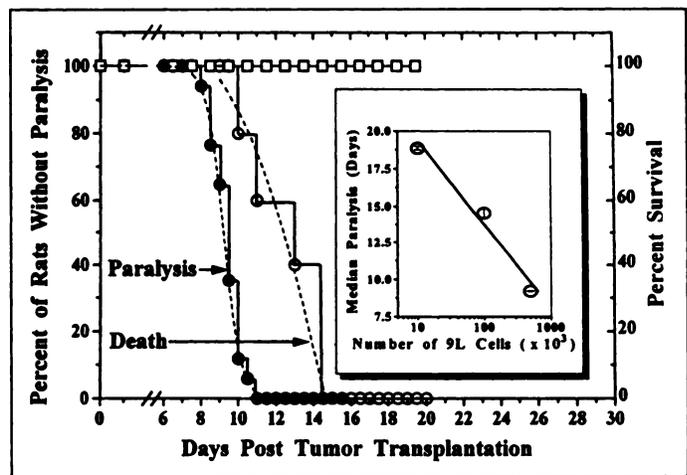
## MATERIALS AND METHODS

A modification of the leptomeningeal metastases model developed by Kooistra et al. (16) was used. Male CDF (Fischer 344) rats, weighing about 300 g, were anesthetized with an intraperitoneal injection of ketamine–xylazine–acepromazine maleate (75 mg–3.9 mg–0.75 mg per kg body weight). The anesthetized rats were secured on a special stand with their heads elevated and the long-axis of the body at a 90° angle. The atlanto-occipital membrane caudal to the external occipital protuberance in the neck region was surgically exposed and punctured using a 20-G needle. Approximately 8 cm of a polyethylene catheter (PE-10 tubing prethinned by stretching) was inserted through the puncture in the atlanto-occipital membrane into the subarachnoid space dorsal to the spinal cord. The external end of the catheter was sealed and tied under the skin before closing the wound in three layers. Rats were observed for 1 wk and only those rats free of any signs of paralysis were used in the study.

Monolayers of exponentially growing 9L gliosarcoma cells (17) were trypsinized, washed and suspended in phosphate-buffered saline, pH 7.2, and  $5 \times 10^5$  cells were implanted intrathecally in rats ( $n = 17$ ) in a bolus of 5- $\mu\text{l}$  volume through the catheter already in place, which was then flushed with 10  $\mu\text{l}$  0.9% saline. The cell suspension was replaced with phosphate-buffered saline, pH 7.2, in the nontumor-bearing rats ( $n = 9$ ). The rats were observed daily for symptoms of paralysis, defined as the inability to walk and to stand on all four limbs. All rats that developed paralysis of the hind limbs were killed except in one experiment where death caused by the growth of intrathecal tumor was recorded. In this experiment, food and water were lowered into the cage so they could be easily accessed by the paralyzed rats. Routine laboratory techniques were used to prepare histology slides of tumor-bearing spinal cords.

Carrier-free  $^{125}\text{IUdR}$  was prepared by a method developed in this laboratory (18), solubilized in 0.9% saline and sterilized by 0.22- $\mu\text{m}$  Millipore filtration before use.

Biodistribution studies using  $^{125}\text{IUdR}$  were performed in tumor-bearing ( $n = 9$ , injected i.t. with  $5 \times 10^5$  9L gliosarcoma cells) and in nontumor-bearing control ( $n = 10$ , injected i.t. with 5  $\mu\text{l}$  PBS) rats. In these experiments, each rat received 10  $\mu\text{Ci}$  of  $^{125}\text{IUdR}$  (a bolus of 5- $\mu\text{l}$  volume, i.t. injection) five days after i.t. administra-



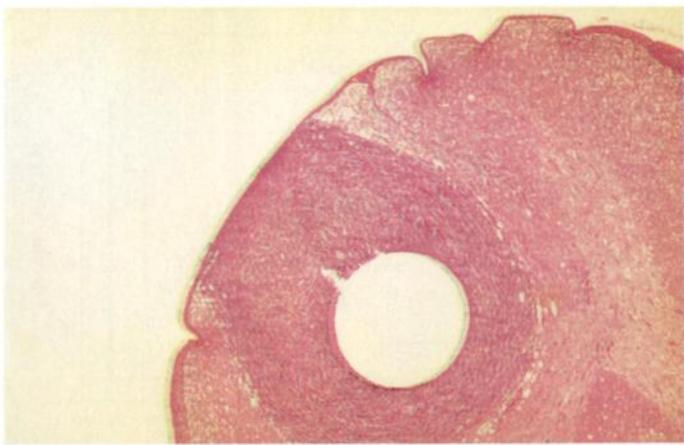
**FIGURE 1.** Paralysis (●) and death (○) of rats ( $n = 17$ ) injected intrathecally with  $5 \times 10^5$  9L gliosarcoma cells. Control rats ( $n = 9$ ) injected intrathecally with saline (□). Insert contains data previously obtained indicating relationship between number of tumor cells injected and onset of paralysis.

tion of the 9L tumor cells or PBS. The rats were killed at 1, 8, 24 and 48 hr after  $^{125}\text{IUdR}$  injection. Various tissues and organs were isolated, blotted and weighed; their radioactive content was determined in a gamma counter, and the results were expressed as percent injected dose per gram of wet tissue. The entire spinal column of each rat was also excised and fixed in 10% buffered formalin before isolating the spinal cord from the surrounding bones and measuring the radioactive content in 1-cm slices. Forty-eight hours after the  $^{125}\text{IUdR}$  i.t. injection, histological sections (6- $\mu\text{m}$  thick) of fixed spinal cords from tumor-bearing rats were also prepared and used for the autoradiographic detection of DNA-incorporated  $^{125}\text{IUdR}$ . To this end, the slides were washed in methanol at  $-20^\circ\text{C}$ , coated in NTB emulsion (Kodak), and stored at  $4^\circ\text{C}$ . After 14 days of storage in total darkness, the slides were developed with Kodak developer D19 for 3 min at  $15^\circ\text{C}$ , fixed with Kodak fixer for 5 min, and washed and stained with hematoxylin-eosin. Finally, the slides were dehydrated and mounted in Permount.

Treatments of i.t. tumors with  $^{125}\text{IUdR}$  were performed in four groups of 11–12 rats with a total dose of 500  $\mu\text{Ci}$  of  $^{125}\text{IUdR}$ /rat administered i.t. as: (a) a single injection (20  $\mu\text{l}$ ), (b) five daily injections (100  $\mu\text{Ci}/20 \mu\text{l/day}$ ) or (c) a continuous 5-day infusion (0.5  $\mu\text{l/hr}$ , 20  $\mu\text{l}$ ) using micro-osmotic pumps. Rats in the control group received 0.9% saline i.t. The treatments were started 3 days after  $5 \times 10^5$  9L gliosarcoma tumor cells were injected i.t. into each rat. All i.t. injections or infusions were performed through the i.t. catheter already in place. Rats were monitored daily for symptoms of paralysis. The median duration for the onset of paralysis in each group of rats was statistically compared using the Mantel-Haenszel (log-rank) test for comparison of survival curves.

## RESULTS

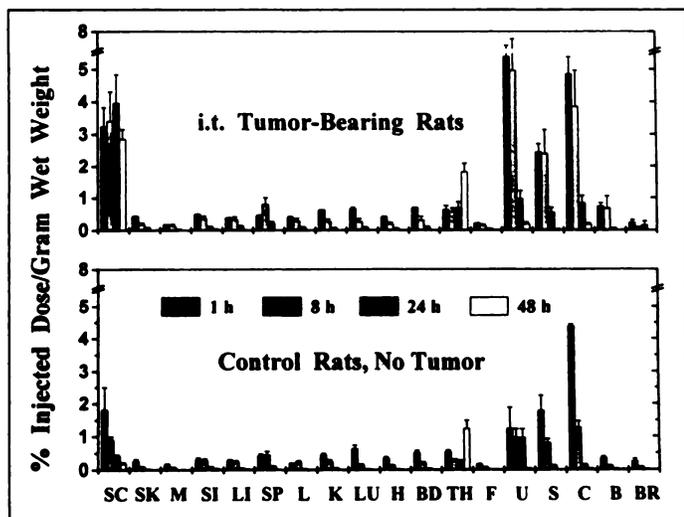
Approximately 95% of rats implanted i.t. with catheters into the subarachnoid space remained free of any sign of paralysis in the immediate period after the surgical procedure. The onset of paralysis in the hind limbs of these rats was caused by i.t. growth of tumor cells and was predictable (Fig. 1). For example, 50% of the rats that received  $5 \times 10^5$  tumor cells i.t. were paralyzed in  $9.20 \pm 0.02$  days and died in  $12.1 \pm 2.1$  days, whereas none of the nontumor-bearing control animals with i.t. catheter and injected i.t. with saline were paralyzed or died during 20 days of observation. Furthermore, paralysis occurred at an earlier time when the number of transplanted tumor cells was increased (insert, Fig. 1).



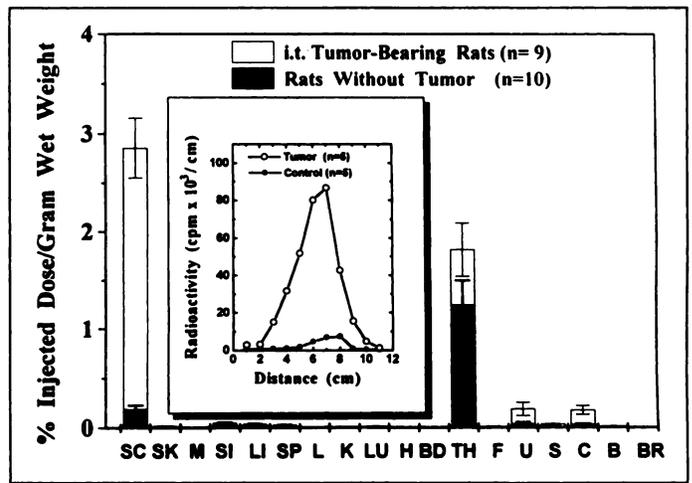
**FIGURE 2.** Photomicrograph of spinal cord of 9L-gliosarcoma-bearing rat showing catheter and surrounding tumor.

Histological examination of spinal cords revealed the bulk of the tumor mass was around the tip of the catheter. The tumor was confined to the subarachnoid space around the catheter and in various places encircled the spinal cord and compressed it (Fig. 2). In a few instances, tumor cells infiltrated the white matter and grew around the blood vessels (data not shown).

In both tumor- and nontumor-bearing rats, the radioactivity from i.t.-administered  $^{125}\text{I}$ UdR was cleared over time from all normal tissues with the exception of the thyroid (Figs. 3 and 4). The percentage of the injected dose in the urine, stomach, and blood of tumor-bearing rats appeared to be higher than that in the controls, possibly reflecting increased leakage of radioactivity into the systemic circulation due to the presence of tumor within the intrathecal space. In tumor-bearing animals, 3%–4% of the injected radioactive dose remained associated with the spinal cord. At 48 hr, the percent injected dose per gram of the spinal cords from tumor-bearing ( $2.94 \pm 0.48$ ) and nontumor-bearing ( $0.23 \pm 0.06$ ) rats was significantly different ( $p \leq 10^{-6}$ , Student's paired t-test). When the radioactive content of 1-cm consecutive sections of spinal cord was plotted as a function of distance from the cranial apex of the excised spinal cord, the radioactivity (area under the curve) in tumor-bearing rats was ~17-fold greater than that of nontumor-bearing rats

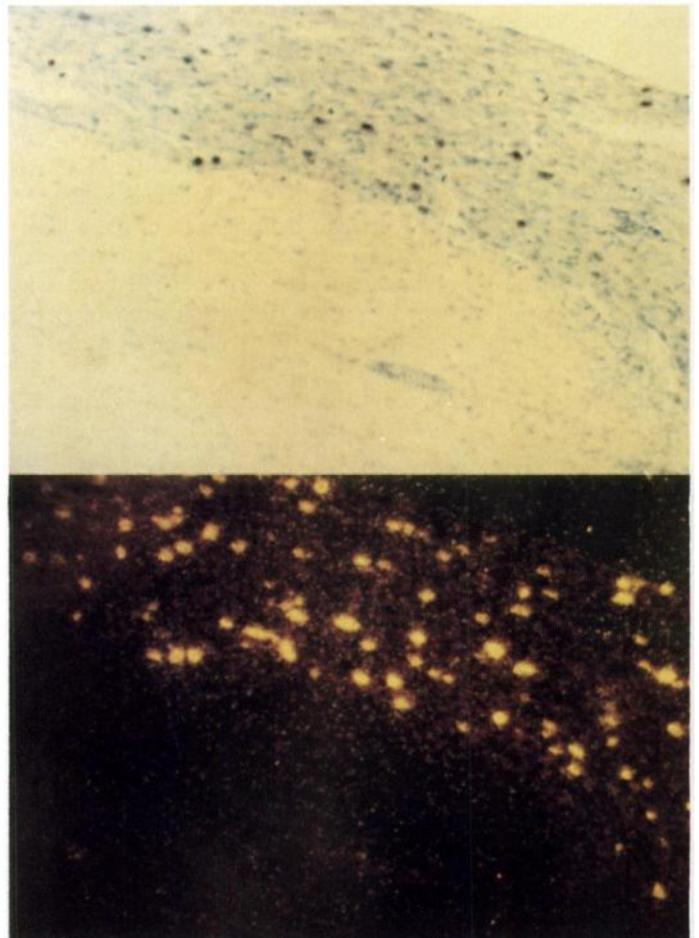


**FIGURE 3.** Biodistribution of  $^{125}\text{I}$  in 9L-gliosarcoma-bearing and control rats after intrathecal administration of  $10 \mu\text{Ci } ^{125}\text{I}$ UdR. SC = spinal cord; SK = skin, M = muscle, SI = small intestine, LI = large intestine, SP = spleen, L = liver, K = kidney, LU = lung, H = heart, BD = bladder, TH = thyroid, F = femur, U = urine, S = stomach, C = stomach contents, B = blood, BR = brain.



**FIGURE 4.** Comparison of biodistribution of  $^{125}\text{I}$  in 9L-gliosarcoma-bearing and control rats 48 hr after intrathecal administration of  $10 \mu\text{Ci } ^{125}\text{I}$ UdR. Insert indicates distribution of  $^{125}\text{I}$  along spinal cord (0 cm = neck region) of 9L-gliosarcoma-bearing (○) and control (●) rats at 48 hr. See Figure 3 for abbreviations.

(insert, Fig. 4). The peak activity seemed to correspond to the position of the catheter tip where the bulk of the tumor mass was located. Autoradiography of the tissue sections from tumor-bearing spinal cords showed no radioactivity associated with normal cells of the spinal cord: silver grains were associated only with tumor cells (Fig. 5). Since any unbound radioactivity was washed away during processing of tissue



**FIGURE 5.** Autoradiograph of thin section from spinal cord of 9L-gliosarcoma-bearing rat 48 hr after intrathecal administration of  $10 \mu\text{Ci } ^{125}\text{I}$ UdR, light (top) and dark (bottom) fields.

**TABLE 1**  
Induction of Paralysis (days) in Rats Bearing Intrathecal Tumors After Intrathecal Administration of 500  $\mu\text{Ci}$   $^{125}\text{I}$ UdR\*

Treatment	No. of animals	Median of paralysis $\pm$ s.e.m.	Range	% Increase in median paralysis	p value <sup>†</sup>
Saline	11	9.0 $\pm$ 0.1	4	0.0	—
$^{125}\text{I}$ UdR					
single injection	12	11.2 $\pm$ 0.1	3	24.4	$\leq$ 0.005
five daily injections	12	12.3 $\pm$ 0.1	6	36.7	$\leq$ 0.005
continuous five-day infusion	11	15.2 $\pm$ 0.4	11	68.9	$\leq$ 0.005

\*Rats were treated three days after i.t. injection of  $5 \times 10^5$  tumor cells. Control rats received i.t. saline.

<sup>†</sup>Values were determined by Mantel-Haenszel (log-rank) test for comparison of survival curves.

sections for autoradiography, these grains reflect DNA-incorporated radionuclide.

Therapy with  $^{125}\text{I}$ UdR in tumor-bearing rats was started 3 days after i.t. inoculation of  $5 \times 10^5$  tumor cells. The results of these experiments are summarized in Table 1 and illustrated in Figure 6. In the experiment shown, 50% of the control rats (with i.t. tumor and treated with i.t. saline injection) developed paralysis on  $9.0 \pm 0.1$  days. In comparison, the onset of paralysis in tumor-bearing rats treated with  $^{125}\text{I}$ UdR (single i.t. administration—500  $\mu\text{Ci}$ , five daily i.t. injections—100  $\mu\text{Ci}/\text{day}$  or 5-day i.t. infusion—500  $\mu\text{Ci}$ ) was significantly prolonged. In this study, the time to median paralysis was  $11.2 \pm 0.1$ ,  $12.3 \pm 0.1$  and  $15.2 \pm 0.4$  days, respectively, for the three experimental groups. The paralysis curves for all three treatments were statistically significant ( $p \leq 0.005$ ) according to the Mantel-Haenszel test. However, all of the tumor-bearing rats eventually developed paralysis and were killed.

## DISCUSSION

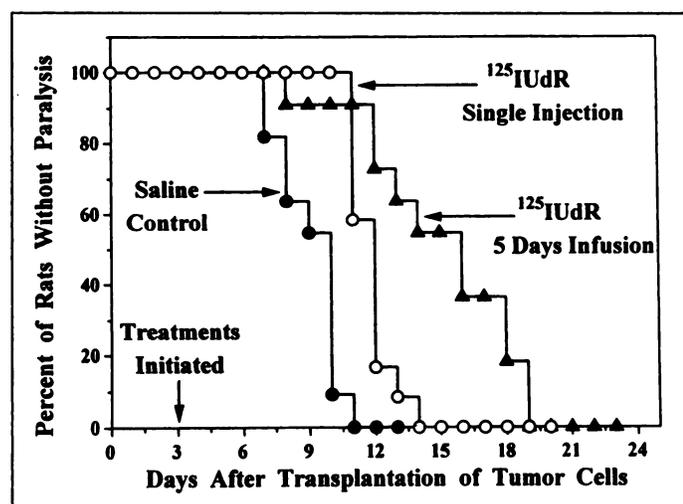
In the past two decades, efforts to develop therapy for leptomeningeal metastases have continued to be unsuccessful. The difficulty lies in the need to treat the entire neuraxis, since tumor cells are disseminated throughout the subarachnoid space in close proximity to neural structures. Consequently, various intrathecal therapies are being explored, including radioiodi-

nated monoclonal antibodies directed against tumor-associated antigens (19), gene therapy (20) and chemotherapy (3,21–23). All these attempts have been only partially effective. Although treatment often provides good local control, leptomeningeal metastases usually occur in the setting of systemic relapse, and patients eventually die of their systemic disease. Parallel efforts are needed to find therapies for systemic disease as well as for leptomeningeal metastases.

The radionuclide  $^{125}\text{I}$  causes predominantly double-strand breaks (dsb) in DNA when  $^{125}\text{I}$  decays in close proximity to DNA (24) or when it is incorporated into nuclear DNA (25–30). While most of these dsb seem to be repaired at the same rate as those observed with gamma rays (31), the decay of this radionuclide after DNA incorporation in the form of  $^{125}\text{I}$ UdR is extremely toxic to cultured mammalian cells (5–7). In comparison, when  $^{125}\text{I}$  decays outside the cell nucleus, it is quite innocuous (5,32,33). In fact, in cultured cells, the relative biological effectiveness of  $^{125}\text{I}$ UdR compared with that of conventional gamma irradiation is  $\sim 8$ -fold greater when  $^{125}\text{I}$ UdR is DNA-incorporated and less than twofold greater when it decays outside the cell nucleus (5,7,33,34). While these biophysical characteristics demonstrate the high toxicity of  $^{125}\text{I}$ UdR and therefore its suitability as a radiotherapeutic agent, the nonspecificity of this cycle-dependent agent (i.e., taken up by all dividing cells) may limit its utility to those cancers where local or regional administration is feasible.

In this report, 3%–4% of the administered radioactivity was associated with the spinal cords of tumor-bearing rats after 48 hr. It is important to note that these percent injected dose per gram values greatly underestimate the actual tumor uptake of  $^{125}\text{I}$ UdR because the tumor mass constitutes only a small fraction of the weighed spinal cord. Moreover, the DNA-incorporated activity can be enhanced by the administration of various thymidylate synthetase antimetabolites. For example, both 5-fluorodeoxyurine (35) and methotrexate (Kassis AI, Adelstein SJ, unpublished results) have been shown to increase the DNA incorporation of  $^{125}\text{I}$ UdR in mammalian cells. Since methotrexate is currently used in the clinic for therapy of tumors within the spinal cord (3,21), the potential of this combined therapy should be explored.

The data suggest the therapeutic effectiveness of intrathecally administered  $^{125}\text{I}$ UdR against intrathecal gliosarcoma tumors in a rat model. However, while the total dose of  $^{125}\text{I}$ UdR was equal in all cases, a direct comparison of the treatments cannot be made. Nevertheless, continuous infusion for 5 days was superior to single or five daily intrathecal injections of  $^{125}\text{I}$ UdR (Fig. 6), an observation that is consistent with the effects of cell-cycle-specific agents. Furthermore, five daily injections of  $^{125}\text{I}$ UdR had greater effect than the single injection ( $p \leq 0.05$ ). However, in the present experiments, there were no long-term survivors. The failure of  $^{125}\text{I}$ UdR to cure these rats may be due to the low growth fraction of intrathecally growing 9L tumor cells. DNA-incorporated  $^{125}\text{I}$ UdR activity was observed throughout the tumor mass (Fig. 5), clearly demonstrating that the low molecular weight IUDR molecule can diffuse throughout the cellular layers of the tumor and be incorporated into the DNA of tumor cells undergoing DNA synthesis. Similar problems may be encountered in patients with leptomeningeal metastases. In contrast, we have observed the survival of  $\sim 20\%$  of rats bearing intracranial 9L gliosarcoma solid tumors and mice with ascites ovarian tumors treated with locoregionally administered  $^{125}\text{I}$ UdR or  $^{123}\text{I}$ UdR (13–15). In the studies with rats bearing brain tumors (15), this may have been due to the fact that  $2 \times 10^4$  9L gliosarcoma cells were transplanted only 24 hr before  $^{125}\text{I}$ UdR intratumoral injection, while the intrathe-



**FIGURE 6.** Induction of hind-leg paralysis after intrathecal administration of 500  $\mu\text{Ci}$   $^{125}\text{I}$ UdR in rats bearing intrathecal 9L gliosarcoma. Data from group having five daily injections not shown.

cal tumors treated in our current studies were initiated with 25-fold more cells than used in the brain tumor model and all treatments were begun after 3 days. Consequently, the tumor burden in the intrathecal studies may have been approximately 75-fold that in the brain tumor studies. Unless the growth fraction of intrathecal tumors is unity (which it certainly is not), more of the tumor cells will escape from the cell-cycle-dependent toxicity of  $^{125}\text{I}$ UdR with an increase in tumor burden, leading to a diminished curability of the disease. Yet,  $^{125}\text{I}$ UdR treatment increased time to paralysis by 69% in our intrathecal tumor model. This fact suggests the therapeutic effectiveness of  $^{125}\text{I}$ UdR against intrathecal tumors that have advanced from the microscopic stage of the disease. Interestingly, the prolongation of survival in animal models observed using either intrathecally administered 4-hydroperoxycyclophosphamide or intrathecal gene therapy is similar in magnitude.

Being a cycle-dependent agent,  $^{125}\text{I}$ UdR incorporates itself into the DNA of any dividing cell and as such does not specifically target tumor cells. However, since (a) very few noncancerous cells within the central nervous system are cycling at any one time period and (b)  $^{125}\text{I}$ UdR is swiftly cleared and rapidly dehalogenated once it has entered the vascular space, the intrathecal administration of this radiopharmaceutical should lead to a high degree of specific uptake by intrathecally-growing tumor cells. Our biodistribution and autoradiography data corroborate these expectations; none of the normal proliferating cells in the tumor-bearing rats (i.e., skin, intestine, spleen, and bone marrow) incorporated  $^{125}\text{I}$ UdR. These results are consistent with those reported earlier after the intracerebral injection of  $^{125}\text{I}$ UdR in rats with brain tumors (36). The rapid accrual of large amounts of radioactivity in thyroid, urine, and stomach contents which is evident in the biodistribution data (Fig. 3) suggests the prompt release of  $^{125}\text{I}$ UdR from the subarachnoid space, its rapid dehalogenation, and the excretion of free radioiodine through urine and stomach.

## CONCLUSION

In the 9L gliosarcoma tumor rat model, intrathecal administration of the DNA precursor  $^{125}\text{I}$ UdR directs the incorporation of the Auger-electron-emitting radionuclide  $^{125}\text{I}$  selectively into dividing tumor cells, which are bathed by cerebrospinal fluid. This radiopharmaceutical is therapeutically effective against intrathecal tumor cells. Normal proliferating cells and tissues within the spinal cord and the remainder of the body escape the extreme toxicity of Auger electrons produced by the decay of  $^{125}\text{I}$  incorporated into DNA.

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