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ACTION OF CARBAMOYLATING AGENTS ON THE UPTAKE OF METABOLITES IN HEPATOMAS AND LIVER

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Abstract—The effects of 2-chloroethylisocyanate, ethylisocyanate and sodium cyanate on the uptake of isotope-labeled thymidine, leucine and H_2O were compared in rat liver and hepatomas. The data suggested that carbamoylating agents may have a common property of inhibiting uptake of compounds in hepatomas under conditions in which there is a smaller effect or no action in the liver of tumor-bearing rats. The distinction between tissues may have been mediated, in part, through effects on tumor circulation and was less apparent when isolated cells were studied *in vitro*. Preferential inhibitory effects of carbamoylating agents on the uptake of leucine and H_2O were also observed with a murine hepatoma, but they were not as great as with rat hepatomas.

Sodium cyanate has been observed to cause a preferential inhibitory effect on the incorporation of precursors into macromolecules of rat tumors relative to normal tissues [1–4]. These effects of sodium cyanate are accompanied by decreased uptake of the precursors [2, 4, 5] and a decreased rate of distribution of water and extracellular markers [6], which suggests that there may be effects of sodium cyanate on circulation in the tumors. Since evidence has been presented for NADPH-dependent metabolism of cyanate to an uncharacterized and unstable metabolite [7], it is not clear whether the actions of sodium cyanate on tumor metabolism were a response to carbamoylation or were secondary to reaction with a cyanate metabolite. In an attempt to determine whether the responses to sodium cyanate are a general feature of carbamoylating agents, we have studied the effects of two organic isocyanates on the distribution and incorporation of compounds in hepatomas.

Although we have seen marked inhibitory effects of sodium cyanate on the incorporation of ^{3}H-labeled thymidine into DNA of B16 melanoma transplanted in mice, we have not been able to demonstrate growth inhibitory effects on this tumor with sodium cyanate administered as a single agent [8]. In order to characterize further the tissue and species specificity of the action of sodium cyanate, we have studied the action of this compound in murine tumors. These data were compared with similar observations that had been made in tumor-bearing rats and suggest that greater effects may be seen in rat hepatomas. Preliminary reports of this work have been presented [9, 10].

MATERIALS AND METHODS

Animals and tumors. Morris hepatomas were transplanted in Buffalo-strain rats. The BH3 hepatoma was transplanted in BUB mice and the B16 melanoma and RIF-1 sarcoma were transplanted in C57BL and C3H mice respectively. The animals were maintained on a 12-hr light–dark cycle with food and water available *ad lib.* Partial hepatectomies were performed by excision of the median and left lateral lobes as described by Higgins and Anderson [11].

Reagents. Ethylisocyanate and 2-chloroethylisocyanate were obtained from the Aldrich Chemical Co., Milwaukee, WI. Aqueous solutions of these isocyanates were used within a few seconds after they were dissolved in ice-cold 0.9% NaCl in water. In preliminary studies we established that carbamoylation of proteins requires preparation of cold solutions for optimal activity and that this is more critical for 2-chloroethylisocyanate than for ethylisocyanate. Sodium cyanate was obtained from ICN Pharmaceuticals, Plainview, NY. L-[4,5-^{3}H]Leucine (44 Ci/mmol), [*methyl*-^{3}H]thymidine (62 Ci/mmol) and [$^3H_2$]O (100 mCi/ml) were purchased from ICN Radiochemicals, Irvine, CA.

Uptake and incorporation of isotope-labeled compounds. In studies on tumor-bearing animals, the rats and mice were killed by decapitation. Tissues were homogenized in water and adjusted to a final concentration of 1.0 N with respect to perchloric acid. The homogenate was centrifuged at 800 *g* for 10 min, and an aliquot of the acid-soluble supernatant fraction was used for liquid scintillation counting. Procedures for measuring incorporation into DNA and protein were performed as previously described [12]. In studies with regenerating liver, the uptake of [^{3}H]thymidine was determined 24 hr after partial hepatectomy.

Hepatoma cells from Morris hepatoma 7288CTC were prepared by collagenase digestion as previously described [13]. Hepatocytes were prepared by Dr. J. S. H. Yoo by a procedure adapted from the methods of Williams *et al.* [14] and Inmon *et al.* [15]. Incubations with [^{3}H]leucine were performed as previously reported [12].

Statistical analysis of data was done by Student's

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Table 1. Effect of 2-chloroethylisocyanate on the uptake of ^{3}H-labeled thymidine in rats bearing 7777 hepatoma

Treatment	Acid-soluble radioactivity (dpm/mg tissue)			DNA radioactivity (dpm/100 μg DNA)	
	Blood	Tumor	Liver	Tumor	Liver
Control (5)	526 ± 60	417 ± 27	1037 ± 56	2030 ± 137	199 ± 36
0.1 mmol/kg 2-chloroethylisocyanate (4)	550 ± 40	304 ± 70	1187 ± 68	1263 ± 550	224 ± 37
0.2 mmol/kg (5)	562 ± 65	40 ± 33*	664 ± 215	29 ± 26*	116 ± 66

Buffalo rats bearing s.c. transplants of 7777 hepatoma received i.p. injections of 0.9% NaCl (controls) or 2-chloroethylisocyanate (0.1 or 0.2 mmol/kg body wt). After 60 min, the rats received s.c. injections of [^{3}H]thymidine (250 μCi/kg) and were killed 20 min after the isotope injection. Data are presented as means ± SE for the number of animals given in parentheses for each treatment.

* $P < 0.05$, relative to controls.

t-test with a probability of less than 5% being considered significant.

RESULTS

Treatment of rats bearing subcutaneous transplants of Morris hepatoma 7777 with 0.2 mmole 2-chloroethylisocyanate per kg body weight caused a 90% inhibition of [^{3}H]thymidine uptake into the acid-soluble fraction of the tumors and a 99% inhibition of incorporation into DNA (Table 1). Under these conditions, a statistically significant effect was not detected in the host livers. The 60-min interval between administration of the isocyanate and injection of the isotope-labeled thymidine was chosen to make the study comparable with earlier work on the effects of sodium cyanate on thymidine uptake in rat hepatomas [1]. The study presented in Table 2 indicates that significant inhibitory effects on the uptake of [^{3}H]thymidine into the acid-soluble fraction of a rat hepatoma were observed when the isotope-labelled material was injected 10 min after administration of 0.2 mmol ethylisocyanate or 2-chloroethylisocyanate per kg body weight. The effect on uptake into the acid-soluble fraction and incorporation into DNA was greater after treatment with 2-chloroethylisocyanate than with ethylisocyanate. The former compound also caused a significant inhibition of uptake into the acid-soluble fraction of host liver under these conditions, but the effect was less than in the tumor. There was a small but significant effect of 2-chloroethylisocyanate on the uptake of [^{3}H]thymidine into the acid-soluble fraction of regenerating rat liver (data not shown). Under the conditions described in Table 2, the mean uptake was 23% less in four rats treated with 2-chloroethylisocyanate than in three control rats when isotope was injected 24 hr after partial hepatectomy.

The inhibitory effect of 2-chloroethylisocyanate on the uptake of [^{3}H]leucine and incorporation into protein of hepatoma 7288CTC was greater than that seen with ethylisocyanate (Table 3). The data in Table 4 suggest that, under conditions in which the two isocyanates caused an inhibition of uptake into the acid-soluble fractions of three hepatomas, there was either no significant effect on radioactivity in blood and host liver or an increase in these tissues. This pattern is similar to that reported previously after treatment of hepatoma-bearing rats with sodium cyanate [2] and confirmed in the present study with rats bearing hepatoma 7777. There were inhibitory effects of the two isocyanates on incorporation of [^{3}H]leucine into liver proteins of rats bearing transplants of hepatoma 7777 but the percent inhibition was less than for the tumors (Table 5). Evidence was obtained for a prolonged effect of 2-chloroethylisocyanate on the uptake of [^{3}H]leucine into the acid-soluble fraction and on incorporation into protein of Morris hepatoma 7777. The data in Tables 4 and 5 indicate 67 and 87% inhibition, respectively, for these variables. In additional studies (data not shown), we found that with a 10-min period between treatment with 2-chloroethylisocyanate and isotope injection the inhibitions were 50 and 62%,

Table 2. Effects of isocyanates on the uptake of ^{3}H-labeled thymidine in rats bearing 7288CTC hepatoma

Treatment	Acid-soluble radioactivity (dpm/mg tissue)			DNA radioactivity (dpm/100 μg DNA)	
	Blood	Tumor	Liver	Tumor	Liver
Control (6)	563 ± 31	253 ± 17	1484 ± 82	486 ± 91	312 ± 92
C_2H_5NCO (4)	783 ± 92	131 ± 52*	1639 ± 118	151 ± 127	171 ± 43
ClC_2H_4NCO (3)	581 ± 23	59 ± 6*	1088 ± 120*	20 ± 3*	201 ± 112

Buffalo rats bearing s.c. transplants of 7288CTC hepatoma received i.p. injections of 0.9% NaCl (controls) or 0.2 mmol/kg of either ethylisocyanate or 2-chloroethylisocyanate. After 10 min the rats received s.c. injections of [^{3}H]thymidine (250 μCi/kg) and were killed 20 min after the isotope injection. Data are presented as means ± SE for the number of animals given in parentheses for each treatment.

* $P < 0.05$, relative to controls.

Table 3. Effect of isocyanates on the uptake of ^{3}H-labeled leucine in rats bearing 7288CTC hepatoma

Treatment	Acid-soluble radioactivity (dpm/mg tissue)			Acid-insoluble radioactivity (dpm/100 μg protein)	
	Blood	Tumor	Liver	Tumor	Liver
Control (4)	703 ± 55	377 ± 41	944 ± 137	48 ± 13	557 ± 44
C_2H_5NCO (3)	766 ± 112	162 ± 18*	998 ± 95	12 ± 7	526 ± 120
ClC_2H_4NCO (3)	741 ± 81	117 ± 17*	966 ± 93	7 ± 3*	534 ± 105

Buffalo rats bearing s.c. transplants of 7288CTC hepatoma received i.p. injections of 0.9% NaCl (controls) or 0.2 mmol/kg of either ethylisocyanate or 2-chloroethylisocyanate. After 10 min, the rats received s.c. injections of [^{3}H]leucine (500 μCi/kg) and were killed 20 min after the isotope injection. Data are presented as means ± SE for the number of animals given in parentheses for each treatment.

* $P < 0.05$, relative to controls.

Table 4. Distribution of ^{3}H-labeled leucine in rats treated with isocyanates

Tumor	Isocyanate	Dose (mmol/kg)	N	Acid-soluble radioactivity (dpm/mg tissue)		
				Blood	Tumor	Liver
7288CTC	Control		5	589 ± 41	379 ± 23	591 ± 42
	C_2H_5NCO	0.4	5	877 ± 77*	80 ± 54*	726 ± 6
	ClC_2H_4NCO	0.1	4	816 ± 44*	36 ± 6*	687 ± 29
7777	Control		13	667 ± 45	439 ± 36	714 ± 51
	C_2H_5NCO	0.2	4	919 ± 54*	163 ± 65*	762 ± 22
	ClC_2H_4NCO	0.1	4	972 ± 27*	146 ± 48*	826 ± 64
	NaOCN	3.8	5	794 ± 103	176 ± 45*	780 ± 137
5123C	Control		6	635 ± 20	789 ± 67	710 ± 27
	C_2H_5NCO	0.2	6	867 ± 62*	685 ± 132	871 ± 58*
	C_2H_5NCO	1.0	2	690 ± 83	60 ± 28*	1251 ± 2*
	ClC_2H_4NCO	0.1	3	1051 ± 53*	72 ± 13*	871 ± 101

Rats bearing s.c. transplants of Morris hepatomas received i.p. injections of 0.9% NaCl (controls) or ethylisocyanate or 2-chloroethylisocyanate at the stated dose. After 60 min, the rats received s.c. injections of [^{3}H]leucine (500 μCi/kg body wt) and were killed 20 min after the isotope injection. Data are presented as means ± SE for the number of animals denoted by N.

* $P < 0.05$, relative to controls.

Table 5. Incorporation of ^{3}H-labeled leucine in rats treated with isocyanates

Tumor	Isocyanate	Dose (mmol/kg)	N	Incorporation (dpm/100 μg protein)	
				Tumor	Liver
7288CTC	Control		5	107 ± 43	669 ± 123
	C_2H_5NCO	0.4	5	11 ± 8	411 ± 89
	ClC_2H_4NCO	0.1	4	4 ± 1	327 ± 29
7777	Control		13	249 ± 28	734 ± 42
	C_2H_5NCO	0.2	4	41 ± 22*	452 ± 34*
	ClC_2H_4NCO	0.1	4	32 ± 21*	371 ± 38*
	NaOCN	3.8	5	48 ± 17*	661 ± 92
5123C	Control		6	391 ± 25	832 ± 113
	C_2H_5NCO	0.2	6	165 ± 49*	668 ± 46
	C_2H_5NCO	1.0	2	4 ± 1*	655 ± 99
	ClC_2H_4NCO	0.1	3	5 ± 1*	463 ± 102

The experimental conditions and presentation of data are as described for Table 4.

* $P < 0.05$, relative to controls.

Table 6. Distribution of $[^3H_2]O$ in rats treated with isocyanates

Tumor	Isocyanate	Dose (mmol/kg)	N	Acid-soluble radioactivity (dpm/100 μg tissue)		
				Blood	Tumor	Liver
7288CTC	Control		6	329 ± 18	177 ± 16	250 ± 22
	C_2H_5NCO	0.4	6	353 ± 23	73 ± 31*	266 ± 21
	C_2H_5NCO	0.8	4	322 ± 32	13 ± 9*	191 ± 26
	ClC_2H_4NCO	0.1	4	341 ± 47	48 ± 40*	243 ± 29
	ClC_2H_4NCO	0.2	3	329 ± 54	2 ± 0*	221 ± 34
7777	Control		7	323 ± 34	220 ± 27	277 ± 22
	C_2H_5NCO	0.4	7	345 ± 25	108 ± 39*	254 ± 23
	C_2H_5NCO	0.8	3	366 ± 67	30 ± 16*	213 ± 44
	ClC_2H_4NCO	0.1	4	301 ± 73	30 ± 11*	248 ± 38
	ClC_2H_4NCO	0.2	3	100 ± 18*	1 ± 1*	61 ± 4*
5123C	Control		6	325 ± 18	290 ± 17	269 ± 14
	C_2H_5NCO	0.4	7	270 ± 40	173 ± 53	192 ± 36
	C_2H_5NCO	0.8	3	321 ± 21	53 ± 14*	211 ± 15*
	ClC_2H_4NCO	0.1	5	333 ± 45	40 ± 14*	224 ± 37
8999	Control		3	291 ± 42	253 ± 37	248 ± 38
	ClC_2H_4NCO	0.2	4	257 ± 18	7 ± 3*	171 ± 10

Rats bearing s.c. transplants of Morris hepatomas received i.p. injections of 0.9% NaCl (controls) or ethylisocyanate or 2-chloroethylisocyanate at the stated dose. After 60 min the rats received s.c. injections of $[^3H_2]O$ (1 mCi/kg body wt) and were killed 10 min after the isotope injection. Data are presented as means ± SE for the number of animals denoted by N.

* $P < 0.05$, relative to controls.

respectively, and with a 5-hr period there was a 67% inhibition of uptake into the acid-soluble fraction and an 89% inhibition of incorporation into protein.

In addition to inhibitory effects of the two isocyanates on the uptake of [^{3}H]labeled thymidine and leucine into acid-soluble fractions of hepatomas, a decreased rate of distribution of $[^3H_2]O$ was observed (Table 6). There was not usually an effect on radioactivity in blood and host livers under these conditions and, when the radioactivity was decreased, the change was less than in the hepatomas.

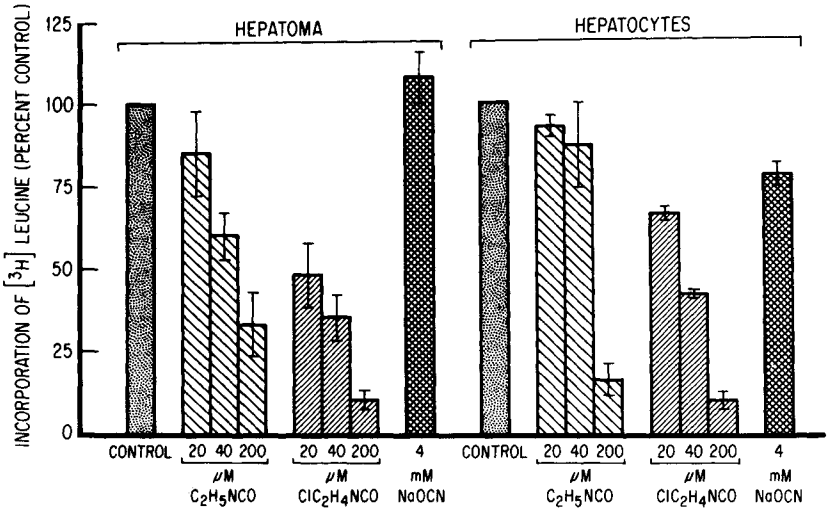

Fig. 1. Effects of isocyanates and sodium cyanate on the incorporation of ^{3}H-labeled leucine in isolated hepatocytes and hepatoma cells. Cells were obtained by collagenase digestion of Morris hepatoma 7288CTC or rat liver. Preincubations for 10 min at 37° in the absence (controls) or presence of carbamoylating agents were followed by an incubation for 20 min with 5 μCi [^{3}H]leucine. The incorporation into acid-soluble material is expressed as a percentage of the control value which was 2494 ± 1020 dpm/10^5 cells for the hepatocytes and 985 ± 287 dpm/10^5 cells for the hepatoma cells. Data are presented as means ± SE for four hepatoma preparations and three hepatocyte preparations. Triplicate incubations were performed for each cell preparation.

Table 7. Effect of sodium cyanate on the uptake of ^{3}H-labeled leucine in mice bearing BH3 hepatoma

Treatment	Acid-soluble radioactivity (dpm/mg tissue)			Acid-insoluble radioactivity (dpm/100 μg protein)	
	Blood	Tumor	Liver	Tumor	Liver
Control (5)	486 ± 63	451 ± 58	593 ± 81	120 ± 31	310 ± 41
NaOCN, 200 mg/kg (3)	516 ± 42	314 ± 75	572 ± 78	61 ± 29	226 ± 55
NaOCN, 250 mg/kg (5)	602 ± 54	310 ± 59	731 ± 51	29 ± 8*	170 ± 20*

BUB mice bearing s.c. transplants of BH3 hepatoma received i.p. injections of 0.9% NaCl (controls) or sodium cyanate (200 or 250 mg/kg body wt). After 60 min the mice received s.c. injections of [^{3}H]leucine (500 μCi/kg) and were killed 30 min after the isotope injection. Data are presented as means ± SE for the number of animals given in parentheses for each treatment.

* $P < 0.05$, relative to controls.

The distinction between hepatomas and liver noted *in vivo* was much less apparent when isolated hepatoma cells and hepatocytes were treated with isocyanates *in vitro* (Fig. 1). The inhibitory effect of the isocyanates at a concentration of 0.2 mM was as great for the incorporation of [^{3}H]leucine in hepatocytes as it was in hepatoma cells. In contrast to the action of sodium cyanate which has been reported to be more inhibitory for tumor protein synthesis *in vivo* than *in vitro* [3], the effects of ethylisocyanate and 2-chloroethylisocyanate on protein synthesis in the isolated cells were similar to those noted in tumor-bearing rats (Table 5) and, in each case, were greater with 2-chloroethylisocyanate than with ethylisocyanate.

When mice bearing transplants of BH3 hepatoma were treated with sodium cyanate (200 or 250 mg/kg) and were killed 30 min after injection of [^{3}H]leucine, a significant effect on uptake of isotope into the acid-soluble fractions of the tumors was not detected (Table 7). Under these conditions there were significant inhibitory effects on incorporation into protein of tumors and liver in rats treated with 250 mg sodium cyanate/kg. With this level of sodium cyanate there were no significant effects on the uptake of [^{3}H]leucine into the acid-soluble fractions of RIF-1 sarcomas and B16 melanomas, but incorporation into protein was inhibited 57 and 39% respectively (data not shown). However, there were significant decreases in the uptake of isotope into acid-soluble fractions of tissues when the mice were killed at 20 min rather than at 30 min after injection of the [^{3}H]leucine (Table 8). The data in Table 8 indicate that in mice treated with 0.2 mmol 2-chloroethylisocyanate per kg there was an inhibitory effect on uptake of isotope label into the acid-soluble fraction and incorporation into protein of hepatoma and liver with a greater percent inhibition in the tumor than in the liver relative to controls.

The mean uptake [$^3H_2$]O was lower in the BH3 hepatoma in mice treated with sodium cyanate (250 mg/kg) but was not statistically different from con-

Table 8. Effects of 2-chloroethylisocyanate and sodium cyanate on the uptake of ^{3}H-labeled leucine in mice bearing BH3 hepatoma

Treatment	Acid-soluble radioactivity (dpm/mg tissue)			Acid-insoluble radioactivity (dpm/100 μg protein)	
	Blood	Tumor	Liver	Tumor	Liver
Control (5)	757 ± 23	561 ± 34	861 ± 27	80 ± 7	261 ± 19
ClC_2H_4NCO, 0.1 mmol/kg (4)	768 ± 57	326 ± 115	806 ± 81	41 ± 27	202 ± 60
ClC_2H_4NCO, 0.2 mmol/kg (3)	713 ± 45	53 ± 12*	584 ± 40*	5 ± 1*	56 ± 13*
NaOCN, 250 mg/kg (3)	417 ± 88*	203 ± 78*	457 ± 122*	17 ± 7*	56 ± 17*

BUB mice bearing s.c. transplants of BH3 hepatoma received i.p. injections of 0.9% NaCl (controls) or 2-chloroethylisocyanate (0.1 or 0.2 mmol/kg) or sodium cyanate (250 mg/kg body wt). After 60 min the mice received s.c. injections of [^{3}H]leucine (500 μCi/kg) and were killed 20 min after the isotope injection. Data are presented as means ± SE for the number of animals given in parentheses for each treatment.

* $P < 0.05$, relative to controls.

Table 9. Effects of 2-chloroethylisocyanate and sodium cyanate on the uptake of $[^3H_2]O$ in mice bearing BH3 hepatoma

Treatment	Acid-soluble radioactivity (dpm/100 μg tissue)		
	Blood	Tumor	Liver
Control (6)	149 ± 7	92 ± 9	132 ± 6
ClC_2H_4NCO, 0.1 mmol/kg (5)	128 ± 15	34 ± 12*	102 ± 18
ClC_2H_4NCO, 0.2 mmol/kg (6)	125 ± 22	19 ± 12*	76 ± 16*
NaOCN, 250 mg/kg (4)	139 ± 17	68 ± 3	119 ± 15

BUB mice bearing s.c. transplants of BH3 hepatoma received i.p. injections of 0.9% NaCl (controls) or 2-chloroethylisocyanate (0.1 or 0.2 mmol/kg) or sodium cyanate (250 mg/kg body wt). After 60 min the mice received s.c. injections of $[^3H_2]O$ (500 μCi/kg) and were killed 10 min after the isotope injection. Data are presented as means ± SE for the number of animals given in parentheses for each treatment.

* $P < 0.05$, relative to controls.

trols (Table 9). There were significant decreases of radioactivity in the hepatomas when 2-chloroethylisocyanate was administered at a level of 0.1 or 0.2 mmol/kg. At the higher dose level, there was a significant 43% decrease in radioactivity in the host livers which was less than the 79% decrease in the tumors.

DISCUSSION

The present studies suggest that the action of alkyl isocyanates in hepatoma-bearing animals may resemble that of sodium cyanate inasmuch as inhibitory effects can be exerted on metabolite uptake and incorporation into macromolecules in tumors under conditions in which there is a less marked, or no, effect in the host liver. In addition, these carbamoylating agents can inhibit the distribution of isotope-labeled water in a manner that is compatible with a preferential effect on tumor blood circulation. Although evidence has been provided for the conversion of sodium cyanate to an active metabolite [2], the relative concentrations required for equivalent effects of sodium cyanate, ethylisocyanate and 2-chloroethylisocyanate in the present work are in line with their respective activities as agents that carbamoylate proteins [9, 16]. The correlation between the carbamoylating activities of these compounds and their effects on metabolite uptake in tumors is circumstantial evidence that these effects are mediated through carbamoylation reactions. Effects on the circulation may be a factor that results in greater tissue specificity *in vivo* than would be seen in isolated cells *in vitro*.

Carbamoylation of macromolecules by isocyanates primarily affects proteins, with little or no modification of DNA [17]. Carbamoylating agents have been shown to inhibit several metabolic processes, including protein synthesis [18, 19], DNA repair [20], and RNA processing [21]. Several enzymes have been shown to be inhibited by carbamoylating agents, including carbamoyl phosphate synthetase [22], papain [23], glutathione reductase [24, 25], DNA polymerase [26], DNA ligase [27] and protein A24 lyase [28]. Much attention has been focused on the release of carbamoylating species from nitrosoureas, although several investigators have concluded that alkylating activity rather than carbamoylating activity is more closely related to the antitumor effects of clinically useful nitrosoureas [29–34].

Amino groups are the most stable sites for carbamoylation of proteins, and comparative studies of carbamoylating activity have used carbamoylation of amino groups as a model [35, 36]. However, alternative sites such as hydroxyl groups and sulfhydryl groups are possible [37]. The instability of the thiocarbamyl adduct at pH 7 has been used as an argument against the involvement of cysteine residues in the carbamoylation of tropomyosin [38]. On the other hand, the conformation of a protein can greatly influence the kinetics and stability of cysteine-residue carbamoylation in comparison with free cysteine [22, 23]. In addition, the low pH in tumors [39] would promote the stability of a cysteine-residue modification. Differences in environmental pH may be a factor in the diminished sensitivity of cultured tumor cells to sodium cyanate relative to the effect *in vivo*.

Much of the toxicological data available for organic isocyanates relates to their respiratory effects [40]. The rapid reaction of many organic isocyanates with water, and the irritant character of these molecules, have limited the study of them as cancer chemotherapeutic agents. Some success was reported for two isocyanates, which were inactive in saline, when the isocyanates were dissolved in peanut oil [41]. In the case of nitrosoureas, a slow release of isocyanates has been reported [36]. Sodium cyanate is less toxic than the organic isocyanates and provides a slowly acting but relatively stable carbamoylating agent. It has been suggested that organic isocyanates liberated from nitrosoureas would be more effective than inorganic cyanate in entering cells [33], but this would be influenced by the ionization of the cyanate. The low pH in tumors would favor the formation of uncharged isocyanic acid and thereby facilitate cellular uptake. Preliminary data indicate that a decrease in pH enhances

the effect of sodium cyanate on macromolecular synthesis in hepatoma cells [42].

The present work has suggested that hepatomas in rats may be more sensitive than tumors in mice to the action of sodium cyanate. Unfortunately, we have found that rats are more sensitive than mice to the toxic effects of chronic administration of sodium cyanate (unpublished observations), and it may not be possible to separate toxic from therapeutic effects.

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