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STUDIES OF THE ENZYMATIC DEAMINATION OF CYTOSINE ARABINOSIDE—III

SUBSTRATE REQUIREMENTS AND INHIBITORS OF THE DEAMINASE OF HUMAN LIVER

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Abstract—Structure–activity relationships of 50 structural analogues of cytosine arabinoside were studied with the pyrimidine nucleoside deaminase found in human liver. The following pyrimidine nucleoside configurations appeared to be required for activity: a 2-keto group, an unsubstituted N-3 ring nitrogen, a C-6 ring carbon, a pentofuranose at pyrimidine position 1, a free 3′-α-hydroxyl group, and a nonphosphorylated 5′-hydroxyl group. The inhibitory activity of different functional groups substituted at position 4, in order of increasing inhibition, was methylamino, keto, hydrazino, thio, amino, and hydroxyamino. The introduction of either a halogen or a methyl group at pyrimidine position 5 did not alter deamination rates markedly; the order of deamination was 5-chloro > 5-bromo > no 5-substitution > 5 > iodo > 5-methyl. 5-Azacytidine was active as an inhibitor but 6-azacytidine was not. The 2′-position was not involved in enzyme–substrate binding, but epimerization at this position altered the 'fit' of the substrate on the active site.

N^4-hydroxy-5-methyl-2′-deoxycytidine was the most active deaminase inhibitor tested. A 50 per cent inhibition in the deamination of cytosine arabinoside was obtained when the concentration of inhibitor was only 4 per cent that of the substrate; the inhibition appeared to be competitive. The measured K_I value for this compound was $1{\cdot}1$–$1{\cdot}25 \times 10^{-5}$ M, and its measured *competitive efficacy* (a newly developed test) was 24. By comparison, cytosine arabinoside had a K_m value of $1{\cdot}2$–$1{\cdot}6 \times 10^{-4}$ M and a competitive efficacy of 1.

EARLY clinical studies,*[1] in cancer patients showed that i.v. administered cytosine arabinoside† (CA)‡ was degraded rapidly in man to a biologically inactive product. This inability to maintain adequate blood levels of CA prompted several studies, one of which was to determine whether this degradation, subsequently shown to be an enzymatic deamination, could be inhibited in a specific manner. This paper reports the results of such a study and indicates further that the reaction leading to the deamination of CA, at least in human liver homogenates, can be inhibited both efficiently and specifically with relatively low concentrations of N^4-OH-5-CH_3-CdR. Subsequent

* R. W. Talley and C. G. Smith, unpublished data.

† 1-β-D-arabinofuranosylcytosine (generic name, cytarabine).

‡ The compounds were numbered as shown in Fig. 1. The abbreviations used are: C for cytosine; *iso*-C for 2-amino-4-oxypyrimidine; T for thymine; U for uracil; A for D-arabinofuranose; Gl for D-glucopyranose; L for D-lyxofuranose; R for D-ribofuranose; dR for 2′-deoxy-R; X for D-xlofuranose; CR, TdR, etc., for cytidine, thymidine and other 1-β-D-sugar-pyrimidines; 3′-CMP for cytidine 3′-monophosphate; CMP, CDP, and CTP for the 5′-nucleotides of cytidine; dCMP, dCDP, and dCTP for the 5′-nucleotides of deoxytidine.

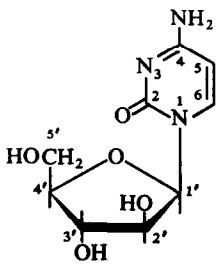

FIG. 1. The structural formula of CA. The numbering system used with pyrimidines, pyrimidine nucleosides, and pyrimidine nucleotides is indicated.

studies by Dollinger *et al.*[2] have confirmed and extended this finding. Previous papers[3, 4] have described the discovery and some of the properties of the human liver deaminase, and the procedures by which its activity was measured.

MATERIALS AND METHODS

Materials

The preparation of human liver homogenates, the composition of KR buffer, and the sources of human liver have been described earlier.[3]

The author gratefully acknowledges gifts of the following compounds: 3-CH_3-CA, 3-CH_3-UA, 3-CH_3-CR, 3-CH_3-CdR, 3-CH_3-CX, 3-CH_3-CL, N-CH_3-CA,*iso*-CA, 5-Cl-CA, 5-Br-CA, 5-I-CA, 5-CH_3-CR, 5-CH_3-CGl, CX, 5-CH_3-CX, and CL from Dr. James H. Hunter of The Upjohn Co.; 1,5-diCH_3-C, N^4-OH-5-CH_3-CdR, and N^4-NH_2-5-CH_3-CdR from Dr. Jack J. Fox, Sloan-Kettering Institute for Cancer Research, Rye, N.Y.; 5-aza-U, 5-aza-orotate, and 6-aza-UR from Dr. Arnold D. Welch, Squibb Institute for Medical Research, New Brunswick, N.J.; 4-thio-UR from Dr. Arthur R. Hanze of The Upjohn Co.; 5-aza-CR (a fermentation product[5]) from Mr. Malcom E. Bergy of The Upjohn Co.

CA and UA were bulk products prepared by The Upjohn Co. U and C were purchased from Nutritional Biochemicals Co., Cleveland, Ohio. CdR and 1-CH_3-C were purchased from Cyclo Chemical Corp., Los Angeles, Calif. dCDP was obtained from Sigma Chemical Co., St. Louis, Mo. CA-^{3}H, CR-^{3}H, and dCMP were purchased from Schwarz Bio-Research, Inc., Orangeburg, N.Y. 5-Br-U, TdR, UR, CR, 5-CH_3-CdR, 6-aza-CR, iso-C, 5-Br-UR, 5-CH_3-C, 3′-CMP, CMP, CDP, CTP, and dCTP were obtained from Calbiochem, Los Angeles, Calif.

Deamination test

Incubation mixtures, which were prepared in 12-ml centrifuge tubes in an ice bath, contained 250 μmole glycylglycine buffer at pH 8·0, 1.0 μmole test compound, 0·2 ml of a centrifuged 25% homogenate of normal human liver prepared in KR buffer, and distilled water to a total volume of 0·5 ml. The tubes were incubated for 30 min at 37°, and their contents were assayed spectrophotometrically as described previously.[3] 5-Cl-CA, 5-Br-CA, and 5-I-CA were assayed at 310 mμ; 5-CH_3-CR, 5-CH_3-CdR, 5-CH_3-CX, and 1,5-diCH_3-C were assayed at 300 mμ; all other compounds were assayed at 290 mμ. The lower limit of the assay sensitivity was 5 per cent deamination.

Competitive efficacy (C.E.) test

The C.E. value of a test compound is a measure of how well that compound is able to compete with CA-^{3}H for the active site of the human liver deaminase under certain specified reaction conditions. The larger the C.E. value, the more competitive the inhibitor. Unlabeled CA (CA-^{1}H) is used in place of test compound in order to construct a standard curve and to standardize test results. The molar ratios of CA-^{1}H to CA-^{3}H can be correlated directly with the amount of deaminase inhibition.

C.E. values are expressed in terms of CA-^{1}H-equivalents/μmole test compound. Thus, a C.E. value of 10 means that 1 μmole of the test compound was as inhibitory as 10 μmole of CA-^{1}H. By definition, CA-^{1}H has a C.E. value of 1·0.

The incubation mixtures, which were prepared in 12-ml centrifuge tubes in an ice bath, contained 1·0 μmole CA-^{3}H (5 μc/μmole), a variable amount of test compound (or CA-^{1}H), 250 μmole glycylglycine buffer at pH 8·0, 0·2 ml of a centrifuged 25% homogenate of normal human liver prepared in KR buffer, and distilled water to a total volume of 0·5 ml. The tubes were incubated for 45 min at 37°, and the tube contents were assayed for radioactivity as described previously.[4]

To calculate the C.E. values, a standard curve was constructed by plotting the $\log_{10}$ of the ratios of the starting concentrations of CA-^{1}H and CA-^{3}H vs. the per cent conversion of CA-^{3}H to UA-^{3}H. With this standard curve, the data indicating the percentage conversions of the test compounds were then translated into equivalents of CA-^{1}H; these equivalents, in turn, were divided by the number of μmole of test compound. The ordinate ratio, which was used to calculate the standard curve, ranged from 0·1 to 10·0 (Fig. 3).

EXPERIMENTAL AND RESULTS

Deamination and C.E. tests

Fig. 2 shows typical time curves for the deamination of both CA and CR. The assay of deamination was very reproducible, and successively obtained assay curves were almost superimposable. In 11 assays run over a period of 2 yr, the 30-min deamination values for CA and CR ranged between 56 and 61 per cent, and between

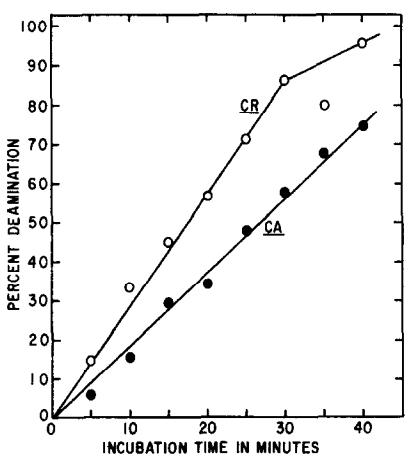

FIG. 2. The deamination of CA and CR as a function of incubation time. Incubation mixtures and conditions were those described in Methods.

77 and 86 per cent respectively. The reproducibility of the assay is believed to stem, in large measure, from the stability of the deaminase to storage.[4]

The deamination of CA and CR was linear past 70–80 per cent depletion of the substrates. This situation probably resulted from the use of an initial substrate concentration which was about 20 times that of the K_m concentration[4] coupled with only a slight product inhibition.[3] The linearity of the deamination rate over such a broad range was very useful in that it permitted the direct comparison of a wide variety of deamination measurements.

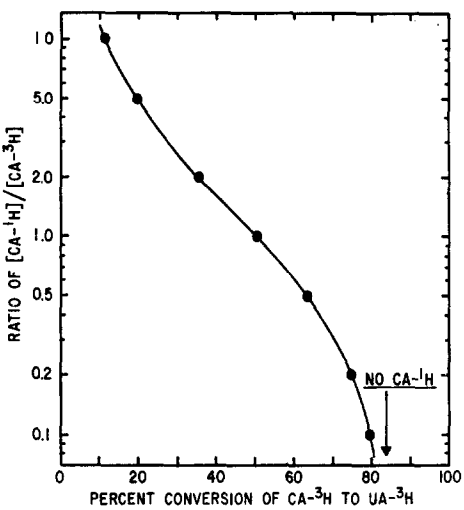

FIG. 3. The competitive inhibition by CA-^{1}H of the deamination of CA-^{3}H by human liver deaminase. C.E. test mixtures and incubation conditions were those described in Methods.

Fig. 3 shows a representative CA standard curve used for the calculation of C.E. values. These curves, like those in the deamination assay, are very reproducible and almost superimposable. The standard deviation of the C.E. assays ranged from 2 to 4 per cent conversion. C.E. values equal to, or less than, 0·1 were called zero.

Ring nitrogen N-3

The data presented in Table 1 for CA, UA, CR, and CdR clearly show that the addition of a methyl group to ring nitrogen N-3 completely prevented the attachment of the compound to the enzyme.

Pyrimidine position 4

Various 4-substituted pyrimidine nucleosides were tested for their ability to inhibit competitively the deamination of CA. The compounds were too few and too diverse, however, to reveal any relationship between structure and activity. As shown in Table 2, the 4-CH_3NH, 4-keto, and 4-hydrazino analogues had either only a small amount of or no competitive activity; the 4-thio nucleoside had a moderate amount of activity. The 4-HONH compound, N^4-OH-5-CH_3-CdR, was the most active compound tested, being 5–7 times more active than CR and 24 times more active

than CA. The fact that this compound was not deaminated probably contributed to its effectiveness as an inhibitor.

The 4-HONH compound appeared to inhibit the CA deamination reaction in a specific and competitive manner. When the inhibitor data were plotted by the double

TABLE 1. THE EFFECT ON DEAMINASE ACTIVITY OF METHYLATION AT RING NITROGEN N-3*

Compound	% Deamination	C.E.
CA	60	1·0
3-CH_3-CA	<5	0
UA		0·18
3-CH_3-UA		0
CR	83	4·0
3-CH_3-CR	<5	0
CdR	68	4·0
3-CH_3-CdR	<5	0

* Deamination and C.E. (competitive efficacy) tests were conducted as described in Methods.

TABLE 2. THE EFFECT ON DEAMINASE ACTIVITY OF SUBSTITUTIONS AT NUCLEOSIDE POSITION 4*

Compound	% Deamination	C.E.
CA	61	1·0
N^4-CH_3-CA	<5	<0·2
UA		0·20
CR	79	3·5
4-Desamino-4-thio-CR†		>1·1†
UR		0·68
5-CH_3-CdR	33	4·5
N^4-OH-5-CH_3-CdR	<5	24·0
N^4-NH_2-5-CH_3-CdR	19‡	0·4
5-CH_3-UdR		0·4

* Deamination and C.E. tests were conducted as described in Methods.

† This preparation was impure.

‡ The significance of this value is not known. The deamination curves for the hydrazino compound were atypical; the peak of the postincubation curve did not show the usual downward displacement toward lower wavelengths that is seen with deaminated analogues.

reciprocal method of Lineweaver and Burk,[6] the resulting straight line curves (Fig. 4) had a common intercept on the ordinate equal to $1/V_{max}$. K_I constants calculated from such plots have ranged from 1·1 to 1·25 $\times$ 10^{-5} M. K_m values for CA, as noted previously,[4] have ranged from 1·2 to 1·6 $\times$ 10^{-4} M. As a check on our procedures, the K_I of CA-^{1}H was found to be identical to the K_m of CA-^{3}H.

Substitutions at pyrimidine position 5

The rate of deamination of cytosine nucleosides varied with the functional group substituted at the 5-position (Table 3). A 5-Cl group increased the deamination of

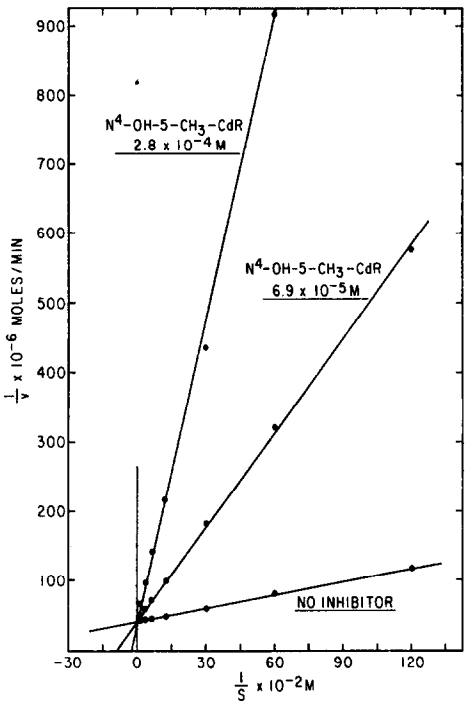

FIG. 4. The competitive inhibition by N^4-OH-5-CH_3-CdR of the deamination of CA-^{3}H by the human liver deaminase. The incubation mixtures were prepared in 12-ml centrifuge tubes in an ice bath. They contained CA-^{3}H (5 μc/tube) in the indicated concentrations, N^4-OH-5-CH_3-CdR in the indicated concentrations, 250 μmole glycylglycine buffer at pH 8·0, 0·2 ml of a centrifuged 25% homogenate of normal human liver prepared in KR buffer, and distilled water to a total volume of 0·6 ml. The tubes were incubated at 37° for 80, 40, 20, 10, 4, 2, and 1 min, respectively, going from left to right in the above figure. Tube contents were assayed for radioactivity as described previously.[4] The reaction velocity for each substrate concentration was calculated from the initial linear portion of each deamination curve. The velocities were then corrected for the lower incubation temperatures that obtained during the first 15 sec of incubation. The measured temperatures of the reaction mixtures were: 0° at 0 sec, 27° at 5 sec, 33° at 10 sec, and 36° at 15 sec.

TABLE 3. THE EFFECT ON DEAMINASE ACTIVITY OF 5-SUBSTITUTED CYTOSINE NUCLEOSIDES*

Compound	% Deamination	C.E.
5-Cl-CA	86	0·82
5-Br-CA	65	1·0
CA	58	1·0
5-I-CA	38	1·3
CR	77	3·7
5-CH_3-CR	50	5·6
CdR	64	3·8
5-CH_3-CdR	33	4·8

* Deamination and C.E. tests were run as described in Methods.

CA about 1·5-fold, but a substitution of either a 5-I or a 5-CH_3 decreased nucleoside deamination 1·5–1·9-fold. The deamination rate of 5-Br-CA was approximately equivalent to that of the unsubstituted (i.e. 5-H) CA. The 5-F analogue[7] was not tested in this study but, based upon the dCMP deaminase data presented by Maley[8] and by Maley and Maley,[9] and upon the data presented here, one would predict that this compound would be deaminated very rapidly.

The data indicating the percentage deamination were inversely proportional to the C.E. values. This result suggested that the primary effect of the 5-substitution was inductive rather than steric.

Aza-substituted and miscellaneous pyrimidines and pyrimidine nucleosides

The 6-aza-compounds uniformly showed no activity with the deaminase. They neither served as substrate nor competed with substrate for the active site of the enzyme (Table 4). In contrast, however, 5-aza-CR showed a moderate amount of

TABLE 4. THE COMPETITIVE EFFICACY OF AZA-SUBSTITUTED AND MISCELLANEOUS PYRIMIDINES AND PYRIMDINE NUCLEOSIDES*

Aza-compounds		Miscellaneous	
Compound	C.E.	Compound	C.E.
CR	3·5	2-NH_2-U (iso-C)	0
		2-NH_2-UA (iso-CA)	0
5-Aza-CR	1·1		
5-Aza-U	0	3-CH_3-CX	0
5-Aza-orotate	0	3-CH_3-CL	0
6-Aza-CR	0	5-Br-U	0
6-Aza-UR	0	5-Br-UR	0

* Deamination and C.E. tests were performed as described in Methods.

activity. Thus, it would appear that the C-6 ring carbon was required for activity, but that the C-5 ring carbon was not; other 6-substituted pyrimidine nucleosides should be tested.

Table 4 also lists the C.E. test data for pyrimidines and pyrimidine nucleosides that have not been reported elsewhere in this paper. None, including 2-NH_2-substituted UA, was active as a competitive inhibitor of the deaminase. This last result would suggest that the enzyme may require a 2-keto group.

Pentose at pyrimidine position 1

The data presented in Table 5 suggest that pentose-containing nucleosides are required as substrates for enzyme activity. When the ribose moiety was removed from both CR and 5-CH_3-CR, the substrate activity of these compounds was completely abolished. Glucose and methyl groups could not replace the requirement for ribose, but other pentoses could do so (see Table 7).

Phosphate substitution at 3′- and 5′-hydroxyls

The deamination patterns observed with nucleotides [CR > CMP > CDP > CTP and CdR > dCMP > dCDP > dCTP (column 2, Table 6)] suggest that these compounds must be dephosphorylated prior to deamination. Independent evidence for the

validity of this hypothesis is furnished by the ratios of competitive efficacy data shown in columns 3 and 4 of the same table. If only a single enzyme is involved in the deamination of nucleotides, then the ratios of C.E. activity for each nucleotide, as compared to CR, should have been approximately the same with both the crude and heat-treated enzyme preparations; the fact that they were not indicates that more than one enzyme activity is involved. Since only cytidine deaminase activity was

TABLE 5. THE EFFECT ON DEAMINASE ACTIVITY OF VARIOUS 1-SUBSTITUTED CYTOSINE COMPOUNDS*

Compound	% Deamination	C.E.
CR	79	3·0
C	<5	0
1-CH_3-C	<5	0
5-CH_3-CR	50	5·2
5-CH_3-C	<5	0
1,5-diCH_3-C	<5	0
5-CH_3-CGl	<5	0

* Deamination and C.E. tests were conducted as described in Methods.

TABLE 6. THE EFFECT ON DEAMINASE ACTIVITY OF PHOSPHORYLATION AT POSITIONS 3′ AND 5′*

Compound	% Deamination	C.E. ratios of test compounds to CR	
		Crude homogenate	Heat-treated homogenate†
CR	79	1·00	1·00
CMP	41	0·40	0
CDP	7	0·05	0
CTP	<5	0	0
3′-CMP‡	<5	0·04	0§
CdR	68	1·18	1·12
dCMP	36	0·26	0
dCDP	16	0·04	0
dCTP	<5	0	0

* Deamination and C.E. tests were conducted as described in Methods.

† Crude homogenate was heat-treated at 80° for 5 min, and the precipitate was removed by centrifugation as described previously.[4]

‡ Commercial preparations of 3′-CMP usually contain a small amount of 2′-CMP.

§ The lower limit of sensitivity for the ratio calculation, <0·03.

present in the heat-treated preparation, then, by difference, phosphatase activities must have been lost during the heat step. It may be concluded that nonphosphorylated hydroxyl groups at position 3′ and 5′ are required for substrate activity.

Positions 2′ and 3′

The deamination and C.E. data presented in Table 7, Part A for CR (2′-α-OH) and CdR (2′-deoxy) show that not only are both compounds effective competitors

for the active site of the enzyme, but also, once bound, they are deaminated rapidly. Similar data also were obtained with the 5-CH_3 analogues of CR and CdR. It may be concluded that the 2′-hydroxyl probably is not involved in the binding of cytosine nucleosides to the active site of the enzyme. The 2′-position is important, however, with regard to the structural 'fit' of the substrate on (or in) the active site. Epimerization of the 2′-α-OH group (to form CA) clearly gave a less desirable substrate structure, as evidenced by the 3–4-fold reduction in competitive efficacy. Interestingly (and unexplainably), this epimerization did not reduce the per cent deamination to a corresponding degree.

TABLE 7. THE EFFECT ON DEAMINASE ACTIVITY OF EPIMERIZATION AND REDUCTION OF THE 2′- AND 3′-HYDROXYL GROUPS*

	Compound	Configuration and groups at positions 2′	3′	% Deamination	C.E.
Part A	CR	α-OH	α-OH	77	3·4
	CdR	deoxy	α-OH	68	4·0
	5-CH_3-CR	α-OH	α-OH	50	5·6
	5-CH_3-CdR	deoxy	α-OH	33	4·8
	CA	β-OH	α-OH	61	1·0
Part B	CR	α-OH	α-OH	79	3·7
	CX	α-OH	β-OH	<5	<0·2†
	5-CH_3-CR	α-OH	α-OH	50	5·6
	5-CH_3-CX	α-OH	β-OH	<5	0
	CA	β-OH	α-OH	58	1·0
	CL	β-OH	β-OH	<5	0

* Deamination and C.E. tests were conducted as described in Methods. Data shown in the table were confirmed from 1 to 6 times.

† Not zero. The CX preparation reproducibly showed the same small C.E. activity on 3 retests.

The data shown in Table 7, Part B indicate that the 3′-α-OH group is required for the binding of the nucleoside substrate to the active site of the deaminase. Epimerization of this group destroyed the ability of CR, 5-CH_3-CR, and CA to compete for the active site. This result is in agreement with the data presented in Table 6, which show the need for a nonphosphorylated 3′-OH group. As would be expected in an 'all or nothing' situation, the correlation between C.E. values and percentage deamination is good.

DISCUSSION

The C.E. test was developed primarily as a technique for ranking test compounds in order of their effectiveness in preventing the deamination of CA. Classically, inhibitors have been ranked by their K_i values, but this method was not used in these studies for two reasons: first, the C.E. test was much faster and easier to perform, and we were thus able to measure the C.E. values for 20 compounds in the same amount of time that it took to determine the K_i value for one compound; second, the C.E.

test provided a direct measure of how well an inhibitor actually was able to spare CA deamination for a finite period of time and this was one of the primary goals of these studies. The K_I determination, by contrast, provided only a measure of the affinity of the enzyme for the inhibitor. As an example of how these two test values can vary, the case of N^4-OH-5-CH_3-CdR and CA is cited. The ratio of the C.E. values for these two compounds was about 24, but their K_I values varied only 10-fold. The difference between these two values represents the fact that the N^4-OH compound was not deaminated and hence was a more effective inhibitor than the K_I value would indicate.

When determining the order of effectiveness of inhibitors using C.E. values, it is important also to examine their ability to serve as substrates for the deaminase (as, for example, with the deamination test). The concentration of a compound that is both an inhibitor of and a substrate for an enzyme is decreased continually during the course of the reaction, whereas the concentration of the 'nonsubstrate' inhibitor remains constant. Thus, a comparison of C.E. values in the absence of deamination data could be very misleading.

The data in Table 1 show the need for an unsubstituted N-3 ring nitrogen. The data do not indicate, however, the reasons for this requirement, but at least three possible reasons can be offered: (1) the unsubstituted N-3 atom is involved directly in enzyme–substrate binding; (2) N-3 substituents interfere with the physical or electronic 'fit' of the substrate molecule on the enzyme; or (3) the N-3 substitution forms a 4-imino group that is not bound by the enzyme. (Brookes and Lawley[10] have shown that 1,3-di-substituted cytosine compounds have a 4-imino configuration.)

The finding of an inverse relationship between per cent deamination and C.E. data (Table 3) suggests that substitutions at position 5 altered deamination rates through inductive rather than steric effects. If the effects of 5-substitutions were inductive, then the different 5-substituted analogues would be expected to 'fit' the active site of the enzyme about equally well. If this were so, then the more rapidly deaminated analogues would compete with CA for shorter periods of time, and the less rapidly deaminated compounds would compete for longer periods of time; hence, one would obtain the inverse relationship seen in Table 3. Had the effect been primarily steric, then the deamination rates of the 5-substituted analogues would have been a direct indication of how well they had 'fit' the active site, and both the C.E. data and the deamination data would have been in the same direction (e.g. less deamination, a smaller C.E. value).

The data derived from 1-methyl and 1-glucose substitution (Table 5) and the 2′,3′-configuration data (Table 7) suggested that the deaminase requires a pentose nucleoside as substrate. This conclusion, however, is quite speculative, since only a limited number of 1-substituted cytosine compounds were tested. Schaeffer's group[11–17] has found, in studies with adenosine deaminase, that various hydroxyalkyls and cyclopentocarbinols are able to substitute for the ribose portion of adenosine. Perhaps an analogous series of compounds could substitute for the ribose portion of cytidine.

The data presented in Table 6 show the need for nonphosphorylated hydroxyl groups at positions 3′ and 5′ of cytidine. The data do not indicate, however, whether free hydroxyl groups are required or whether phosphate groups interfere with enzyme action by virtue of their electrical charge or size. Subsequently, it was shown (see Table 7) that a free hydroxyl group at position 3′ is required in enzyme–substrate

binding. Since no comparable 5′-analogues were available for testing, it still is not known whether a free hydroxyl group is required at position 5′.

Comparison of the substrate specificity data reported here (for the nucleoside deaminase) with those reported by Maley,[8] and by Maley and Maley[9, 18] (for dCMP deaminase), showed several interesting similarities. Both enzymes had fairly specific substrate requirements, and both enzymes were inhibited to about the same degree by 4-HONH analogues of their respective substrates.* Neither enzyme catalyzes the deamination of the 4-N-methyl derivatives of their substrates, but the enzymes were not inhibited by the presence of these derivatives. Both enzymes showed similar deamination patterns with their 5-substituted substrates (Table 8), and it is concluded, with both enzymes, that the 5-substituents act inductively (rather than sterically) in determining the deamination rates for these analogues.

TABLE 8. A COMPARISON OF THE ACTIVITIES OF THE CA AND dCMP DEAMINASES TOWARD 5-SUBSTITUTED CA AND dCMP SUBSTRATES

Human liver deaminase data from Table 3		dCMP deaminase data reported by Maley[8]	
Compound	μmole deaminated/ml mixture	Compound	μmole deaminated/10 min
5-Cl-CA	1·72	5-F-dCMP	1·26
5-Br-CA	1·30	5-Br-dCMP	0·96
CA	1·16	dCMP	0·90
5-CH_3-CA	0·70*	5-CH_3-dCMP	0·77
5-I-CA	0·76		

* Estimated from the data in Table 3, since the compound was not available for testing.

TABLE 9. A COMPARISON OF THE ACTIVITIES OF HUMAN LIVER AND MOUSE KIDNEY DEAMINASES TOWARD 5-SUBSTITUTED CA, CR, AND CdR

Human liver deaminase data from Table 3		Mouse kidney deaminase data reported by Creasey[19]			
Compound	μmole deaminated/ml of mixture	Compound	μmole deaminated/hr	Compound	μmole deaminated/hr
		5-F-CR	9·54	5-F-CdR	6·66
5-Cl-CA	1·72	5-Cl-CR*		5-Cl-CdR	4·94
5-Br-CA	1·30	5-Br-CR	8·93	5-Br-CdR	2·68
CA	1·16	5-I-CR	4·23	5-I-CdR	2·20
5-I-CA	0·76	CR	2·78	CdR	1·57
5-CH_3-CA	0·70†	5-CH_3-CR	1·05	5-CH_3-CdR	0·78

* Not reported. Estimated to be approximately 9–9·5 μmole/hr.

† Estimated from the data in Table 3, since the compound was not available for testing.

Creasey[19] has reported on some substrate requirements for the pyrimidine nucleoside deaminase found in mouse kidney. These data, when compared to those reported here for the human liver deaminase, show several interesting similarities and differences. Neither enzyme could deaminate 5′-nucleotides to any appreciable extent and with one exception, neither enzyme could deaminate cytosine or cytosine derivatives to any extent. The one exception was 5-$HOCH_2$-C, for which Creasey found a moderate amount of deamination.

* N^4-OH-5-CH_3-CdR, $K_i = 1·1–1·25 \times 10^{-5}$ M; N^4-OH-dCMP, $K_i = 1·3 \times 10^{-5}$ M.

The comparative deamination patterns found for the two nucleoside deaminases with various 5-substituted substrates are shown in Table 9. Although both enzymes showed essentially the same patterns of deamination, there were two significant differences. First, the relative order of deamination rates for the different compounds was different with the two enzymes. With the mouse kidney deaminase, the 5-halogenated nucleosides were deaminated much more rapidly than were the nonhalogenated nucleosides; with the human liver deaminase, only the 5-Cl nucleoside was deaminated substantially faster than was its nonsubstituted counterpart. Second, the relative rates of deamination for the 5-substituted substrates were also different with the two enzymes. With the kidney enzyme, the ratios of the deamination rates for the 5-Cl nucleosides as compared to the 5-CH_3 nucleosides were 6–9; for the liver enzyme, the ratio was only 2·5. By comparison with the dCMP deaminase, the ratio of deamination rates for 5-F-dCMP to 5-CH_3-dCMP was 1·6 (Table 8).

Another significant difference between the two enzymes was the relative rates at which CdR and CA were deaminated as compared to CR. With the human liver deaminase these values were 82 and 74 per cent, respectively, of the rate of deamination of CR, but the mouse kidney deaminase the values were only 56 and 6 per cent respectively. This last result suggests that kidney deaminase from different strains of mice may be different. Camiener and Smith[3] have reported on a kidney deaminase from an Upjohn line of Swiss mice that had a very high activity toward CA.

Note added in proof. 2-SH-CR and 2-SH-UR (courtesy of Dr. J. H. Hunter of The Upjohn Company) had C.E. values of 0·95 and <0·1, respectively. These data reinforce the suggestion that a 2-keto group is a preferred configuration for deaminase activity.

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