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STUDIES OF THE ENZYMATIC DEAMINATION OF CYTOSINE ARABINOSIDE—I.

ENZYME DISTRIBUTION AND SPECIES SPECIFICITY

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Abstract—Pyrimidine nucleoside deaminase activities that convert cytidine to uridine, and cytosine arabinoside to uracil arabinoside, have been demonstrated in human liver and kidney. Investigations of the distribution of these enzyme activities showed marked differences among four tissues and twelve animal species tested. Preliminary studies of the properties and inhibitors of the enzymatic activity in human liver are reported. The significance of these observations and their possible chemotherapeutic applications are discussed.

DURING blood level studies in human cancer patients,* it was found that intravenously administered cytosine arabinoside (CA)† disappeared very rapidly from the blood, as measured by a biological assay.[1] Since the rate of disappearance could not be accounted for by urinary excretion, it was postulated that the drug was being metabolized to some inactive product. This hypothesis was tested by incubating CA with homogenates of human liver and then determining the reaction products. The experiments established that CA is metabolized under these conditions to an inactive product, and that the main reaction involves a deamination of CA, with the formation of uracil arabinoside (UA).

Since certain cytosine nucleosides are of interest as potential chemotherapeutic agents in the cancer and virus fields, the presence of nucleoside deaminase activity in man could represent a serious obstacle to the systemic use of such agents. Accordingly, studies in these laboratories were undertaken with two objectives in mind—one aimed at finding experimental test animals that closely resemble man, with respect to both the activity and tissue distribution of this enzyme activity, and the other aimed at partially characterizing the enzyme and determining whether its effects could be negated.

The present paper describes the methods used for following the deamination of CA and CR, the species and tissue distributions of the enzyme, the identification of the deamination product, and preliminary characterization and inhibitor studies with the crude enzyme. Additional papers‡ will describe in more detail certain properties of the human liver deaminase and its inhibition by a variety of compounds.

* Dr. R. W. Talley, Henry Ford Hospital, in collaboration with The Upjohn Co.; unpublished.

† The abbreviations used are: CR for cytidine; CA and cytosine arabinoside for 1-β-D-arabinofuranosylcytosine (Cytarabine, registered trade name, The Upjohn Co.); UR for uridine; UA and uracil arabinoside for 1-β-D-arabinofuranosyluracil.

‡ G. W. Camiener, in preparation.

MATERIALS AND METHODS

Materials. Cytosine arabinoside and uracil arabinoside were prepared in the Upjohn laboratories. Cytidine and uridine were purchased from Calbiochem, Los Angeles, Calif. Cytosine and uracil were purchased from Nutritional Biochemicals Co., Cleveland, Ohio. EDTA was obtained from Eastman Organic Chemicals. Potassium penicillin G and streptomycin sulfate were bulk products produced by The Upjohn Co. Human tissues were kindly supplied by Dr. P. S. Rutherford and Dr. F. Cox (Borgess Hospital, Kalamazoo, Mich.), Dr. E. Hubbard and Dr. A. Russcher (Bronson Methodist Hospital, Kalamazoo, Mich.), Dr. H. VanderKamp (Veterans Administration Hospital, Battle Creek, Mich.), and Dr. E. Sproul (Roswell Park Memorial Institute, Buffalo, N.Y.). Monkey tissues were obtained commercially from Asiatic Imports Co., San Francisco, Calif.; all other animal tissues were obtained from Upjohn laboratories.

Krebs-Ringer (KR) buffer. This solution is a single-strength, modified Krebs-Ringer, medium low in bicarbonate[2] which is supplemented, per ml, with 100 μg each of potassium penicillin G and streptomycin sulfate.

Assays. Spectrophotometric, radioisotopic, cytotoxic,[1] and paper-chromatographic techniques have been used to determine whether CA or UA, or both, are present in nucleoside deaminase reaction mixtures. Three of these assays are described below; the radioisotope assay will be presented elsewhere.*

The spectrophotometric assay is based upon the fact that, under acidic conditions, cytosine-containing compounds generally absorb u.v. light at higher wavelengths than do their corresponding uracil analogues; thus, at sufficiently high wavelengths, decreasing optical densities can be correlated with increasing deaminase activity.

The spectrophotometric assay procedure consists of (1) stopping the enzyme reaction with 3 volumes of iced, 5% (w/w) trichloroacetic acid, (2) removing the precipitate by centrifugation; and (3) measuring the optical density of the diluted supernatant solution at a suitable wavelength. When CA and CR were used as substrates, the optical density measurements were made at 290 mμ. At this wavelength, CA and CR retain approximately 70% to 75% of their maximum (280 mμ) optical densities, whereas UA and UR retain less than 3% of their maximum (262 mμ).

In the paper-chromatographic assay, deproteinized reaction mixtures were spotted on Whatman No. 1 or No. 40 chromatography paper, and the papergrams were developed by the descending technique. After drying, the papers were sprayed with 0·01 N HCl and redried, and the locations of all u.v.-absorbing zones were determined, their absorption spectra were obtained, and their *total* optical opacities were measured at the appropriate wavelengths by a Cary recording spectrophotometer modified for paper-strip scanning. In this manner, u.v.-absorbing materials were characterized by R_f values, absorption spectra, and optical densities.

Tissue collection and storage. Attempts were made to collect tissues as quickly as possible after death. With most experimental animals, collection usually was accomplished within a few minutes, but with human tissues, as much as 12 to 24 hr often elapsed between the time of death and tissue collection. Locally obtained animal tissues were chilled quickly in waterproof plastic bags in an ice bath, trimmed, frozen

* G. W. Camiener, in preparation

in dry ice, and stored in liquid nitrogen. Except as indicated, monkey and human tissues were frozen and shipped to us in dry ice; upon arrival, the tissues were thawed, trimmed, repackaged in more suitable containers, refrozen in dry ice, and stored with the other animal tissues in liquid nitrogen. Trimming procedures consisted of decapsulating the kidneys, removing fat, blood vessels, and gallbladders from livers, discarding blood vessels and rinsing blood from hearts, trimming fascia from striated muscle, and carefully rinsing strips of ileum in cold tap water.

Preparation of homogenates. All tissue homogenates were prepared fresh in KR buffer at 0° in a Kontes* Kel-F† homogenizer, Potter-Elvehjem style. Twenty-five per cent homogenates were prepared by using 3 ml KR buffer/g frozen tissue; 20% homogenates were prepared by using 4 ml/g. The term *centrifuged homogenate* refers to the supernatant material siphoned from 12-ml conical centrifuge tubes after a 1,000 *g*, 15 min centrifugation at 2° to 4° in an International clinical-style centrifuge.

Preparation of bacteria-free deaminase. Approximately 5 ml of a cold, freshly prepared, 25% tissue homogenate was centrifuged for 30 min at 4° in a Beckman model L-2 centrifuge at 25,000 rpm in a No. 40.2 head ($\sim$ 40,000 *g*). The supernatant solution was forced through a sterile, Swinny-type filter-holder containing a type HA Millipore filter‡ (0·45 μ), and the filtrate was collected in a sterile injection vial (at 0°) and used as soon as possible.

Bacterial counts. Freshly prepared homogenates, or sterile-filtered enzyme preparations (see above) were serially diluted in sterile, 0·9% solution of NaCl at 100-fold dilution increments, and 1-ml aliquots from each dilution level were added (in duplicate) to 9-ml portions of sterile, 1·1 × -strength, 50° Difco-brain heart infusion agar and Difco thioglycollate agar, USP. As the saline samples were added to the tubes of agar, the tube contents were briefly swirled and poured into sterile plastic petri dishes (8·5 cm diameter). After agar solidification, the plates were inverted and incubated at 37°, and the number of bacterial colonies was counted after 24 and 48 hrs' incubation. The levels of the antibiotics present in KR buffer were selected, in part, so that dilution of the homogenate, coupled with the use of highly nutrient bacterial growth media, would overcome the growth-inhibitory effects of the antibiotics at the 10^2-dilution level.

RESULTS

Detection of pyrimidine nucleoside deaminase in human tissues. The data in Table 1 clearly show that homogenates of human liver, and to a lesser extent human kidney, were able to convert CA rapidly to noncytotoxic material(s). The actual amount of CA converted corresponds, on a weight basis, to the degradation by a human liver of about 5 g of CA in less than 1 hr. The maximal dose of CA normally administered to man is 50 mg/kg. Although these experiments did not indicate whether there were any additional mechanisms for detoxifying CA *in vivo*, they did indicate that the liver nucleoside system acting alone could probably account for the observed disappearance of CA from blood.

* Kontes Glass Co., Vineland, N.J.

† Registered trademark, M. W. Kellogg Co.

‡ Millipore Filter Corp., Bedford, Mass.

TABLE 1. DETOXIFICATION OF CA BY HOMOGENATES OF HUMAN TISSUE*

Reaction mixture	Human tissue homogenate	[CA] in reaction mixtures (μg/ml)	Apparent [CA] remaining after incubation† (μg/ml)
1	none	1,000	>900
2	liver	none	< 60
3	liver	1,000	< 60
4	kidney	none	< 60
5	kidney	1,000	460

* The incubation mixtures were prepared in 25-ml Erlenmeyer flasks in an ice bath, and they contained, in a total volume of 4·0 ml of KR buffer: 2·0 ml of a freshly prepared 20% homogenate of adult human tissue (as shown) plus 4·0 mg of CA hydrochloride (where indicated). The flasks were incubated for 2 hr at 37° on a Dubnoff shaker with a 4-cm stroke, operating at 120 cycles per minute; the reactions were stopped by heating at 100° (water bath) for 3 minutes. Protein was removed by centrifugation and aliquots of the supernatant solutions were stored in a frozen state. Zero-time controls, not shown in the table, are discussed in the text.

† As determined by cytotoxicity assays against KB cells in culture.[1]

Identification of the products of the detoxification of CA was accomplished by spectrophotometric and paper-chromatographic techniques. For the spectrophotometric identification, deproteinized reaction mixtures prepared as shown in Table 1, together with various "zero-time" controls, were diluted to appropriate volumes with 0·01 N HCl, and the diluted solutions were examined in a Cary recording spectrophotometer. The curves obtained from the zero-time and 2-hr liver samples showed spectral shifts consistent with the hypothesis that CA had been converted completely to UA: (a) maximal wavelength values decreased from 280 mμ to 262 mμ; (b) maximal absorbancy values decreased 25%; (c) optical density ratios at 250, 260, 270, 280 and 290 mμ were the same as those of authentic UA. The spectral data obtained from the kidney samples, however, were not nearly as clear-cut as those of the liver; the results suggested that perhaps only a portion of the CA substrate had been converted during the incubation. It should be noted that this conclusion is in agreement with the cytotoxicity data presented in Table 1, and it is supported also by the chromatographic studies reported below.

The paper-chromatographic identification of UA as the primary product of the process of detoxification of CA was accomplished according to the procedures given in Fig. 1. In these experiments, all the u.v. absorbing materials were characterized by both R_f values and u.v.-absorption spectra. Although the data for only one solvent system are shown in Fig. 1, mobility and spectral comparisons were determined in two additional solvent systems—isobutyric acid : water : 25% NH_4OH, 100 ml : 52 ml : 0·1 ml; isopropanol : conc. HCl : water, 170 ml : 41 ml : water to 250 ml.[3] In all cases, it was found that the enzymatic product and authentic UA exhibited the same spectra and had identical R_f values.

In addition to the identification of UA, the data presented in Fig. 1 provide two other interesting suggestions. First, in agreement with earlier conclusions, it can be seen that the kidney homogenate only partially converted the CA to UA, while the

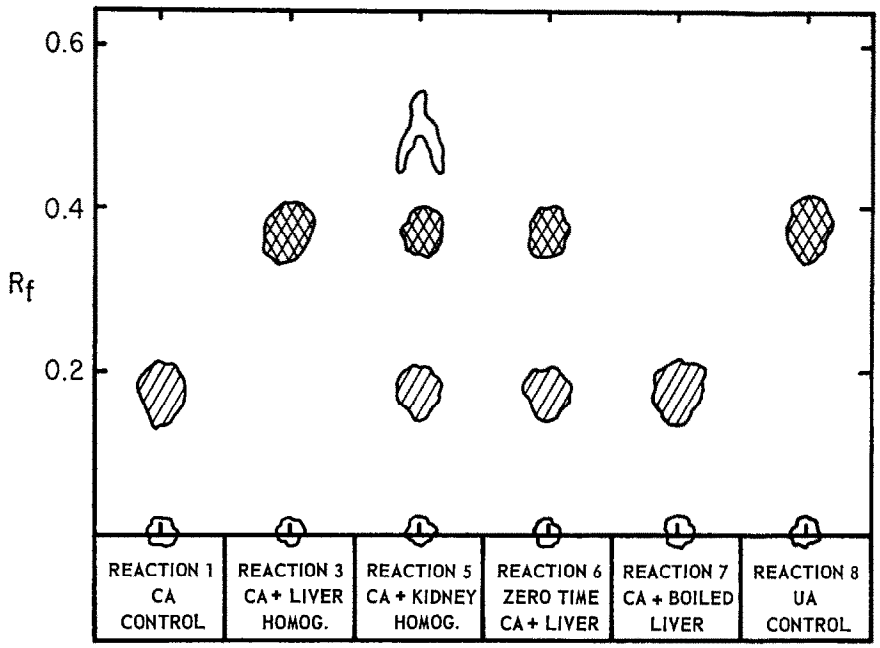

FIG. 1. A composite drawing of paper chromatograms showing the location and type of ultraviolet-absorbing materials present in CA reaction mixtures. Reaction mixtures 1, 3 and 5 are those described in Table 1. Reaction mixture 6 is the same as 3 except that the reaction was stopped at zero-time. Reaction mixture 7 is the same as 3 except that boiled homogenate was substituted for fresh homogenate. Reaction mixture 8 is the same as 1 except that CA was replaced by an equal amount of UA. A total of 100 μlitres of each reaction mixture was spotted on Whatman no. 1 paper, and the chromatograms were developed by the descending technique for 26 hr at room temperature, with an *n*-butanol : water solvent system 84 : 16, v/v). Ultraviolet-absorbing zones on the paper were located and characterized as described in Methods. Cross-hatched zones had u.v.-spectra characteristic of uracil-containing compounds, while the shaded zones had spectra characteristic of cytosine-containing compounds. The open zones shown at the origin were buffer zones, and the open zone at $R_f \simeq 0{\cdot}5$ is a nonpyrimidine, u.v.-absorbing material present in the kidney homogenate.

liver homogenate effected a complete conversion (reaction mixtures 3 and 5). Stoichiometric calculations, in agreement with this finding, show that >95% of the starting amount of CA could be accounted for as UA.

The second suggestion to be derived from the data involves the very active nature of the human liver deaminase. Under no circumstances was it possible to obtain a *true* zero-time control when the heat inactivation step was performed *after* enzyme was added to the substrate. Apparently, appreciable deamination can occur even within the short time it takes to destroy the enzyme activity. Proper zero-time controls are obtained, however, when the homogenate is heat-inactivated first (reaction mixtures 6 and 7).

Although little mention has been given thus far to the deamination of CR to UR by homogenates of human tissue, it should be noted that all of the reported work (excepting cytotoxicity assays) also has been carried out with CR as a substrate. The conclusions from this series of experiments were exactly the same as those reported for CA.

Proof of endogenous origin of human liver deaminase. Since it had been shown that bacterial systems are capable of deaminating a variety of cytosine-containing compounds,[4–7] it became important to determine whether the deaminase activity present in human liver homogenates was of human or bacterial origin. To this end, it was found that sterile-filtered enzyme solutions prepared from two samples of fresh, *unfrozen*, human liver tissue obtained at autopsy had excellent deaminase activity (800 mμmoles converted in 30 min), although there were present fewer than 100 organisms/ml. In a negative approach, it should be noted that homogenates of rat livers, although grossly contaminated with intestinal bacteria (ca. 10^4 organisms/ml), contained negligible deaminase activity under the conditions employed (see Table 2).

TABLE 2. DISTRIBUTION OF PYRIMIDINE NUCLEOSIDE DEAMINASE ACTIVITY IN VARIOUS ANIMAL TISSUES*

Species	Liver	Kidney	Heart	Striated muscle
Postnatal man†	5+‡	3+	1+	—
Prenatal man	—	2+§	—	—
Rhesus monkey	5+	4+	4+	4+
Squirrel monkey	4+	—	—	—
Rabbit (New Zealand white)	2+	—	—	—
Rat (Wistar)	—	—	—	—
Dog (beagle)	1+	—	—	—
Guinea pig	—	5+	—	—
Mouse (Swiss)	—	5+	—	—
Frog (*R. pipiens*)	1+	not run	—	—
Pigeon (White King)	>3+	not run	—	—
Cat‡	2+	—	—	—
Pig‡	—	5+	—	—

* The incubation mixtures were prepared in 12-ml centrifuge tubes in an ice bath; they contained 0·2 ml of 1·25 M glycylglycine buffer at pH 8·0, 0·1 ml of either CA or CR prepared in KR buffer, and 0·2 ml of a 25% centrifuged homogenate of the indicated tissue. The amount of substrate used in the reaction mixtures was varied in the manner described in the text. The tubes were incubated for 1 hr at 37°, and their contents were assayed spectrophotometrically at 290 mμ, as described in Methods.

† Eight months to 75 years of age.

‡ The symbols refer to the average number of mμmoles of UA formed from CA under the assay conditions described above: —, <50; 1+, 50–100; 2+, 100–200; 3+, 200–400; 4+, 400–800; 5+, >800. The omission from the table of the data obtained with CR is discussed in the text.

§ These data were derived from the tissues of only one specimen. All other data were obtained from two or more animals.

The reason for using fresh, unfrozen tissues in this study was to avoid bacterial disruption and consequent enzyme liberation during the freezing step. Enzyme activity and bacterial numbers were determined simultaneously in order to measure the number of bacteria actually present at the time of deaminase assay. The antibiotics present in KR buffer inhibit bacterial growth but do not inhibit the deaminase activity of either pre-existing organisms or enzyme.

Characteristics of the crude enzyme. The initial enzyme studies showed the human liver deaminase to be both soluble and quite stable—retaining considerable activity after 2-hr incubation at 37°. The deamination reaction is linear with time for at least

1 hr (Fig. 2), and the rate of product formation is proportional to enzyme concentration (Fig. 3). No co-factor requirements have been demonstrated, but enzyme preparations are still crude. The deamination products, UA and UR, are effective inhibitors

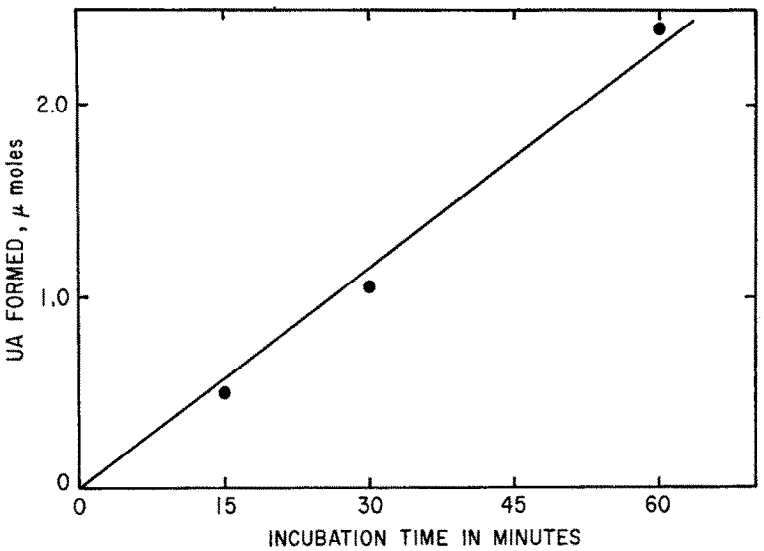

FIG. 2. The enzymatic formation of UA as a function of time. The reaction mixtures and incubation conditions were those described in Table 2, while incubation times are shown in the figure. The substrate, CA, was present in the reaction mixtures in 5·0-μmole amounts.

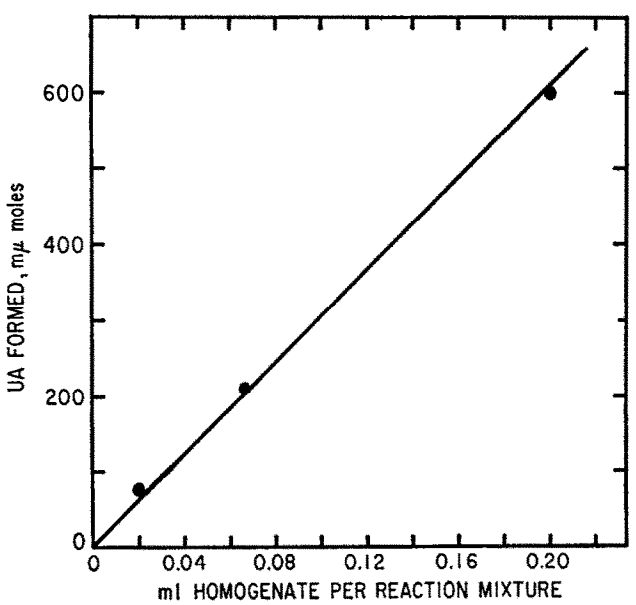

FIG. 3. The formation of UA from CA as a function of enzyme concentration. The reaction mixtures and incubation conditions were those described in Table 2, while the time of incubation was 15 min. The reaction mixtures contained, per ml, 2·5 μmoles CA. The amounts of centrifuged homogenates were those shown in the figure, and the reaction mixtures were brought to 0·5 ml with KR buffer.

of the deamination reaction itself (Fig. 4) and, as a consequence, their concentrations must be kept low in kinetic studies. Similar inhibition of the deamination of CA by PPLO-contaminated KB cells also has been reported.[8] Finally, the enzyme shows a broad pH optimum between pH values of 7 and 10.

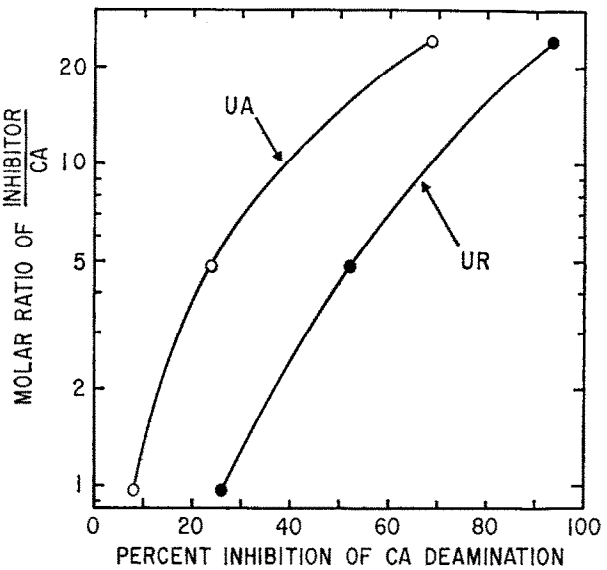

FIG. 4. The inhibition of the deamination of CA by either UA or UR. The reaction mixtures and incubation conditions were those described in Table 2; the time of incubation was 30 min. The reaction mixtures each contained 625 mμmoles CA. UA and UR were used at the levels described in the figure.

Survey studies. A broad survey of four tissues from each of twelve animal species was undertaken in an effort to determine the species and tissue distributions of the pyrimidine nucleoside deaminase activity. In these experiments the tissue homogenates, prepared as described in Methods, were tested first against 250-mμmole amounts of CA and CR substrates in order to determine their relative orders of enzyme activity. Tissues with low activities (i.e. <150 mμmoles deaminated) were retested under the same conditions to confirm the observation, and the substrate level was not readjusted. Tissues that showed higher activities were retested at increasingly higher concentrations of substrate until a point was reached at which approximately half the starting amount of substrate was deaminated during the time of observation. Although this objective was not achieved in every case, it was established with several of the tissues that approximate conditions of enzyme saturation prevailed at the substrate level that was finally employed.

The results of these enzyme survey studies are summarized in Table 2. A statistical analysis of a portion of the data was carried out by J. G. Lee of the Upjohn Company in order to rank certain species and tissues within a species, and these results are presented in Tables 3 and 4. The data for CR have not been included in these tables since they so closely paralleled the data on CA. In every tissue checked, the ratio of activity of CR, as compared to that of CA, fell between 1·0 and 4·0, with most of these ratios occurring between 1·0 and 2·0.

The data in Table 2 show interesting and unexpected distributions of the pyrimidine nucleoside deaminase activity. For example, tissues of the rhesus monkey had a very high deaminase activity in all cases studied, including blood (not presented in the table), whereas with those of man, a high activity was found only in the liver, with a lower activity in the kidney. Interestingly, with respect to distribution and level of enzyme activity, the squirrel monkey (with no activity in the blood) more closely resembles the rabbit and dog than either the rhesus monkey or man. The tissues of the mouse, pig and guinea pig exhibited a high order of activity only in the kidney, while no measurable activity was found in the other three tissues. The rat, on the other hand, had no measurable activity in any of the tissues tested.

TABLE 3. STATISTICAL COMPARISON OF THE DISTRIBUTION OF PYRIMIDINE NUCLEOSIDE DEAMINASE ACTIVITY AMONG TISSUES WITHIN A SPECIES*

Species†	Organ	No. of individuals	Response, mμmoles UA*			P values‡		
			Median	Average	Range	Kidney	Heart	Muscle
Postnatal man	Liver	8	1,150	1,180	710–1,800	<0.01	<0·01	<0·01
	Kidney	6	220	210	66– 340		<0·05	<0·01
	Heart	5	70	76	50– 110			<0·05
	Muscle	5	< 50	< 50	<50§–50			
Rhesus monkey	Liver	4	760	860	620–1,300	>0·05	>0·05	>0·05
	Kidney	4	730	770	600–1,000		>0·05	>0·05
	Heart	4	610	620	570– 690			>0·05
	Muscle	4	460	460	330– 640			
Squirrel monkey	Liver	4	> 290¶	> 400¶	200–690	<0·05	<0·05	<0·05
	Kidney	4	< 50	< 50	< 50		N.A.	N.A.
	Heart	4	< 50	< 50	< 50			N.A.
	Muscle	4	< 50	< 50	< 50			
Rabbit	Liver	5	120	120	76– 190	0·01	<0·01	<0·01
	Kidney	5	< 50	< 50	< 50		N.A.	N.A.
	Heart	5	< 50	< 50	< 50			N.A.
	Muscle	5	< 50	< 50	< 50			

* See Table 2 for the experimental procedures. The omission from the table of data derived from CR is discussed in the text.

† Not included in these analyses are the data from 3 groups of 3 rats each (9 rats total) which showed <50 mμmoles in the 4 tissues tested.

‡ The statistical analyses were performed by J. G. Lee of the Upjohn Co., using a Mann-Whitney U test. "P" values are reported as <0·01 and >0·001, <0·05 and >0·01, or >0·05; N.A. indicates that no analysis was performed.

§ For ranking purposes, values of <50 mμmoles were considered to be 49 mμmoles.

¶ Because of insufficient tissue, one of the four values used to calculate this average could be recorded only as having a response of >250 mμmoles.

Although the number of prenatal and newborn specimens is too low to permit valid statistical comparisons, it is interesting to note the existence of very high levels of deaminase activity in postnatal human livers, in contrast to its apparent absence in prenatal livers; however, the levels of enzyme activity in the renal tissues were fairly constant. It is conceivable therefore, that the kidney enzyme is constitutive, whereas that of the liver is induced shortly after birth.

TABLE 4. STATISTICAL COMPARISON OF THE DISTRIBUTION OF ORGAN DEAMINASE ACTIVITY AMONG SPECIES*

Organ	Species	No. of individuals	Response, mμmoles UA			P values		
			Median	Average	Range	Rh. monkey	Sq. monkey	Rabbit
Liver	Man	8	1,150	1,180	710–1,800	>0·05	<0·01	<0·01
	Rh. monkey	4	760	860	620–1,100		>0·05	<0·05
	Sq. monkey	4	> 290	< 400	200– 690			<0·05
	Rabbit	5	120	120	76– 190			
Kidney	Man	6	220	210	66– 340	<0·05	<0·05	<0·01
	Rh. monkey	4	730	770	600–1,000		<0·05	<0·05
	Sq. monkey	4	< 50	< 50	< 50			N.A.
	Rabbit	5	< 50	< 50	< 50			
Heart	Man	5	70	76	50– 110	<0·05	<0·05	<0·05
	Rh. monkey	4	610	620	570– 630		<0·05	<0·05
	Sq. monkey	4	< 50	< 50	< 50			N.A.
	Rabbit	5	< 50	< 50	< 50			
Muscle	Man	5	< 50	< 50	< 50	<0·05	>0·05	>0·05
	Rq. monkey	4	460	460	330– 640		<0·05	<0·05
	Sg. monkey	4	< 50	< 50	< 50			N.A.
	Rabbit	5	< 50	< 50	< 50			

* See Table 3 for data, procedures, and explanations.

DISCUSSION

The initial studies with homogenates of human liver were conclusive in showing that the disappearance with time of either CA or CR was the result of a deamination reaction effected, most probably, by an endogenous enzyme. The actual mechanism whereby this reaction occurs is presumed to consist of a hydrolytic cleavage of the 4-amino group of the cytosine-containing materials to yield ammonia and the corresponding uracil derivatives. Since analyses for ammonia have not been carried out, however, the precise stoichiometry of the reaction must remain speculative.

The question of whether PPLO contamination of liver tissue was responsible for the observed deamination of CA was considered to be both unlikely and too difficult to test. First, for such a situation to exist, there would have to be a substantial amount of PPLO contamination which was specifically and closely associated with only liver tissue, since the other human tissues tested were much less active. Second, even if such a situation did exist, it would be extremely difficult, if not impossible, to differentiate between liver deaminase activity, and that present in the liver-associated PPLO. Finally, in light of the widespread distribution and relatively constant levels of human liver deaminase activity in the population, the source of the deaminase becomes relatively unimportant.

The survey studies presented in this paper show very interesting species and tissue differences with respect to the distribution of pyrimidine nucleoside deaminase activities. Data such as these may have important applications in the selection of animal species for preclinical toxicology studies and for the selection of routes of administration. Thus, it would appear to be therapeutically wiser to administer CA parenterally than orally, either to humans or to other mammalian species with high deaminase

activities in the liver and, similarly, it might be possible to prolong CA blood levels in man by administering CA parenterally, together with an inhibitor of deaminase activity given orally.

Based on the observation that rat tissues lack deaminase activity, it was predicted that CA should be more toxic to rats than to mice. Such was not the case, however, as was shown by subacute toxicity tests in both species, in which 0/5 mice survived i.p. injections of CA, 1,000 mg/kg given daily for 5 days, while 5/5 rats survived such dosage without signs of toxicity. The reasons for this result are not known at present, but several possible explanations can be postulated: (1) the rat may lack the kinases required to anabolize CA; (2) the rat may excrete CA more rapidly than the mouse; and (3) the rat might degrade CA by a pathway not involving an immediate deamination.

Previous studies by several investigators have shown the presence of pyrimidine nucleoside deaminase activity in *Escherichia coli*[6, 7] and in various animal tissues.[9–12] It is entirely possible that the enzyme investigated in this study is either similar to or identical with those investigated previously, but specific characteristics are not as yet available, and such comparisons cannot be made at this time.

The patterns of distribution of deaminase activities reported by Creasey,[12] who used 5-iododeoxycytidine (ICdR) as a substrate, are in partial agreement with those reported in this paper with CA and CR. Both studies showed poor deaminase activity in rat and rabbit tissues, and good activity in mouse kidney. Creasey found a small amount of activity in mouse liver not noted in the present study, while the opposite result was obtained in rabbit livers. A more important difference is Creasey's finding of a moderate amount of ICdR-deaminase activity in human ileum. In four separate attempts, we have been unable to show CA–CR deaminase activity in this tissue under the conditions used. Whether or not the inclusion of antibiotics in our incubation medium or some other circumstance is responsible for this result cannot be ascertained at present.

The above investigations, as well as those of Calabresi and Papac,* suggest that the deamination of CA to form UA is a major catabolic route in man and that this enzyme activity may be responsible for considerable loss of CA from the blood stream during and immediately subsequent to its intravenous administration.

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* P. Calabresi and R. Papac, unpublished observations.

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