Nucleoside Aminohydrolase, an Enzyme Involved in the Degradation of Deoxycytidine

Deamination of deoxycytidilate has been studied extensively[1-3], since this is one of the key reactions for DNA synthesis in cells in S phase. On the contrary, direct deamination of deoxycytidine has only been mentioned casually[4]. Recently, we have demonstrated[5] that deaminating activity for deoxycytidine is high in human liver and much lower in rat and pig liver, and that this pattern is related to differences in deoxycytidine excretion by these species. In the present investigation, the distribution of nucleoside aminohydrolase activity was determined in different organs from hamster and in the liver of various strains of mice and the results were related to the excretion of deoxycytidine in urine. Moreover, data are presented on the specificity of the enzyme for different substrates, the pH activity, the Michaelis constant and on the methods of a partial purification.

Methods. The different nucleosides used were purchased from Calbiochem. Deoxycytidine-2-C^{14} (spec. activity 42.3 mCi/m*M*) was obtained from the C.E.N. and deoxyadenosine-6-T (spec. activity 1.54 Ci/m*M*), cytosine 5.6-T (spec. activity 5 Ci/m*M*), cytidine-2-C^{14} (spec. activity 77 mCi/m*M*) and deoxycytidilate-C^{14} (spec. activity 65 mCi/m*M*) from Amersham.

Liver was homogenized in 9 vol. of 0.1 *M* phosphate buffer pH 7, and 0.5 ml of the homogenate was incubated at 37°C with 0.1 ml of 30 m*M* substrate solution (deoxycytidine unless otherwise stated) and 10 µl of labelled substrate (e.g. 0.5 µCi deoxycytidine) for 30 or 60 min. The reaction was terminated by immersing the tubes in boiling water for 2 min, and the precipitated proteins removed by centifugation. The enzymatic product (0.1 ml of a 30 m*M* solution of deoxyuridine, for example) was added to facilitate detection of the substance and an aliquot of the mixture was applied as a streak on 5/40 cm strips of Whatmann 3 MM paper. Separation was carried out in a Camag high voltage electrophoresis unit in 0.1 *M* formic acid at 4500 volt for 20 min. After localization under UV light, the metabolites were excised, eluted in the dioxane fluorsystem and their radioactivity counted. Blank values were obtained from samples immediately heated after addition of the substrate. The enzymatic activity is expressed in nmoles converted per min by 1 g tissue (or mg protein for the purified enzyme). When labelled substrates other than deoxycytidine were used, the assay was carried out in a corresponding manner using the appropriate reference compounds. Substrates for which labelled material was not available were assayed by measuring the changes in UV absorption[6] in the assay medium deproteinized with perchloric acid (methyldeoxycytidine at 290 nm, AMP and ATP at 260 nm). The protein content was determined by the biuret reaction or from the absorption at 280 nm[7].

Deoxycytidine in urine was isolated as described elsewhere[8] and its amount measured by microbiological assay with *Lactobacillus leichmannii*. Labelled deoxycytidine was added to the urine to correct for losses during isolation.

Table I. Distribution of nucleoside aminohydrolase in different organs from starved hamsters

	Enzyme activity	
	nmole deoxycytidine/ g tissue/min	nmole deoxyadenosine/ g tissue/min
Liver	370	2300
Kidney	1030	700
Spleen	42	
Testis	16	
Intestine	500	
Brain	28	
Muscle	20	

Results and discussion. Highest activities of nucleoside aminohydrolase among different organs of the hamster are found in kidney followed by liver and intestine, whereas lymphoid organs, brain and muscle contain only traces of enzymatic activity (Table I). The ability to degrade deoxycytidine varies also in the liver of different strains of mice (Table II). It is relatively small in the C3H and greater in the other strains. Apparently, the enzymatic activity is higher in females than in males and also increases in starvation, but the variations between experiments carried out on different days was too large to permit the statistical proof of these changes. Ability to degrade deoxycytidine and excretion of deoxycytidine are inversely correlated (Table II). This becomes even more evident when other species are taken for comparison. Thus, enzymatic activity is very high and deoxycytidine excretion very low in man, whereas the inverse is true for rats[5]. In species such as man, hamster, etc., direct degradation of deoxycytidine to deoxyuridine may, therefore, represent an efficient bypass to the deoxycytidilate aminohydrolase and make deoxyuridine available for synthesis of thymidine. On the other hand, it is difficult to explain why this deamination of deoxycytidine occurs mainly in organs having but little cell division

[1] F. Maley and G. F. Maley, J. biol. Chem. *235*, 2968 (1960).
[2] V. R. Potter, H. C. Pitot, T. Ono and H. P. Morris, Cancer Res. *20*, 1255 (1960).
[3] F. Scarano, Biochim. biophys. Acta *29*, 459 (1958).
[4] J. Shejbal, J. Kostir and F. Smid, 5th FEBS Prague (1968), abstr. 872, p. 218.
[5] B. Zicha and L. Buric, Science, in press (1969).
[6] S. P. Colowick and N. O. Kaplan, *Methods in Enzymology* (Academic Press, New York 1955), vol. II, p. 469.
[7] S. P. Colowick and N. O. Kaplan, *Methods in Enzymology* (Academic Press, New York 1957), vol. III, p. 450.
[8] G. B. Gerber and J. Remy-Defraigne, Radiat. Res., in press (1969).

Table II. Activity of nucleoside aminohydrolase in the liver of different strains of mice and of hamster and its relation to urinary excretion of deoxycytidine

Strain	Enzyme activity[a] (nmole/g per min) Male Fed	Male Starved[b]	Female Fed	Female Starved[b]	Excretion of deoxycytidine nmole/mg creatinine
Hamster		370 (2300)			
Mouse CBA	35 (800)	45 (1140)	53 (1300)	85 (1300)	18
C3H	5 (400)	14 (450)	25 (950)	40 (980)	31
C57Bl	43	67 (1250)	59	92 (1200)	12
C^+	65	101 (1300)	58	137 (1500)	10

[a] Substrate deoxycytidine, values in parenthesis for deoxyadenosine as substrate are indicated only were carried out. [b] Starved for 24 h before assay.

(liver, kidney). The significance of this pathway for DNA synthesis therefore remains to be elucidated.

Several attempts were made to characterize the enzyme further. The pH activity curve (Figure 1) shows a very broad peak from pH 5–7, with the maximum around pH 6. The Lineweaver Burk plot for the determination of the Michaelis constant for deoxycytidine as substrate yields a value of K_m 20 mM. This value is significantly different from that of deoxycytidilate deaminase (K_m 4.1 mM)[9] measured in the absence of dCTP and Mg^{++}. The enzyme may not be specific for deoxycytidine but appears also to react with other nucleosides and nucleic bases containing amino groups (Table III). For this assay a partially purified enzyme preparation was used (see above). Highest activities are found for deoxyadenosine and adenosine, lower ones for deoxycytidine and cytidine, and still lower ones for methyldeoxycytidine, guanosine and cytosine. Nucleotides were not attacked to a significant degree.

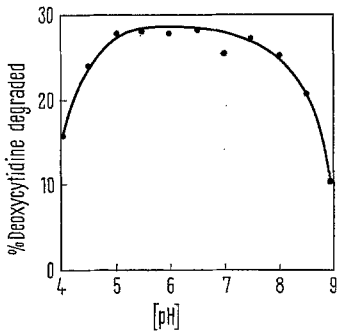

Fig. 1. pH dependence of nucleoside aminohydrolase activity (substrate deoxycytidine) in liver homogenate from hamster; liver tissue was homogenized with 5 volumes of 0.9% NaCl and diluted with 1 volume of veronal buffer of different pH values.

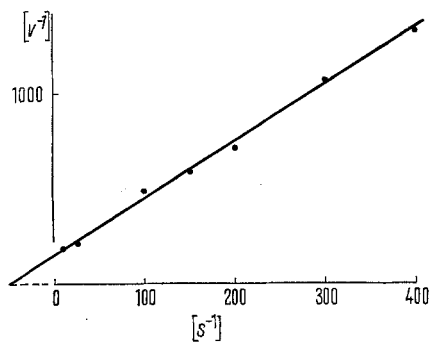

Fig. 2. Lineweaver-Burk plot of nucleoside aminohydrolase (substrate deoxycytidine) in liver homogenate of hamster ($K_m = 20.8$ mM).

Table III. Reaction of partially purified nucleoside aminohydrolase with different substrates

Substrate (final concentration and activity added)	Activity (nmole/mg protein/min)
Cytosine (5 mM, 5 μCi C-5,6-T)	4
Deoxycytidine-5′-monophosphate (5 mM, 0.5 μCi dCMP-C^{14}	$\leqslant 0.5$
Cytidine (5 mM, 0.8 μCi CR-C^{14})	9.0
Deoxycytidine (5 mM)	8.0
Methyldeoxycytidine (1 mM)	2.0
Deoxyadenosine (1 mM, 2 μCi dA H^3)	18
Adenosine (1 mM)	15
AMP (1 mM)	$\leqslant 0.5$
ATP (1 mM)	$\leqslant 0.5$
Guanosine (1 mM)	3.0

Partial purification of the nucleoside amino hydrolase was carried out starting from liver homogenates of hamsters. The enzyme is present in the supernatant of the homogenate centrifuged at 105,000 g for 1 h. Precipitation of the enzymatic activity occurs at 50% saturation with ammonium sulphate. The material was then dialysed and separated on a column of Sephadex G150, eluted with phosphate buffer 0.1 M pH 7. Most of the enzymatic activity was not retained by the column under these conditions. By these procedures, the specific activity of the enzyme could be increased by a factor of 4 compared to liver homogenate. Experiments are now in progress to improve this separation further[10].

Zusammenfassung. Hohe Nukleosidaminohydrolase-Aktivitäten finden sich in Niere, Leber und Darm von Hamstern. Bei Mäusen hängt die Aktivität vom Tierstamm ab und steht in Beziehung zur Deoxycytidinausscheidung.

B. ZICHA[11], G. B. GERBER and J. DEROO

Euratom and Département de Radiobiologie, Centre d'Etude de l'Energie Nucléaire Mol (Belgium), 6 June 1969

[9] G. F. MALEY and F. MALEY, J. biol. Chem. *239*, 1168 (1964).

[10] This publication is No. 436 of the Euratom Biology Division (Contract No. 059-65-7 BIO). A grant of the A.I.E.A. made it possible for Dr. ZICHA to carry out this work at Mol.

[11] Present address: Biophysics Institute, Charles University, Praha (CSSR).

Recording and Analysis of Sounds Produced by Human Lungs During Respiration

The activity of certain human organs, like the lungs or the heart, is associated with the emission of characteristic sounds (respiration sounds, heart beats).

By hearing these sounds the physician obtains information on the condition of these organs. This so-called auscultation method, although affected by a certain degree of subjectivism, is widely used in practical medicine. Inspite of this, very little is known about the physical characteristics of the sounds produced within the human body. This is true especially for respiration sounds. Taking this into account, we started a detailed investigation of the sounds emitted by normal and pathological lungs during the respiratory cycle, with the purpose of finding some objective criteria for evidencing deviations from the normal, associated with special morbid changes. Such criteria could lead, we think, to a better interpretation of the information provided by the respiration sounds and to the elaboration of more objective diagnostic methods in certain respiratory diseases.

As a first step, we carried out the recording and analysis of the respiration sounds of 2 randomly chosen