

ratio of *cis* to *trans* isomer in the mixture was 40:60.

The infrared spectrum of the separated *trans*-propylure showed a medium band at  $965\text{ cm}^{-1}$  (*trans* HC=CH) that was absent in the spectrum of the *cis* isomer, and the compound exhibited a retention time of 13.7 minutes by gas-liquid chromatography. *cis*-Propylure showed an inflexion at  $740\text{ cm}^{-1}$  on its infrared spectrum and a chromatographic retention time of 13.2 minutes. The *cis* isomer could be converted readily into the *trans* isomer by ultraviolet irradiation of its hexane solution containing a trace of iodine.

Laboratory bioassays were conducted with the isomers and their mixtures by exposing caged male moths to the air from pipettes containing the vapors of their methylene chloride solutions (1, 17). Of 100 males used in each test, approximately 75 showed sexual excitement when exposed to *trans*-propylure, whereas only 3 or 4 moths responded to *cis*-propylure, the isomeric 9-propyl-5,9-tridecadienyl acetate, or methylene chloride. Males exposed to the *trans* isomer within 15 minutes after exposure to the *cis* isomer failed to respond, but a complete response was obtained each time in consecutive exposures to the *trans* isomer alone. Vapors of *trans*-propylure containing 10 percent of the *cis* isomer elicited a response in only 15 percent of the moths, and mixtures containing at least 15 percent *cis* isomer failed to cause a response.

Thus, *cis*-propylure acts as an inhibitor or masking agent for *trans*-propylure, but the mechanism through which this occurs is not yet understood.

Presumably, the vapors of the *cis* isomer act on the antennal sensory system to block temporarily the nerve endings responsible for sex pheromone detection.

MARTIN JACOBSON

Entomology Research Division,  
U.S. Department of Agriculture,  
Beltsville, Maryland 20705

#### References and Notes

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15. The chromatography was carried out on an F&M model 500 instrument equipped with a model 1609 flame-ionization attachment on stainless steel columns packed with 5 percent SE-30 on Chromosorb W (3.05 m by 0.31 cm) at  $185^{\circ}\text{C}$ ; the nitrogen flow rate was 25 ml/min.
16. Adsorbosil-ADN-1, containing 10 percent calcium sulfate binder and 25 percent silver nitrate, obtained from Applied Science Laboratories, State College, Pennsylvania.
17. I thank Dr. H. M. Graham, U.S. Department of Agriculture, Brownsville, Texas, for supplying the insects used in the bioassay tests.

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## Deoxycytidine and Radiation Response: Exceedingly High Deoxycytidine Aminohydrolase Activity in Human Liver

**Abstract.** *The activity of deoxycytidine aminohydrolase in the liver of various species including man was investigated. An enormously high activity was found in human liver. This fact explains the extraordinarily low level of deoxycytidine in human urine in comparison with that of other tested species. The suitability of deoxycytidinuria as a biochemical indicator of postirradiation damage in man remains, therefore, an open question.*

Pařízek *et al.* (1) reported the excretion of deoxycytidine in the urine of rats after irradiation with doses ranging from 10 to 600 r, in amounts proportional to the radiation damage. The suitability of that early biochemical test for radiation exposure was confirmed also for the dog, mouse, rabbit,

pig, sheep, and monkey (2, 3). In these studies, however, deoxycytidine was measured by the cysteine-sulfuric acid reaction described by Dishe (see 4). Besides deoxycytidine there are also other deoxypyrimidines, as thymidine or deoxyuridine, which contribute to this reaction, but in the rat deoxycytidine

remains, in normal as in irradiated animals, at least the main catabolite, which is excreted by the urine. Thus it seems that deoxycytidine is really the final biotransformation product of deoxycytidine nucleotide.

It would be of great importance if the deoxycytidinuria could be detected and made use of in irradiated human beings. But by means of the usual chemical analyses it was practically impossible to identify deoxycytidine in human urine. A very sensitive specific method was elaborated, including ion exchange chromatography and microbiological testing with the aid of *Lactobacillus acidophilus* (5). Arient *et al.* (6), using this method, have found that the excretion of deoxycytidine within 24 hours varies from undetectable amounts up to  $1.5\text{ }\mu\text{g}$ , with the average about  $0.5\text{ }\mu\text{g}$  per day. Only in one case of acute myeloblastic leukemia was a detectable amount of deoxycytidine found ( $24\text{ }\mu\text{g}$  in a 24-hour specimen) (6). Similarly, Berry *et al.* (7) could not detect any deoxycytidine in specimens collected before irradiation. After irradiation, however, a high level of deoxycytidine was found in two followed-up humans receiving therapeutic irradiation of the whole body for malignancies (7). Such a high level could not be confirmed by Arient *et al.* (6). As far as we know there are no more data about excretion of deoxycytidine in irradiated man.

There is obviously a different transformation or different degree of this transformation between rats and human beings. What is the cause of such a different level of deoxycytidine in rat and human urine? One possible explanation could be a different activity of deoxycytidine aminohydrolase, which deaminates deoxycytidine to deoxyuridine. Thus the activity of this enzyme was followed up in liver tissue of various species. Liver sample was homogenized with a 10-fold greater volume of tris buffer ( $\text{pH } 8$ ;  $0.05\text{M}$ ). To  $0.5\text{ ml}$  of homogenate,  $0.1\text{ ml}$  of deoxycytidine solution was added (final concentration of  $5\text{mM}$ ), and the system was enriched with  $0.5\text{ }\mu\text{C}$  of deoxycytidine- $2\text{-C}^{14}$  (specific activity  $211\text{ mc/mmole}$ ; Amersham, England). The system was incubated for 60 minutes at  $37^{\circ}\text{C}$  and inactivated in a boiling water bath for 2 minutes;  $600\text{ }\mu\text{g}$  of deoxyuridine in  $0.1\text{ ml}$  was added as carrier and the supernatant was separated electrophoretically on Whatman No. 3 paper (formic acid  $0.1\text{M}$ , 400 volts, 90 minutes). The radioactive

Table 1. Activity of deoxycytidine aminohydrolase in liver (millimicromoles of deoxyuridine formed per gram of wet liver tissue per 30 minutes; mean  $\pm$  standard deviation).

Species	Deoxycytidine aminohydrolase	% of deoxyuridine	N*
Pig 1	25	0.1	1
Pig 2	25	.1	1
Rat	65 $\pm$ 55	.26	8
Guinea pig	292 $\pm$ 150	.93	7
Rabbit	600 $\pm$ 285	2.44	8
Dog	675 $\pm$ 147	2.70	7
Mouse	1,307 $\pm$ 257	5.23	11
Calf 1	3,312	13.25	1
Calf 2	1,995	7.98	1
Golden hamster	4,742 $\pm$ 960	18.97	6
Man 1 (age 66), cholelithiasis	19,625	78.50	1
Man 2 (age 44) ulcer ventriculi	22,315	89.20	1
Man 3 (age 30), cholelithiasis	18,900	75.60	1
Man 4 (age 56), tumor intestini	14,575	58.30	1

\*Number of species in each group.

spots were cut out and counted directly in the liquid scintillation computer Mark I (Nuclear-Chicago). The activity of deoxycytidine aminohydrolase was expressed in millimicromoles of deoxyuridine formed per gram of wet liver tissue per 30 minutes.

The results are given in Table 1. The activity in human liver is about 300 times higher than in rat liver. The lowest activity was found in rat and pig liver. It is of some interest that a high activity was observed in calf and golden hamster liver. We could not find any literature data concerning the deoxycytidine aminohydrolase except the recent report of Shejbal *et al.* (8).

As can be shown from Table 1, in human beings about 60 to 90 percent of substrate has been transformed to deoxyuridine, and therefore it was probable that the deoxyuridine formed would influence the enzymatic activity by negative feedback. So it can be proposed that the initial activity of this enzyme was really much higher. In order to prove this, we followed up this activity by using more dilute homogenate (1:30), and for only 10 minutes' incubation time. By using a spectrophotometric method, at 290 nm we found a linear response of enzyme activity to incubation time. The initial activity calculated from these data is

153,036 m $\mu$ mole at 5 mM final substrate concentration and 41,112 m $\mu$ mole at 1 mM, that is, activity about six times higher than that shown in Table 1. The great difference in rat and human activity was also confirmed by means of thin layer chromatography of incubated homogenates (silica gel, *n*-butanol-formic acid-water, 77:10:13). Practically no deoxycytidine is still present in incubated human liver after 60 minutes.

To summarize our preliminary findings, we can say that an enormously high deoxycytidine aminohydrolase activity was found in human liver. As to other species tested, only golden hamster and calf liver have a quite high activity. In all other tested species the activity of enzyme is very low, especially in the rat and pig.

There is a reciprocal relationship between the activity of deoxycytidine aminohydrolase and the level of deoxycytidine excreted in human and rat urine: human beings: activity in liver, 22,315 m $\mu$ mole; deoxycytidinuria, 0 to 1.5  $\mu$ g (5); rats: activity in liver 65 m $\mu$ mole; deoxycytidinuria, 90 to 600  $\mu$ g (9). The activity in human liver is so high that deoxycytidine is not expected to be found in human urine, and the experimental findings are fully in agreement with this proposal.

Owing to their high activity, golden hamster and calf liver could be suitable for further study of deoxycytidine metabolism in irradiated organisms especially for judging the validity of this indicator of radiation damage for human beings.

BLAHOŠLAV ZÍCHA  
LUBOMÍR BUŘIČ

*Institute of Biophysics, Faculty of General Medicine, Charles University, Prague 2, Czechoslovakia*

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## Phenethyl Alcohol and Tryptophol: Autoantibiotics Produced by the Fungus *Candida albicans*

**Abstract.** *Two autoantibiotics produced by the fungus Candida albicans have been identified, with the aid of mass spectrometry, as 2-phenylethanol and 3- $\beta$ -hydroxyethylindole. The cyclo-dipeptides, cyclo(proline-leucine) and cyclo(proline-phenylalanine) were also isolated from cultures of Candida albicans, but did not inhibit the growth of the fungus.*

*Candida albicans*, a yeastlike fungus that produces some mycelium, infects almost every part of the human body (1). Healthy individuals are not generally susceptible to *C. albicans*, but it causes a serious disease (candidosis) in newborn babies, pregnant women, the elderly, the bedridden, and others in a condition of low resistance.

Substances which accumulate in the culture medium and inhibit or significantly retard the growth and development of the organisms producing them have been referred to as autoinhibitors, self-inhibitors, and autoantibiotics (2).

The presence of autoantibiotic substances in the cultures of *C. albicans* first became apparent when the fungus was streaked (Fig. 1) and then incubated at either 25° or 37°C for 3 to 7 days. Pseudomycelia failed to develop along the margins of the inner streaks but did appear along the outer edges of the side streaks. This "edge effect" or reciprocal inhibition (3) was also observed on dilution plates. When each plate contained about ten colonies, pseudomycelia could be seen along the edges; with 100 or more colonies per plate, only yeast-like growth occurred. We studied the self-inhibitory metabolites excreted by *C. albicans* to explain this inhibition.

Cultures of *C. albicans* were grown by shaking them in Sabouraud sucrose broth at either 25° or 37°C. After 1 week, the cells were separated from the broth (2.1 liter) by centrifugation and washed with 400 ml of water. The washings were added to the broth, and the latter was then extracted with chloroform (four times, 75 ml each time). The combined extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated at 37°C in a vacuum (10 mm-Hg). The resulting residue (470 mg) was assayed both in Sabouraud sucrose broth and on agar plates which had been seeded with *C.*