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

Species	Deoxycytidine aminohydrolase	% of deoxyuridine	N*
Pig 1	25	0.1	1
Pig 2	25	.1	1
Rat	65 ± 55	.26	8
Guinea pig	292 ± 150	.93	7
Rabbit	600 ± 285	2.44	8
Dog	675 ± 147	2.70	7
Mouse	1,307 ± 257	5.23	11
Calf 1	3,312	13.25	1
Calf 2	1,995	7.98	1
Golden hamster	4,742 ± 960	18.97	6
Man 1 (age 66), cholelithiasis	19,625	78.50	1
Man 2 (age 44) ulcus 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 mμmole at 5 m*M* final substrate concentration and 41,112 mμmole at 1 m*M*, 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 mμmole; deoxycytidinuria, 0 to 1.5 μg (*5*); rats: activity in liver 65 mμmole; deoxycytidinuria, 90 to 600 μg (*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 futher study of deoxycytidine metabolism in irradiated organisms especially for judging the validity of this indicator of radiation damage for human beings.

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5 August 1968

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-β-hydroxyethylindole. The cyclodipeptides, 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.

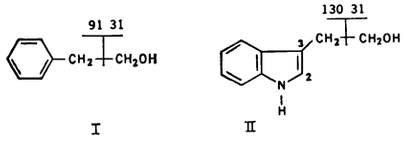
Substances which accumulate in the culture medium and inhibit or significantly retard the growth and development of the organisms producing them have been referred to as autoinhibitors, self-inhibitors, and autoantibiotics (*2*).

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.*

albicans; it was toxic to the fungus at a concentration of 200 μg/ml.

The crude extract (650 mg) was separated into its components by column chromatography on silica gel (Merck 30 to 70 mesh). Compound I (305 mg), a colorless oil, with boiling point (12 mm-Hg) of 98° to 100°C, was obtained by elution with a mixture of petroleum ether and ethyl ether (4 : 1) and was the major component of the mixture. An infrared spectrum ($CHCl_3$) of compound I contained bands at 3600 cm^{-1}, 1600 cm^{-1}, and 1495 cm^{-1}, indicating the presence of both a hydroxyl group and aromatic ring. A high resolution mass spectrum of compound I exhibited a molecular ion at *m/e* (mass/charge) 122.1124, having the elemental composition $C_8H_{10}O$. Abundant fragment ions at *m/e* 92 $(C_7H_8)^+$, 91 $(C_7H_7)^+$, and 31 $(CH_2OH)^+$ suggested that compound I was 2-phenylethanol (phenethyl alcohol). This assignment was confirmed by comparison of the infrared and mass spectra of compound I with those of an authentic sample of phenethyl alcohol.



I II

Compound II (76 mg) was obtained as a light-sensitive oil by eluting the column with a mixture of petroleum ether and ethyl ether (3 : 2). Recrystallization of the oil from the solvent afforded colorless leaflike crystals (melting point, 59° to 60°C), the infrared spectrum of which exhibited bands ($CHCl_3$) at 3600 cm^{-1} (OH), 3490 cm^{-1} (NH), 1600 cm^{-1}, and 1500 cm^{-1} (aromatic ring). By high resolution mass spectrometry, compound II was shown to have a molecular weight of 161.0840 corresponding to an elemental composition of $C_{10}H_{11}NO$. Abundant fragment ions were observed at *m/e* 130 $(C_9H_8N)^+$, 103 $(C_8H_7)^+$, and 77 $(C_6H_5)^+$. This latter series of ions is characteristic of indoles having alkyl substituents at either carbon 2 or carbon 3 (*4*). Since compounds derived from the tryptophan are commonly found in biological systems, an alkylindole derivative substituted at carbon 3 was suspected for compound II. The fragment ion at *m/e* 130 differs from the molecular ion only by the elements, CH_2OH; therefore, compound II was tentatively assigned the structure of 3-β-hydroxyethylindole (tryptophol). A comparison

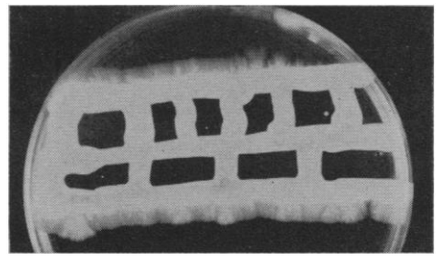
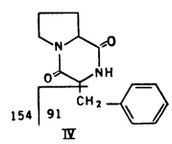


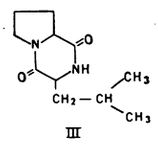
Fig. 1. Opposing streak cultures of *Candida albicans* showing self-inhibition of filamentous development. The fungus developed as a yeast would within the streaks and along the inner opposing edges. Filaments were formed extensively along the exposed outer edges of the streaks (⅘ actual size).

of the infrared and mass spectra of compound II with those of an authentic sample of tryptophol showed them to be identical.

Compounds III (35 mg) (melting point 134° to 135°C) and IV (31 mg) (m.p. 116° to 117°C) were eluted from the column with a mixture of petroleum ether, ethyl ether, and acetone (2 : 1 : 1). Both compounds were initially obtained as oils but crystallized to colorless solids when they were triturated with petroleum ether. Strong bands in the infrared ($CHCl_3$) at 1660 to 1670 cm^{-1} indicated that both compounds III and IV contained an amide group. The high-resolution mass spectrum of compound IV showed a molecular ion at *m/e* 244.1211 ($C_{14}H_{16}N_2O_2$) and abundant fragment ions at *m/e* 153 $(C_7H_9N_2O_2)^+$, 91 $(C_7H_7)^+$, and 70 $(C_4H_8N)^+$. The latter two ions generally occur in molecules containing benzyl and prolyl groups. When the elements contained in the ions at *m/e* 91 and 70 are substracted from the composition of the molecular ion, only the atoms C_3HNO_2 remain, and this group must contain at least one and probably two amide linkages. The presence of both the prolyl and benzyl groups along with two amide bonds suggested that proline and phenylalanine might be involved and led to the assignment of compound IV to the dimer cyclo(proline-phenylalanine).



IV



III

The mass spectrum of compound III contained a very weak molecular ion at *m/e* 210.1362 ($C_{11}H_{18}N_2O_2$) and abundant fragment ions at *m/e* 154 ($C_7H_{10}N_2O_2$)$^+$ and 70 $(C_4H_8N)^+$. Since the infrared spectra of compounds III and IV were quite similar, and the heteroatom composition of both compounds was identical, compound III was also assumed to be a dimer of two amino acids. As in compound IV, the abundant ion at *m/e* 70 indicated that one of the amino acids was proline. The ion at *m/e* 154, which is derived from the molecular ion by loss of C_4H_8, suggested that the other amino acid was leucine. Compound III was therefore formulated as the dimer cyclo(proline-leucine).

Authentic samples of both cyclic dimers were prepared by thermal dehydration of the dipeptides 1-prolyl-1-phenylalanine and 1-prolyl-1-leucine at 200°C (0.1 mm-Hg); mass spectra of the synthetic dimers were identical in every respect with those obtained for compounds III and IV.

Of the above four compounds, phenethyl alcohol and tryptophol inhibited the growth of *C. albicans* at concentrations of 20 and 40 μg/ml, respectively. Commercial samples of phenethyl alcohol and tryptophol (*5*) were similarly toxic to the microorganism. No inhibitory effect on the fungus was manifested by the cyclic dipeptides. Extracts of uninoculated media did not yield the inhibitors. Inhibitors were isolated from media after culturing *C. albicans*. Therefore, these are metabolic products of *C. albicans*.

Tryptophol is a growth hormone in plants (*6*). Phenethyl alcohol is a common constituent of rose and other fragrant essential oils and is an antibiotic (*7*). It also inhibits synthesis of nucleic acids (*8*) and functions in the dimorphism of *Mucor rouxii* (*9*). It is hoped that the autoantibiotics isolated from *Candida albicans* might have some medicinal value in the treatment of candidosis.

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10. Supported by PHS grant AIO7121 and a faculty fellowship from the College of the Holy Cross. We thank Dr. T. Robinson, A. Gawienowski, I. S. Fagerson, and E. Bell for suggestions and encouragement. The mass spectrometric work was supported by PHS training grant (GM01523) in a PHS-sponsored mass spectrometry facility of M.I.T. (FR-00317).

30 August 1968; revised 30 October 1968

6-Phosphogluconate Dehydrogenase: Hemizygous Manifestation in a Patient with Leukemia

Abstract. *In a study of 41 patients with chronic myelocytic leukemia, two were found to have the 6-phosphogluconate dehydrogenase heterozygous phenotype A-B, and two had the phenotype characteristic of* Pd^B *homozygosity. Since one of the two with* Pd^B *homozygosity was the mother of two children with the A phenotype, it was presumed that she carried a* Pd^A *gene not expressed in her blood cells. This was confirmed by electrophoretic analysis of her fibroblasts, which had the A-B phenotypic pattern. Gene deletion is considered to be the most likely explanation.*

In patients with chronic myelocytic leukemia (CML), the myelogenous cells have the Philadelphia[1] (Ph^1) chromosome, a G-group chromosome with a deletion of the long arm involving about a third of its DNA (*1*). Cytogenetic (*2*) and genetic evidence (*3*) support a clonal origin of red cells and granulocytes in patients with this disease; thus, both of these cell types from CML patients can be used for deletion mapping of the involved G chromosome. During such a study of markers for 15 autosomal loci in 41 patients with CML (*4*) and 29 members of their families, we found one patient with a 6-phosphogluconate dehydrogenase (6PGD) (E.C. 1.1.1.44) anomaly which forms the basis for this report.

The starch-gel electrophoretic pattern (Fig. 1) of 6PGD in granulocytes of normal subjects homozygous for either the common allele, Pd^A (*5–7*) or its less common allele, Pd^B, shows a single band with characteristic mobility (*8*). The electrophoretic pattern of the heterozygous phenotype has both A and B enzyme bands as well as a band with intermediate mobility which probably represents a hybrid of A and B subunits (*8*). Of the 41 patients with CML, 37 had A, two had A-B, and two had B phenotypes indistinguishable from those of normal subjects. Comparison of the phenotype frequencies in this small sample with those in the general population is difficult because the sample was drawn from a very heterogeneous population.

In the family of one of the patients (Mrs. I), the husband and both children had the A phenotype typical of Pd^A homozygosity. Nevertheless, both the red cells and granulocytes of Mrs. I had the B phenotype typical of Pd^B homozygosity. The same results were obtained from two subsequent analyses at 2-month intervals. Mrs. I, in complete remission on busulfan therapy, had never undergone transfusion. The results of other genetic marker tests supported her assertion that she was the mother of her children. Thus, she had to be a carrier of the Pd^A gene, even though this gene was not expressed in her granulocytes or red cells. That she was the carrier was confirmed by analysis of her cultured skin fibroblasts, which revealed an A-B phenotype characteristic of cells from a Pd^A/Pd^B heterozygote (Fig. 1).

No other examples of anomalous inheritance were found in our study. In the one other patient with the blood cell B phenotype, the same type was found in cultured fibroblasts; also, her children had type A-B. She was therefore presumed to be a Pd^B homozygote.

There are several possible explanations for the discordance of 6PGD types between the myelogenous and nonmyelogenous cells of Mrs. I. For example, the electrophoretic migration of the enzyme might be altered as a consequence of CML or its treatment. However, none of the other patients on busulfan therapy had an unexpected isozyme pattern. Thus, it seems more likely that Mrs. I's anomaly is due to a genetic event in a stem cell, occurring early in embryogenesis or later in the single stem cell from which the leukemia arose.

There have been no examples of unusual 6PGD inheritance in studies of several hundred families of nonleukemic subjects (*7–9*). Therefore, since the association of CML and discordant 6PGD phenotype in Mrs. I is apparently not fortuitous, the responsible genetic event probably occurred in the leukemic stem cell. This event could have been somatic mutation or recombination, or genetic inactivation, or—more likely—deletion.

From the observations reported here, the site of the assumed deletion cannot be determined. If it is part of the deleted segment of Mrs. I's Ph^1 chromosome, then one must account for the presence of the blood cell A-B pheno-

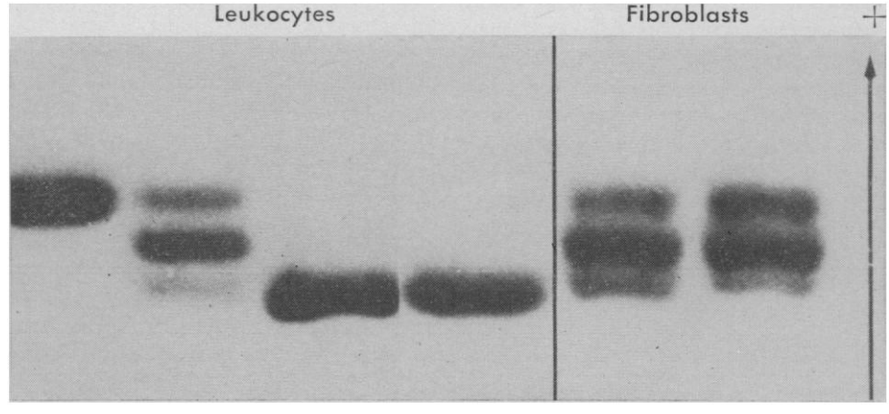

Fig. 1. Starch-gel electrophoresis of 6-phosphogluconate dehydrogenase. From left: slot 1, granulocytes from a normal subject homozygous for the Pd^A gene; slot 2, granulocytes from a normal subject heterozygous for the Pd^A and Pd^B genes; slot 3, granulocytes from a normal subject homozygous for the Pd^B gene; slot 4, granulocytes from Mrs. I; slot 5, cultured skin fibroblasts from Mrs. I; slot 6, cultured skin fibroblasts from a normal subject heterozygous for the Pd^A and Pd^B genes.