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Typical hyperaminoaciduria after high doses of 6-azauridine triacetate

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THE BIOCHEMICAL effect of 6-azauridine triacetate (6-AzUR-TA) consists of an interference with the biosynthesis of the pyrimidine nucleotide components of the nucleic acids. The site of blockade is the enzyme which decarboxylates orotidine 5′-phosphate; to accomplish this 6-AzUR-TA is converted to 6-azauridine 5′-phosphate, which is the specific inhibitor of orotidine 5′-phosphate decarboxylase. Large amounts of orotic acid and orotidine excreted in urine of animals and patients after application of 6-azauridine seem to be caused by this blockade.

However, Bono *et al.*[1] found, using ^{14}C-orotic acid administered intravenously, that patients treated with 6-azauridine have in some instances an increased production of uridine 5′-phosphate. Accumulation of uridine 5′-phosphate in patients treated with 6-azauridine might result in an excretion of β-alanine as the degradative product of uracil formed from uridine 5′phosphate. Also, carbamyl aspartic acid, found in an increased amount in the urine of patients treated with 6-azauridine,[1] could be considered as the source of β-alanine.[2] Furthermore, it was possible that a relationship existed between these changes and the metabolism of certain amino acids. These questions were considered to be important in view of the clinical application of 6-AzUR-TA for the treatment of tumors,[3, 4, 5] viral infections,[6] *mycosis fungoides*[7] and psoriasis.[8, 9]

Four patients aged 43–53, suffering from a generalized form of psoriasis, were treated *per os* with 400 mg of 6-AzUR-TA per kg of body weight per day for 3 weeks. The amino acids in blood and urine were isolated quantitatively by two-dimensional paper chromatography after dilution and filtration of the sample through a column of Dowex-50 × 8 according to the method of Hyánek[10] and detected with ninhydrin. The determinations were carried out in duplicate from a 24-hr sample of urine before and after acid hydrolysis. β-Alanine was identified by paper chromatography by using a known compound as a standard in various elution systems: *n*-butanol: acetic acid: water

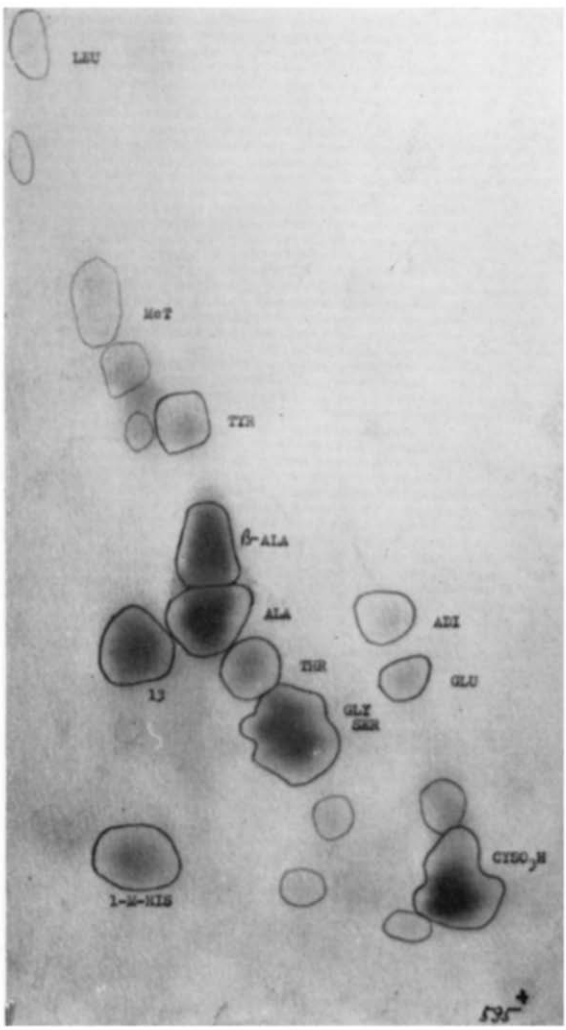

FIG. 1. Representative two-dimensional chromatogram of the aminoacids excreted in the urine of a patient with psoriasis after 2 weeks of treatment with 6-AzUR-TA.

(4:1:5), $R_F = 0{\cdot}53$; phenol:water:96% ethyl alcohol (2:1:1) with 0·05% oxyquinoline (the chromatography was carried out in an atmosphere saturated with ammonia), $R_F = 0{\cdot}46$; isopropyl alcohol: water (4:1), $R_F = 0{\cdot}44$. β-Alanine could not be isolated in a quantitative manner after acid hydrolysis, whereas the concentration of α-amino acids was not substantially altered.

The metabolites of 6-AzUR-TA in the urine were determined by paper chromatography according to the method of Grafnetterová *et al.*[11] None of the urinary metabolites of 6-AzUR-TA gave a ninhydrin-positive reaction in preliminary experiments.

The 24-hr samples of urine from patients treated with 6-AzUR-TA subjected to paper chromatography and detected with ninhydrin showed an increased elimination of ninhydrin-positive compounds. Of the α-amino acids, mainly glycine, serine, L-alanine, cystine derivatives and some unidentified ninhydrin-positive substances were present in the urine in amounts greater than normal. A particularly pronounced increase in the concentration of β-alanine was found in the urine of patients receiving 6-AzUR-TA, which only occurred in trace amounts in normal urine under physiological conditions. After administration of 6-AzUR-TA, an analogous increase in β-alanine excretion was also found in rabbits and in patients suffering from other diseases, such as *mycosis fungoides* and rheumatoid arthritis. A typical two-dimensional chromatogram of the amino acids excreted in the urine of a patient with psoriasis after 2 weeks of therapy with 6-AzUR-TA is shown in Fig. 1. A quantitative measurement of the most frequent amino acids is shown in Table 1. All of these data were obtained before acid hydrolysis. The cystine derivatives were measured after oxidation to cysteic acid. The urine of 6-AzUR-TA-treated patients gave a positive Brandt test at a 1:32 dilution. The content and spectrum of the serum amino acids were not substantially different from the normal; however, α-amino nitrogen content of the urine was increased up to 204 mg/day.

TABLE 1. AVERAGE VALUES OF EXCRETION OF FREE AMINO ACIDS BY THE URINE IN PSORIATIC PATIENTS BEFORE AND AFTER TREATMENT WITH 6-AZUR-TA

Patient	β-Alanine (m-moles/24 hr)		Cysteic acid (m-moles/24 hr)		α-Alanine (m-moles/24 hr)		Glycine + serine (m-moles/24 hr)	
	Before	After	Before	After	Before	After	Before	After
V.V.	none*	1·14	traces†	0·57	0·29	0·49	0·70	1·07
K.K.	none	1·29	none	3·40	0·32	0·99	0·49	0·96
S.E.	none	0·92	traces	0·96	0·23	0·36	0·83	1·10
V.J.	none	0·90	traces	0·26	0·33	0·93	0·63	2·50

* None was found in the sample.

† No quantitative determination was possible.

6-AzUR-TA in high doses blocks the enzyme orotidine 5′-phosphate decarboxylase.[3] Because of this inhibition pyrimidine precursors, which are synthesized before the site of blockade, will accumulate,[1] including carbamyl aspartic acid.[1] Carbamyl aspartic acid could be cleaved by carbamyl aspartate decarboxylase to carbon dioxide and carbamyl β-alanine;[2] the latter metabolite is further catabolized by β-ureidopropionase to yield β-alanine, carbon dioxide and ammonia.[12]

β-Alanine is a part of pantothenic acid and thus also of coenzyme A, as well as of anserine and carnosine. Whether its presence might be one of the factors participating in the favourable therapeutic effect of 6-AzUR-TA in the treatment of psoriasis will be studied in the future. In addition, the mechanism of the increased excretion of cystine derivatives and of other amino acids requires a detailed explanation.

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Effects *in vivo* and *in vitro* of nonsteroidal anti-inflammatory drugs on (rat stomach) histidine decarboxylase*

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Several acidic nonsteroidal drugs, e.g. indomethacin, phenylbutazone, acetylsalicylic acid and fluphenamic acid have been successfully employed in the treatment of inflammatory diseases. Several investigators[1, 2] have shown that these compounds, as well as other salicylates,[3] inhibit *in vitro* the enzyme, histidine decarboxylase (HD), which catalyzes the decarboxylation of histidine to form histamine. The available data[1, 2] suggest that the anti-inflammatory action *in vivo* of there drugs parallels their HD inhibitory activity *in vitro*.

The present study confirms the inhibition *in vitro* by these drugs of specific HD from rat glandular stomach (RS)[4–6] and mouse mastocytoma (MMCT),[7, 8] but demonstrates that anti-inflammatory acids increase HD activity *in vivo*.

METHODS AND MATERIALS

Fundic stomach from CD male rats (250–300 g) and mastocytomas from LAF_1 female mice (25–30 g) were employed in experiments *in vitro*, whereas only the former tissue was used in obtaining data *in vivo*. *In vivo* and *in vitro* in this paper refer to the method of evaluation of drug action. In experiments *in vivo*, the compounds were administered to rats for various periods of time before tissues were removed and assayed for HD activity. With testing *in vitro*, drugs were dissolved in the buffer of the enzyme assay system. MMCT were harvested 1 month after subcutaneous implantations of tumor tissue. The original solid mastocytomas were taken from LAF_1 female mice provided by Dr. J. P. Green of the Department of Pharmacology, Mount Sinai School of Medicine, New York, N.Y.

All tissue was removed after the animals were killed by cervical dislocation. The MMCT's were pooled and frozen until homogenized, but RS homogenates were prepared from fresh tissue and immediately assayed for HD activity.

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