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# Relationship between Urinary β-Aminoisobutyric Acid and Transfer RNA Turnover in Cancer Patients[1]

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## SUMMARY

The excretion of β-aminoisobutyric acid, a thymine catabolite, was investigated in 46 patients with cancer. The dual origin of β-aminoisobutyric acid, indicating both transfer RNA-thymine and DNA-thymine as precursors, has been studied. Comparison of β-aminoisobutyric acid excretion with that of pseudouridine revealed a positive correlation of β-aminoisobutyric acid to pseudouridine excretion in 68% of the patients. Another group of the patients (24%) showed an excretion pattern constituting an increased excretion of pseudouridine and a normal β-aminoisobutyric acid excretion. Furthermore, it was possible to distinguish the probable genetic high excretors of β-aminoisobutyric acid who have an elevated β-aminoisobutyric acid excretion related to a normal pseudouridine and urate. This excretion pattern was the same in different clinical states. The excretion of urate was often found to parallel that of pseudouridine. However, in most cases the urate excretion was within the normal range. In addition, the excretion patterns following major surgery are illustrated. These showed a parallel increase in β-aminoisobutyric acid and pseudouridine on the 2nd and 3rd postoperative days and in pseudouridine alone on the 6th to 9th postoperative days. The results indicate a positive correlation of urinary β-aminoisobutyric acid to transfer RNA turnover in cancer patients.

## INTRODUCTION

The urinary excretion of β-AIB,[2] a thymine catabolite, has previously been investigated in humans. Comparisons have been drawn between "normal" and various pathological conditions, including cancer (reviewed in Refs. 11, 13, 17). In these studies, variations in the excretion of β-AIB were found to be correlated to changes in the patient's clinical state, probably in contrast to the genetically determined high excretors of β-AIB. These persons are the homozygotes for a recessive allele (5, 22).

It has been suggested that increased β-AIB excretion is due to incomplete degradation of thymine and related to a breakdown of DNA, both in genetic high excretors (20) and in pathological conditions (cancer). The demonstration of a dual origin of β-AIB (12), however, indicates that both DNA-thymine and tRNA-thymine are precursors of urinary β-AIB. This is likely to account for the observed high excretion of β-AIB in cancer patients, since the turnover of DNA is known to be slow compared to that of tRNA. Recently, tRNA-thymine was demonstrated to be available for new DNA-thymine syntheses in rapidly growing tumors and regenerating liver of rats (14).

The present report deals with the occurrence of β-AIB in urine from cancer patients with a view to the breakdown of DNA and tRNA. The excretion of β-AIB has been compared with that of $m^7G$, ψ-uridine, and urate.

## MATERIALS AND METHODS

**Urine Collection.** Twenty-four-hr urine samples were used for quantitative estimation of the nucleic acid catabolites. The urine samples were collected and stored without a preservative at −20° until examination. No dietary restrictions were imposed.

**Urinary β-AIB, ψ-Uridine, $m^7G$, and Urate.** β-AIB was isolated by thin-layer chromatography of the dinitrophenyl derivative by using Merck's precoated plates, 0.25 mm Silica Gel 60, and chloroform:pyridine:glacial acetic acid (100:100:2) as the solvent system. Spots containing dinitrophenyl-β-AIB were scraped off and quantified spectrophotometrically at 366 nm (for details see Ref. 11). DL-β-AIB, purchased from Sigma Chemical Co., St. Louis, Mo., was used as a reference.

ψ-Uridine and $m^7G$ were isolated and quantified by 2-dimensional thin-layer chromatography, using Merck's precoated plates, and 0.25 mm Silica Gel 60, with fluorescein indicator. The urine samples were desalted by pretreatment with Dowex 1-X8 and Dowex 50-X8. Fifty μl of desalted urine were applied, and chromatographed 15 cm in the 1st solvent system, isopropyl alcohol:water (120:20). Then the plate was dried at 110° for 10 min and chromatographed at right angles to the former dimension, 15 cm in the 2nd solvent system, 1-butanol:ammonia, 10% (120:20). After drying again, the spots containing ψ-uridine and $m^7G$ were scraped off in UV light (254 nm) and eluted with 1.5 ml of 0.05 N HCl. ψ-Uridine was quantified spectrophotometrically at 263 nm, and $m^7G$ was quantified at 250 nm in a Beckman DU spectrophotometer. All analyses of 24-hr urine samples were performed as duplicate assays, and the results represent mean values. Internal standards and blanks were used. The recovery

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[2] The abbreviations used are: β-AIB, β-aminoisobutyric acid; $m^7G$, 7-methylguanine; ψ-uridine, pseudouridine.

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values of authentic standards for the total method were 80% for ψ-uridine and 65% for $m^7G$. Similar values have been obtained by other workers (6). The coefficients of variation were 10 and 15% for ψ-uridine and $m^7G$, respectively. By this method ψ-uridine was well separated from uracil and uridine, and $m^7G$ was separated from 1-methylguanine and guanine as well as from other purines. The reference substances were obtained from Sigma Chemical Co.

The excretion of urate was determined by using a uricase-UV method modified from the originally described method (16). Uricase was purchased from Leo Pharmaceutical Products, Ballerup, Denmark.

**Patients.** Forty-six patients with cancer were included in this study. Thirty-nine had transitional-cell carcinoma in the urinary tract, 5 had prostatic carcinoma, 1 had a hyperne-phroma, and 1 had a colon carcinoma. Thirty-three (72%) were males and 13 (28%) were females. The average age of the patients was 67, with the youngest being 48 years old and the oldest, 87. The urine specimens were collected at different stages of the patients' diseases. The patterns of excretion were correlated with the presence of a clinically detectable cancer.

To preclude any interference by the treatment, all examinations were done either before any kind of treatment or more than 3 months after the latest intervention. For patients subjected to more than 1 examination, the intervals were from 2 weeks to 2 years. Twenty-two had intervals between 3 months and 1 year; 6, from 2 weeks to 3 months; and 2, more than 1 year.

## RESULTS

The reference values used for this study are based upon results from our own laboratory and from previous reports (1, 4, 6, 9, 10, 15, 19). β-AIB is normally excreted by humans in amounts less than 0.25 mmole/24 hr (26 mg/24 hr), ψ-uridine < 0.33 mmole/24 hr (80 mg/24 hr); $m^7G$ < 0.04 mmole/24 hr (7 mg/24 hr), and urate < 5.80 mmoles/24 hr (976 mg/24 hr).

Chart 1 shows the excretion data of a control.

Six patients (13%) displayed a "genetic pattern." These patients are probably genetic high excretors of β-AIB. This genetic pattern is illustrated in Chart 2, demonstrating an increased urinary β-AIB correlated to normal ψ-uridine excretion. No elevated $m^7G$ excretion was found. This pattern remained unchanged in different clinical states.

Fifteen other patients, 33%, showed an increased β-AIB. excretion, and 25 patients, 54%, showed an increased ψ-uridine excretion on 1 or more of the examinations.

Table 1 presents the different patterns noted for 15 patients subjected to only 1 examination.

Table 2 shows the results for 12 patients with identical excretion patterns.

The last 13 patients displayed variations in their patterns of excretion. Eight patients had clinically detectable cancer. Of these, 3 showed parallel variations in excretion of β-AIB and ψ-uridine, as illustrated in Chart 3. The remaining 5 had variations (Chart 4), demonstrating a normal excretion of β-AIB correlated to increased ψ-uridine excretion. Three patients with no clinical signs of cancer on the examinations showed parallel variations (Chart 3). In the last 2 patients, one

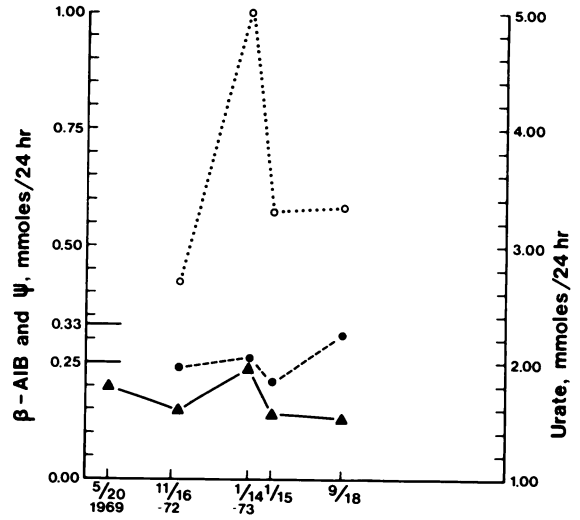


Chart 1. Correlation of urinary β-AIB, ψ-uridine (ψ), and urate from a control person. The excretion of $m^7G$ was less than 0.02 mmole/24 hr on all examinations. ▲, β-AIB; ●, ψ-uridine; ○, urate.

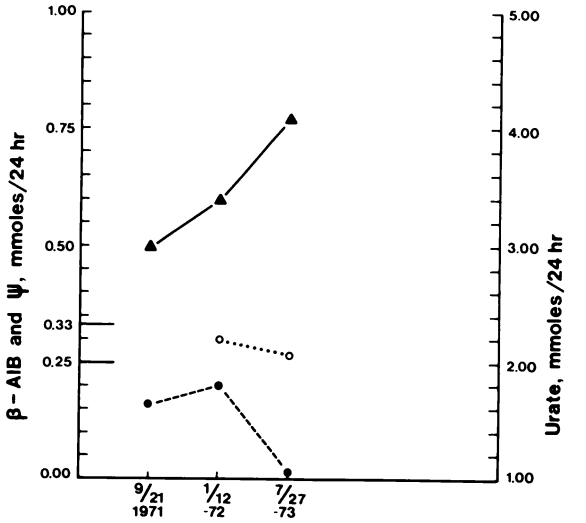


Chart 2. Correlation of urinary β-AIB, ψ-uridine (ψ), and urate from a probable genetic high excretor of β-AIB (a 75-year-old male patient with a transitional-cell carcinoma in the bladder). October to November 1971, treated by high-voltage irradiation (6000 R). The excretion of $m^7G$ was less than 0.02 mmole/24 hr on all examinations. ▲, β-AIB; ●, ψ-uridine; ○, urate.

with and the other without clinically detectable cancer, the excretion pattern was obscure, being presumably of a partially genetic character.

Parallel variations of urate and ψ-uridine excretions were demonstratable in many cases. However, all but 3 patients showed urate excretions varying only slightly within the normal range.

Chart 5 illustrates the estimated excretions of β-AIB, ψ-uridine, and urate in a cancer patient before and after surgical intervention (resection of a colon cancer). Identical patterns of excretion were found in 4 cases (2 malignant and 2 nonmalignant) of major abdominal and urological surgery. Chart 5 shows the parallel increase in excretion of β-AIB and ψ-uridine on the 2nd and 3rd postoperative days and also shows an increase in ψ-uridine excretion alone on the 6th to

Table 1

*Urinary patterns of excretion for β-AIB and ψ-uridine among 15 cancer patients with only 1 examination performed*

| | Urinary β-AIB | | | | | |
|---|---|---|---|---|---|---|
| | Normal (<0.25 mmole/24 hr) | | | Increased (≥0.25 mmole/24 hr) | | |
| Urinary ψ-uridine | Cancer | No cancer | Total | Cancer | No cancer | Total |
| Normal, <0.33 mmole/24 hr | 5 | 4 | 9 | | | 0 |
| Increased, ≥0.33 mmole/24 hr | 2 | 2 | 4 | 1 | 1 | 2 |

Table 2

*Urinary patterns of excretion for β-AIB and ψ-uridine among 12 cancer patients with 2 or more examinations performed without changing their pattern*

| | Urinary β-AIB | | | | | |
|---|---|---|---|---|---|---|
| | Normal (<0.25 mmole/24 hr) | | | Increased (≥0.25 mmole/24 hr) | | |
| Urinary ψ-uridine | Cancer | No cancer | Total | Cancer | No cancer | Total |
| Normal, <0.33 mmole/24 hr | 2[a] | 5 | 7 | 0 | 1[b] | 1 |
| Increased, ≥0.33 mmole/24 hr | 1 | 0 | 1 | 1 | 2[c] | 3 |

[a] Cancer patients in terminal phase.
[b] Patient with diabetes mellitus.
[c] One patient with diabetes mellitus.

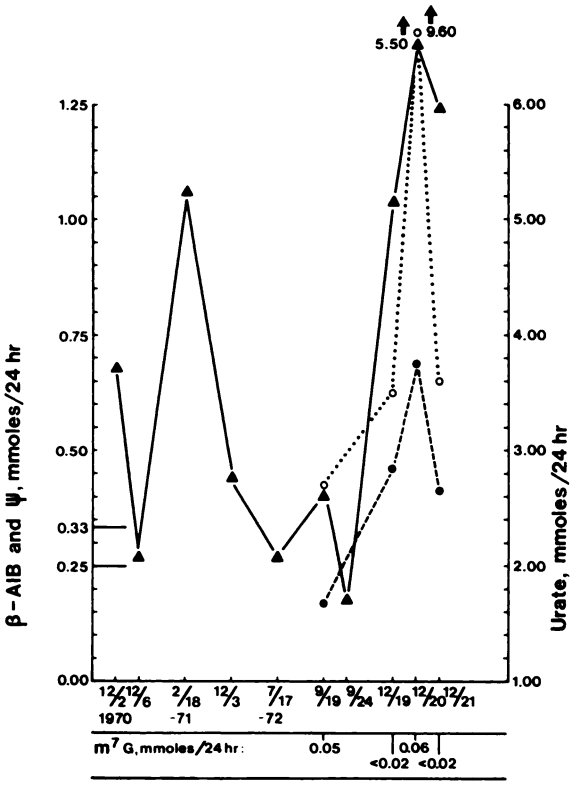


Chart 3. Correlation of urinary β-AIB, ψ-uridine (ψ), $m^7G$, and urate from a cancer patient showing parallel variation (a 58-year-old male patient with a transitional-cell carcinoma in the bladder). January 1971, treated by transurethral resection. Died December 26, 1972. Autopsy: adenocarcinoma pancreatis with metastases. No recurrence of the bladder cancer. ▲, β-AIB; ●, ψ-uridine; ○, urate.

9th postoperative days. Parallel variations of urate and ψ-uridine excretions were also observed.

## DISCUSSION

The metabolism of tRNA and the increased excretion of its components such as ψ-uridine and methylated purines in tumor-bearing organisms have been reviewed by Borek and Kerr (2). ψ-Uridine has been found in rRNA and in low-molecular-weight nRNA, but is particularly abundant in tRNA. Since ψ-uridine is catabolized in neither humans nor animals (3, 21), its excretion reflects mainly the overall rate of breakdown of tRNA. High excretion of β-AIB from tRNA-thymine as well as high excretion of methylated purines might be due either to a more rapid turnover of tRNA or to an increased amount of methylated bases in the tRNA-molecule or both.

The elevated urinary excretion of β-AIB has been investigated in relation to deficiency of D-β-aminoisobutyrate:pyruvate-aminotransferase, which is lacking in the liver of genetic high excretors of β-AIB (7, 18). The demonstration of a so-called genetic pattern of excretion in 6 (13%) of our cancer patients seems to be in accordance with the deficiency of this degradating enzyme of β-AIB.

The patterns of excretion shown in Chart 4 are still uninterpretable.

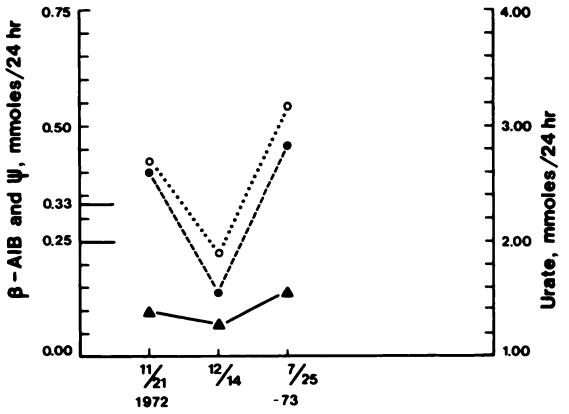


Chart 4. Correlation of urinary β-AIB, ψ-uridine (ψ), and urate from a cancer patient showing discrepancy in variations of β-AIB and ψ-uridine excretion (a 73-year-old male patient with a transitional-cell carcinoma in the bladder). During the following 8 months there were many recurrences. The primary carcinoma and the recurrences were treated by transurethral resections. The excretion of $m^7G$ was less than 0.02 mmole/24 hr on all examinations. ▲, β-AIB; ●, ψ-uridine; ○, urate.

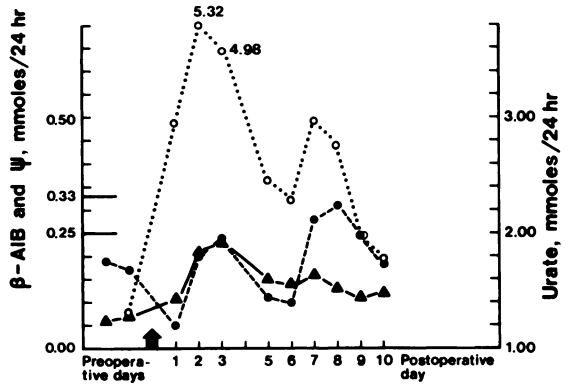


Chart 5. Correlation of urinary β-AIB, ψ-uridine (ψ), and urate from a patient before and after resection of a colon carcinoma. The excretion of $m^7G$ was less than 0.02 mmole/24 hr on all examinations. ▲, β-AIB; ●, ψ-uridine; ○, urate.

Surgical intervention was in none of our cases followed by a β-AIB increase to such a high level as previously described (8). In previous reports, only the increases of β-AIB (8) and ψ-uridine (10) on the 2nd and 3rd postoperative days are stated. The increased excretion (Chart 5) of ψ-uridine on the 6th to 9th postoperative days without corresponding β-AIB excretion may be explained by increased DNA and protein syntheses in relation to wound healing.

Of our cancer patients, 27 (68%) showed a positive correlation of urinary β-AIB to ψ-uridine excretion. Since ψ-uridine excretion is predominantly a reflection of tRNA turnover, and ribothymidine has been found in tRNA only, the results achieved from our investigations indicate a positive correlation of urinary β-AIB to tRNA turnover in most cancer patients.

The clinical significance and usefulness of such a correlation has not yet been clarified. The influence of cellular turnover on the excretion of β-AIB and ψ-uridine is obscure, since the excretion of urate was mainly within the normal range. This is in accordance with other workers' demonstration (6) of a poor correlation between the excretions of ψ-uridine and urate. In our study among patients with urinary tract carcinomas, we found a statistically significant correlation between increased urinary β-AIB and high-grade tumor cell dysplasia. Such a correlation was not observed in relation to the clinical tumor stage (TNM system) unpublished observation.

The possible influence of the established hypermethylation of tRNA in tumor tissues (2) on the excretion of β-AIB has not been clarified in this investigation.

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