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THE DETERMINATION OF BETA-AMINOISOBUTYRIC ACID IN URINE OF NORMAL AND CANCER PATIENTS

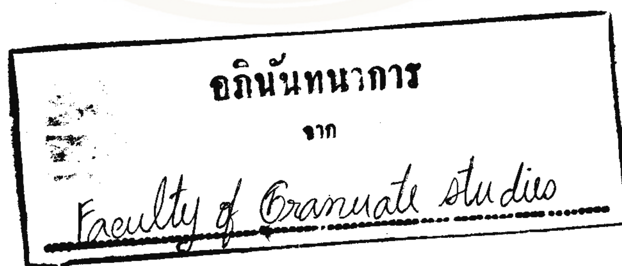
PRANEE CHUNTAPET

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

(BIOCHEMISTRY)



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Thesis

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OF NORMAL AND CANCER PATIENTS

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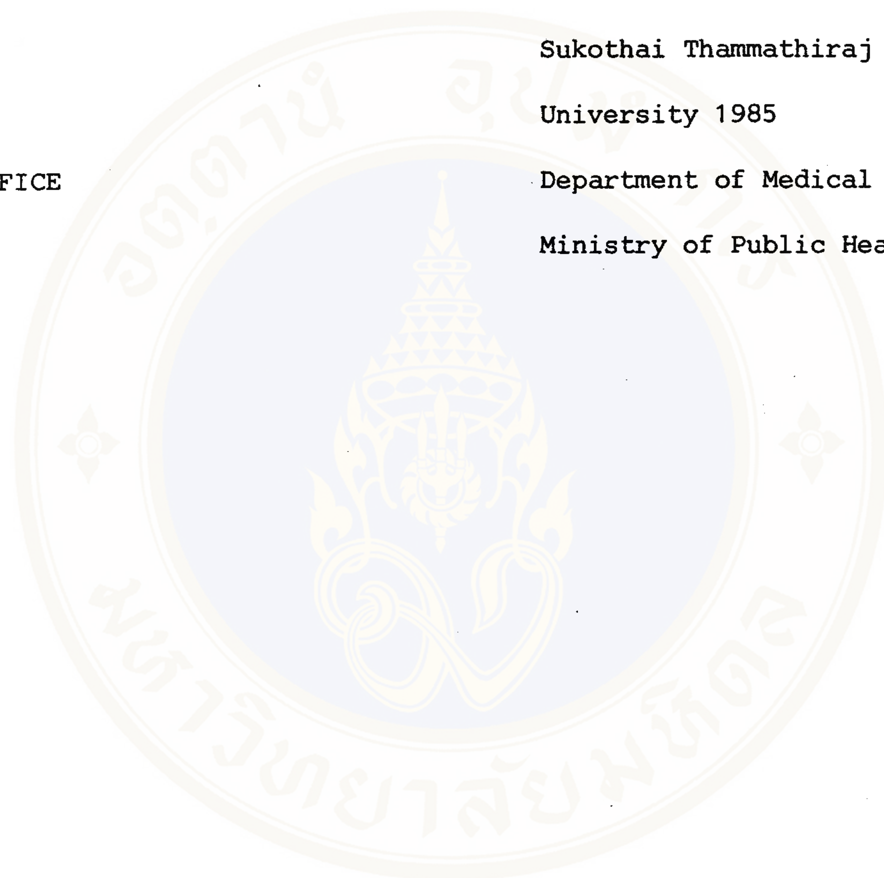

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Pranee Chuntapet

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ชื่อวิทยานิพนธ์	การวิเคราะห์หากรดเบต้าอะมิโนไอโซบิวทีริคในปัสสาวะคนปกติและผู้ป่วยมะเร็ง
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บทคัดย่อ

กรดเบต้าอะมิโนไอโซบิวทีริคผลิตผลที่ได้จากการสลายของไธมีนใน ดี เอ็น เอ และ อาร์ เอ็น เอขนย้ายจากปัสสาวะคนปกติ 65 คน และผู้ป่วยมะเร็งของอวัยวะต่าง ๆ 60 คน ซึ่งวิเคราะห์ด้วยวิธีโครมาโตกราฟีแบบชั้นบาง พบว่าผู้ป่วยมะเร็งมีระดับการขับถ่ายสารนี้สูง (146.79 ± 137.64 นาโนโมลต่อไมโครโมลครีเอตินีน) เมื่อเทียบกับการขับถ่ายในคนปกติ (74.01 ± 25.81 นาโนโมลต่อไมโครโมลครีเอตินีน) การขับถ่ายที่เพิ่มสูงนี้อาจจะแสดงถึงการเปลี่ยนแปลงในเมตาบอลิสมของ ดี เอ็น เอ หรือ การสับเปลี่ยนของ อาร์ เอ็น เอ ขนย้ายในเนื้องอกเพิ่มขึ้น และจากการศึกษาการเปลี่ยนแปลงของระดับการขับถ่ายกรดเบต้าอะมิโน-ไอโซบิวทีริคในผู้มะเร็งก่อนและภายหลังการรักษาด้วยรังสี พบว่าระดับการขับถ่ายลดลงในผู้ป่วยที่อาการดีขึ้น แต่จะยังคงสูงในผู้ป่วยซึ่งอาการของโรคยังคงอยู่ ผลเช่นเดียวกันนี้ยังพบได้จากการติดตามหาระดับการขับถ่ายสารนี้ในผู้ป่วยมะเร็งไข่ปลาอุกระหว่างรับการรักษาด้วยยา ดังนั้นการหาปริมาณกรดเบต้าอะมิโนไอโซบิวทีริคในปัสสาวะจะใช้เป็นตัวบ่งชี้ได้ดีในการติดตามผลของการรักษาโรคมะเร็ง

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Thesis Title: The determination of beta-aminoisobytyric acid in urine of normal and cancer patients

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ABSTRACT

Beta-aminoisobutyric acid (βAIBA), a degradation product of thymine in both deoxyribonucleic acid (DNA) and transfer ribonucleic acid (tRNA), was determined in single urine specimens from 65 normal healthy subjects and 60 patients with cancer of various organs by thin layer chromatography. The normal control value from both males and females was 74.01 $\pm$ 25.81 nmol βAIBA/μmol creatinine. The excretion levels were significantly increased in patients with several tumor type studied (146.79 $\pm$ 137.64 nmol βAIBA/μmol creatinine). Increased levels of βAIBA excretion may reflect changes in DNA metabolism or increased turnover of tRNA in tumor.

Moreover, the changes of βAIBA excretion levels were also studied in cancer patients before and after radiotherapy. The levels returned to normal when they were remission but remained high in patients whose clinical conditions were not improved. The same results were also found in patients with choriocarcinoma during chemotherapy.

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Therefore, the determination of urinary βAIBA level could be used as a potential biological marker for monitoring the effectiveness of therapy in malignancies.

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LIST OF ABBREVIATIONS

βAIBA	=	beta-aminoisobutyric acid
DNA	=	deoxyribonucleic acid
t RNA	=	transfer ribonucleic acid
NADPH	=	β-nicotinamide adenine dinucleotide phosphate (reduced form)
$NADP^+$	=	β-nicotinamide adenine dinucleotide phosphate (oxidized form)
M	=	molar
g	=	gram
ml	=	millilitre
mmol	=	millimole
μmol	=	micromole
nmol	=	nanomole
L	=	liter
mg	=	milligram
kg	=	kilogram
Fig	=	figure
nm	=	nanometre
H^+	=	Hydrogen ion
°C	=	degree celcius
et al	=	et alii
h	=	hour

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CHAPTER I

INTRODUCTION

Beta-aminoisobutyric acid (βAIBA) is a catabolic product of thymine in either DNA or tRNA. Thymine, a pyrimidine base, is catabolized in the liver via the reduction by NADPH and the reduced ring is opened in a hydrolytic reaction to form carbamoyl derivative (β-ureidoisobutyric acid). The carbamoyl group is then eliminated as ammonia and carbon dioxide, yielding β-aminoisobutyric acid. This βAIBA is transaminated to yield methylmalonate semialdehyde. (Fig. 1) The production of dihydrothymine from thymine and β-ureidoisobutyric acid from dihydrothymine proceed reversibly, and further irreversibly hydrolyze to βAIBA (1,2).

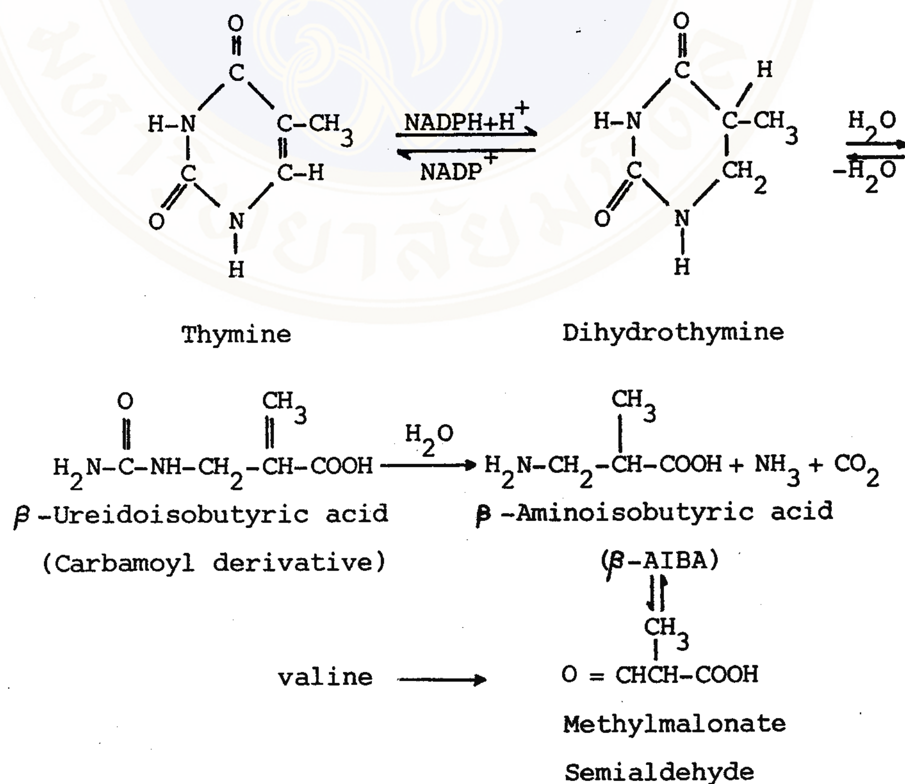

Fig. 1. The pathway of thymine degradation to β-AIBA

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Thymine is present in tRNA as well as in DNA, but the pathway of synthesis in the 2 macromolecules is different. Thymine in DNA is derived from uridine-monophosphate by methylation with $N^{5,10}$-methylene-tetrahydrofolic acid whereas the methylene group is derived from formate (3). On the other hand, the methyl group of thymine in tRNA originated from the methyl group of s-adenosylmethionine which is transferred intact to a uridine by the action of a specific enzyme, tRNA methylase (4). Consequently, the methyl group of DNA-thymine and tRNA-thymine can be labeled with ^{14}C and ^{3}H, respectively.

The dual origin of βAIBA was also demonstrated in animal models by the injection of [^{14}C] formate and [methyl ^{3}H] methionine, and the pure isolated βAIBA contained both labels. Therefore, βAIBA can be used as a probe for the metabolism of DNA and tRNA (5).

Besides thymine, valine had been reported to be a precursor of βAIBA (6). The amount of βAIBA produced from valine from tolerance tests was very small, about 10 percent of that originating from thymine (1).

βAIBA was first isolated and identified in human urine by paper chromatographic method (7,8). It was produced not only in human but also in rats (9). In healthy person βAIBA, was normally excreted in the urine in small amount (0.2-0.4 mg/kg/day) with a relatively constant rate for each individual, but excreted in widely varying concentration from person to person. Some persons habitually excreted a large amount of βAIBA (2-3 mg/kg/day) (10,11).

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Harris (12) reported that about 9.6% of British population excreted consistently high urinary levels of βAIBA. Similar reports were found by family studies on Italian and New York populations (13,14). The high excretion of βAIBA was observed in Mongoloids and Oriental populations ; Chinese, Japanese and Thai, about 60% and 40% respectively, while only 10% of Caucasoid were found to be high excretors. These included the Apache Indians of Arizona and the Black Caribs of British Honduras (15,16,17,18,19,20). These investigators concluded that the variability between individuals in excretion rate was under genetic control. βAIBA excretion was comparable between the sex and was not altered by diet (12,14,15).

βAIBA was known to be enzymatically degraded by hepatic aminotransferase. The decrease of this degradation process might result in the increase of urinary βAIBA concentration. The activity of human hepatic enzyme obtained from an autopsy showed that high excretors lacked the ability to break down D(-)- βAIBA since they had a reduced activity of the enzyme D(-)-β-aminoisobutyrate : pyruvate aminotransferase, an enzyme that catalyzed a reversible transmination between D-β-aminoisobutyrate and pyruvate, in the liver (15,21,22).

<u>The structure of tRNA in normal and malignant tissues</u>

Transfer RNA is a cardinal component of every cells. It is one of the most complex biomacromolecules in both structure and function. The first function discovered is the transport of amino acid to the assembly site of protein. However, it was subsequently demonstrated as a regulatory molecule at different levels (23). It can regulate gene expression at the transcription level and also

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regulate translation of mRNA during protein synthesis including the regulation of biosynthesis of its cognate amino acid. Furthermore a tRNA molecule is required for reverse transcriptase, which transcribes RNA to DNA.

In order to perform its various functions, tRNA is endowed with an extraordinarily complex structure. Its primary sequence consists of about 80 of the four major bases : adenine, cytosine, guanine, and uracil. Not only these major bases, tRNA also contains a large variety of modified bases that are unique to it. The modifications of tRNA bases may be as simple as a methyl group addition or may be much more complex.

The total synthesis of tRNA is extraordinarily complex. The synthesis of its primary sequence probably requires only one enzyme, DNA-dependent RNA polymerase. The completion of its structure, for effectiveness and efficiency in its variety of functions, is extremely intricate. Of the over 50 modifications that are known, in some cases only one enzyme is required, such as for the introduction of a methyl group to a specific base. However, even these methylations can require several enzymes because the enzymes are specific for bases, positions in bases, and indeed positions in tRNA. As many as 200 different enzymes are needed for the completion of the structure of tRNAs.

Of these enzymes, the tRNA methylases are a group of enzymes catalyzing the transfer of the methyl group from s-adenosyl-L-methionine to purine and pyrimidine base of tRNA. Methylation of tRNA can be characterized by (a) measuring the rate of tRNA methylation, (b) measuring extent of tRNA methylation when tRNA is limiting, and (c)

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measuring the methylated bases themselves. Whereas rate is a measure of total activity of tRNA methylases, extent is a measure of the total number of sites on tRNA that a given group of tRNA methylase can recognize. Therefore, differences in extent of methylation between different tissues may reflect qualitative differences in their tRNA methylases.

The tRNA methylase turned out to be quantitatively aberrantly hyperactive and qualitatively different from the normal tissue counterpart in all malignant tumor tissues (24,25,26,27,28,29). The only exceptions are some benign tumors of human ovary, the tRNA methylating enzymes are normal (30). In addition, the amounts of methylated tRNA bases in specific animal tumors were elevated when compared with those in their normal tissues (31).

In turn, every malignant tumor contains a few tRNAs which are different in structure from those in the normal tissue. Furthurmore, different tRNAs are also found in the same or various tumor (29,32).

It had been known for a long time that all normal subjects excreted several modified purines and pyrimidines in their urine (33,34). Since modified bases must be positioned with extraordinary accuracy by highly specific enzymes, therefore, in order to prevent their reinsertion into forbidden sites after release by tRNA trunover, the kinases needed to convert the modified bases nucleoside or deoxy-nucleoside into the appropriate nucleoside triphosphate are lacking from mamalian cells and these bases which are very stable and cannot be metabolized or recycled must be excreted.

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Since βAIBA is most likely a breakdown product of nucleic acid catabolism, it is probable that in certain pathological conditions, among which are cancer diseases, a marked change in the excretion rate of βAIBA could take place.

The excretion of βAIBA by individual patient with neoplastic disease was reported to be increased markedly and to bear some relationship to the neoplastic process (8). Similar finding were reported in patients under the treatment for urothelial tumors, Burkitt's lymphoma, and in leukemic patients after the nitrogen mustard therapy (33,35,36). The degree of elevation of βAIBA excretion before, during, and after antitumor chemotherapy in patients with Burkitt's lymphoma appeared to be related to the size of tumor mass present (37).

Using amino acid analyzer (38) the βAIBA levels in the 24 hour urine samples were measured from patients with malignant tumors of various organs in order to determine whether βAIBA was significantly related to neoplasms and observe the changes of urinary βAIBA levels due to radiotherapy since this βAIBA was also known to be released in abnormally high amounts in irradiated men (39). The percentage of patients with neoplasms who had an increased in βAIBA excretion was 43.8 (35 out of 80 patients). After radiotherapy the βAIBA levels were markedly decreased as tumors disappeared or marked regressed in all cases and the patients' clinical conditions were improved but the levels again increased markedly when the patients developed new tumor. The investigators suggested that the changes in the urinary βAIBA content were almost paralleled with the cancer process and could not accurately determine whether βAIBA was significantly related to any given type of neoplasm because the number of cases was so small. Their observations

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also indicated that daily fractionated irradiation to a small part of the body may not lead to an elevated urinary βAIBA excretion. Moreover, 26 of 27 patients with cancer of different organs had the elevated levels of one or more of the following 7 products : pseudouridine, β-aminoisobutyric acid, 1-methyladenosine, 1-methylinosine, N^2-methylguanosine, N^2, N^2-dimethylguanosine, and 2-pyridone-5-carboxamide-N'-ribofuranoside. The elevated of these markers correlated with the stage of the cancer, but the relative levels of various nucleosides appeared to be an attribute of the tissue site of the tumor (40).

The urinary excretion patterns of those modified nucleosides in patients with malignant mesothelioma and in asbestos workers without this neoplasm were used as a possible approach for the early detection of the disease by using high performance liquid chromatography (41,42, 43). Since it is difficult to diagnose mesothelioma at early stage. Several nucleosides in urine of persons with diagnosed mesothelioma were found to be elevated. The extreme elevation of all nucleosides was found in patients whom chemotherapy had failed. Therefore, the pattern of urinary modified nucleosides excretion may serve as a useful diagnostic tool for malignant disease (44), and it may also apply to occupationally and environmentally induced neoplasms such as mesothelioma. Also, the data on asbestos-exposed individual at high neoplastic risk indicated that the measurement of urinary nucleosides may have a predictive value in addition to facilitating diagnosis. Similar patterns of urinary modified nucleosides excretion were also observed in advanced cases of small cell carcinoma of the lung (45).

In order to studies on the usefulness of these nucleosides as

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tumor markers, the nucleoside patterns were also determined during therapy of patients with trophoblastic discase (46). Most of the patients with hydatidiform mole and invasive mole had elevation of these markers. Furthermore, the patients with very high elevations of βAIBA subsequently developed choriocarcinoma. After uterine curettage, the nucleoside markers returned to normal soon after effective therapy which indicated the absence of residual disease while the usual marker, hCG, was still markedly elevated. For 2 year follow up, the women were found to remain symptom-free. The source of these markers was cleared more rapidly than that of hCG ; therefore, these nucleoside markers may spare the patient of painful and needless therapy (47,48).

It would be a clinical usefulness of βAIBA as another biologic marker to diagnose malignancies and to follow up the recurrence or responsiveness of the treatment while the other detection are unavilable and laborious.

In the present study, sensitive thin layer chromatographic method was used to determine β-aminoisobutyric acid levels in the urine from normal subjects and patients with cancer to include a spectrum of several different types of malignancy before and after radiotherapy and also to follow up βAIBA excretion in patients with choriocarcinoma during chemotherapy for 3 months.

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CHAPTER II

MATERIALS AND METHODS

Materials :

I. Subjects

The subjects were divided into two groups, the first group was sixty-five normal control subjects and the other was sixty cancer patients.

1. Normal control subjects

The normal control subjects were students of medical laboratory technician of Faculty of Medicine, Siriraj Hospital, Mahidol University, and the others.

There were thirty-three men and thirty-two women, aged ranging from 17 to 64 years.

2. Cancer patients

The study was conducted in sixteen men and forty-four women aged ranging from 25 to 90 years with cancer of various organs newly diagnosed during the period February 1988 to April 1988 at Siriraj Hospital. According to the histopathological records, three major groups of the cancers were squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma. A total of 60 cases were 28 cervical carcinomas, 9 carcinomas of breast, 4 laryngeal carcinomas, 2 carcinomas of rectum, 2 esophageal carcinomas, 2 carcinomas of pyriform, 2 carcinomas of tonsil, 1 carcinoma of gum, 1 thyroidal carcinoma, 1 ovarian carcinoma, 1 carcinoma of bladder, 1 nasopharyngeal carcinoma, 1 carcinoma of vagina, 1 carcinoma of postcricoid, 1 carcinoma of floor

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of mouth, 1 carcinoma of buccal mucosa, 1 carcinoma of base of tongue and 1 metastatic carcinoma of soft tissue anterior chest wall.

II. Specimens

Random urine samples were collected from normal control subjects and cancer patients in plastic containers without preservatives. The samples were stored frozen at -20° C until analysis since it was determined earlier that nucleosides in urine were stable therefore no special precaution or preservatives were needed.

III. Apparatus

The apparatus used in this study were as follows :

- Analytical balance : AINSWORTH Type 24 N U.S.A.
- Automatic pipet : Pipetman Gilson, France.
- Centrifuge
- pH meter : ORION, U.S.A.
- Shaking water bath
- Spectrophotometer : The SHIMADZU UV-VIS RECORDING SPECTROPHOTOMETER UV-160, JAPAN.
- Suction pump : THOMAS INDUSTRIES INC. ENGLAND.
- Thin layer chromatographic plate : Whatman LK6 Linear-k Siliga gel, U.S.A.
- Vortex mixer : CURTIN SCIENTIFIC CO., TEXAS, U.S.A.

IV. Chemicals and Reagents

1. The chemicals obtained from Sigma Chemical co. St. Louis, U.S.A. were beta-aminoisobutyric acid and 2,4 dinitro-I-

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fluorobenzene. The following chemicals were obatined from Merck, Darmstadt, Germany : chloroform, creatinine, diethyl ether, ethanol absolute, glacial acetic acid, hydrochloric acid, picric acid, pyridine, sodium bicarbonate, sodium carbonate, sodium hydroxide, and tetrahydrofuran.

2. Reagents for β-aminoisobutyric acid and creatinine determination

2.1 Reagents for β-aminoisobutyric acid determination

2.1.1 Working standard β-aminoisobutyric acid

Dissolve 0.04 g. of β-aminoisobutyric acid in 100 ml distilled water.

2.1.2 Carbonate-bicarbonate buffer 0.6 M pH 9.3

Dissolve 50.4 g. of sodium bicarbonate, anhydrous in 1000 ml distilled water. (Solution A)

Dissolve 63.56 g. of sodium carbonate, anhydrous in 1000 ml distilled water. (Solution B)

Mix 200 ml solution A and 60 ml solution B, adjusted pH to 9.3

2.1.3 2,4 Dinitro-I-Fluorobenzene 1% (v/v)

1 ml of 2,4 dinitro-I-fluorobenzene was added to 100 ml of absolute ethanol.

2.1.4 Hydrochloric acid 3M

25.5 ml of conc. hydrochloric acid (36.5% by weight, density 1.18 gm/ml) in 100 ml distilled water.

2.1.5 Chloroform : pyridine : glacial acetic acid 50 : 50 : 1 (v/v)

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50 ml of chloroform, 50 ml of pyridine, and 1 ml of glacial acetic acid were mixed together.

2.1.6 Glacial acetic acid 10%

10 ml of glacial acetic acid was added to 100 ml distilled water.

2.2 Reagents for creatinine determination

2.2.1 Working standard creatinine (1 mg/dl)

1.0 mg of creatinine was dissolved in 100 ml 0.1 N hydrochloric acid.

2.2.2 Picric acid 0.04 M

Dissolved picric acid in distilled water until the solution saturated.

2.2.3 Sodium hydroxide 0.75 M

3 g. of sodium hydroxide were dissolved in 100 ml CO_2 free distilled water.

V. Determination of β-aminoisobutyric acid in urine by thin layer chromatographic method with some modifications (49).

Principle

The β-aminoisobutyric acid in urine was determined as dinitrophenyl derivative by reaction with 2,4 dinitro-I-fluorobenzene (DNFB) in alkaline buffer pH 9.3 at 37 °C. The yellow DNP-βAIBA was identified by thin layer chromatography. The DNP-βAIBA was scraped off from the plate, which dissolved with acetic acid and then measured at 355 nm.

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Procedure

1. Dilute 1 ml of urine with 1 ml of distilled water.

2. Pipet 0.5 ml of diluted urine, 0.5 ml of 0.6 M bicarbonate-carbonate buffer, and 0.5 ml of 1% 2,4 dinitro-I-fluorobenzene solution, mix and incubate at 37° C for 2 hours with frequent shaking.

3. Add 10 ml of ether and extracted excess DNPB for 1 hour.

4. Discard the ether phase.

5. Add 0.3 ml of 3N hydorchloric acid to the aqueous phase and mixed.

6. Add 7.5 ml of ether and extracted 1 hour.

7. The ether phase was pipetted into a test tube and evaporated to dryness.

8. Dissolve the dry materials in 0.25 ml tetrahydrofuran and 50 μl aliquot was spotted on thin layer chromatographic plate.

9. Separations were attained by ascending chromatography in chloroform : pyridine : acetic acid (50 : 50 : 1).

10. The spots corresponding to DNP-βAIBA were detected in ultraviolet light, scraped off and extracted with 3 ml of 10% glacial acetic acid for 1 hour.

11. The solutions were centrifuged at 1380 g. for 10 minutes.

12. The absorbance of the supernatants were measured at 355 nm using extraction of an equal unspotted area as a blank.

13. Read the concentration of β-AIBA from calibration curve or calculated from the formula :

$$\text{concentration of } \beta\text{AIBA} = \frac{\text{absorbance of unknown}}{\text{absorbance of standard}} \times \text{concentration of standard}$$

μg/0.1 ml or $\frac{\text{mg/L}}{0.1}$

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The conversion from mg/L of βAIBA to nmol/L of βAIBA was calculated as follows :

Molecular weight of βAIBA is 103.1

$$\frac{mg/L}{0.1 \times 103.1} = mmol/L$$

$$mmol/L \times 1{,}000{,}000 = nmol/L$$

Calibration curve for βAIBA

1. Prepare a series of working standard β-aminoisobutyric acid by diluting the dilution standard with distilled water as follows :

Tube	Working standard βAIBA (μl)	distilled water (μl)	concentration of βAIBA (μg/0.1 ml)
1	0	500.00	0
2	31.25	468.75	5
3	62.50	437.50	10
4	93.75	406.25	15
5	125.00	375.00	20

2. Substitute these standard solutions with the unknown urine, measured the absorbance for each solution as outlined under procedure.

3. Plot the absorbances against the corresponding βAIBA concentrations.

VI. Determination of creatinine in urine by Jaffe reaction. (50)

Principle

Creatinine in urine reacted with picric acid in alkaline

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solution and the red complex of alkaline creatinine picrate occurred. The absorbance was measured at 520 nm.

Procedure

1. Pipet 3 ml of distilled water as a blank, 0.5 ml of distelled water and 2.5 ml of working standard creatinine as a standard, 2.975 ml of distilled water and 0.025 ml of unknown urine.

2. Add 1 ml of 0.04 M picric acid in each tube.

3. Add 1 ml of 0.25 M sodium hydroxide in each tube.

4. Mix and stand for 20 minutes.

5. Read the absorbance at 520 nm.

6. Calculate the concentration of creatinine from calibration curve or the formula :

concentration of creatinine

$$= \frac{\text{absorbance of unknown}}{\text{absorbance of standard}} \times \text{concentration of standard gm/L}$$

The conversion from mg/L of creatinine to μmol/L of creatinine was calculated as follows :

Molecular weight of creatinine is 113

$$\frac{\text{mg/L}}{113} = \text{mmol/L}$$

$$\text{mmol/L} \times 1000 = \mu\text{mol/L}$$

Calibration curve for creatinine

1. Prepare a series of working standard creatinine by diluting with distilled water as follows :

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Tube	Working standard creatinine (ml)	distilled water (ml)	concentration of creatinine (g/L)
1	0	3.0	0
2	0.5	2.5	1.66
3	1.0	2.0	3.33
4	1.5	1.5	5.00
5	2.0	1.0	6.66

2. Follow the precedure steps 2-5 page 15.

3. Plot the absorbance against the concentration of creatinine.

Statistical analysis of βAIBA determination

Data on the βAIBA levels were expressed as mean value ± standard deviation. Differences between means were assessed by student's t test.

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CHAPTER III

RESULTS

The effect of increasing incubation time on the formation of DNP-βAIBA is shown in Figure 2. The normal urine sample was incubated with buffer and dinitrofluorobenzene at 37°C for various peroids of time. The amount of βAIBA gradually increased with increasing incubation time up to 2 hours and rapidly decreased when incubation time were 2.5 and 3 hours. Therefore, an incubation time of 2 hours was chosen as the standard incubation time since the maximum formation of DNP-βAIBA ocurred.

In order to determine the effect of temperature on DNP-βAIBA, normal urine sample was incubated with buffer and dinitrofluorobenzene at various temperatures (Fig. 3) for 2 hours. The βAIBA levels increased with increasing temperature up to 37°C and then declined at temperatures of 40°C and 45°C. From these data, the incubation at temperature of 37°C was chosen for subsequent experiments.

Figure 4 illustrates typical separation of authentic βAIBA and βAIBA in human urine by the thin layer chromatcgraphic analysis. It was cleared that both standard and urine sample showed identical separation.

Figure 5 illustrates the absorption spectrum of DNP-βAIBA. The concentration of βAIBA at 15 μg/0.1 ml was processed as described in procedure. The DNP-βAIBA in acetic acid solution was scanned from 300 to 400 nm. The maximum absorption of DNP-βAIBA was at 355 nm.

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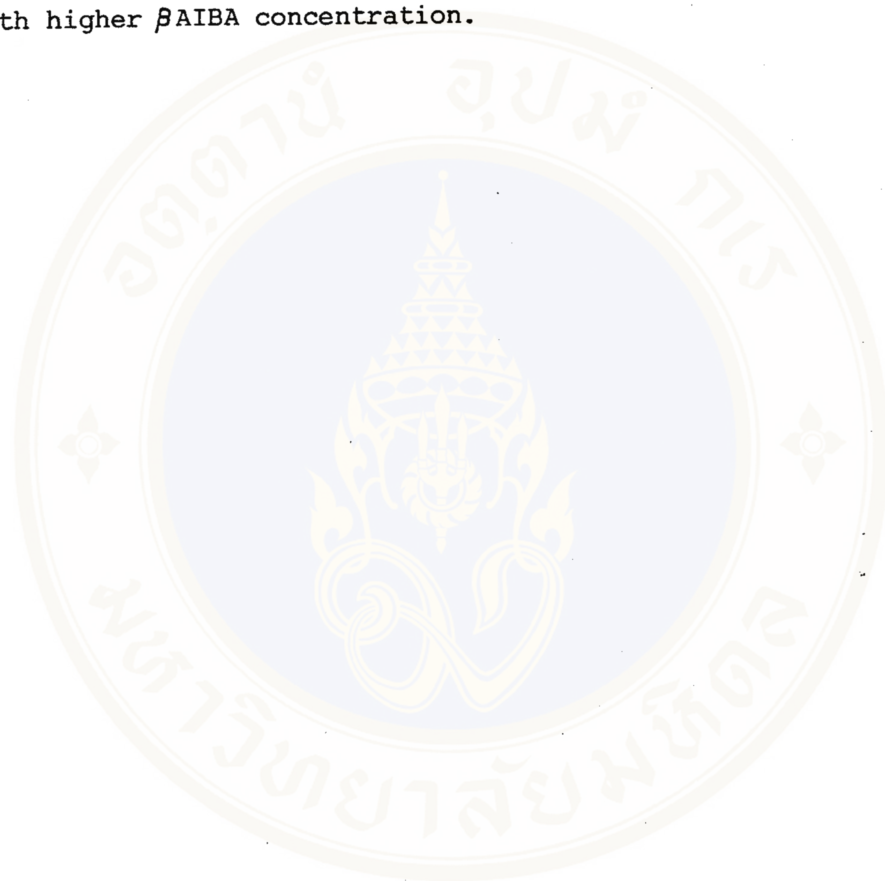
Figure 6 shows the relationship between the amount of DNP-βAIBA and absorbance in the standard samples. A linear relationship existed between the amount of βAIBA up to at least 20 μg and the absorbance at 355 nm. The correlation coefficient (r) was 0.9997 as determined by linear regression analysis. No assays were conducted with higher βAIBA concentration.



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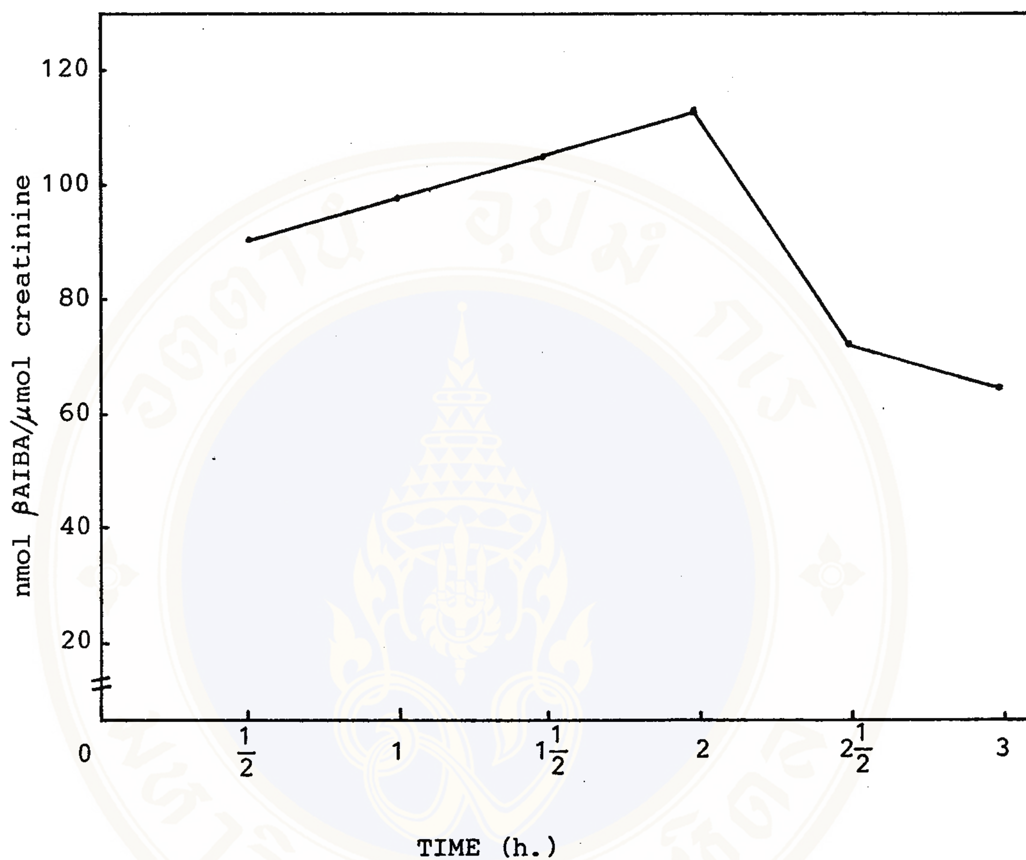

Fig. 2 Effect of time course on the formation of DNP-βAIBA. The incubation mixture contained 0.5 ml of diluted urine, buffer 0.5 ml, and dinitrofluorobenzene 0.5 ml. Each point represents the average value for duplicate determinations.

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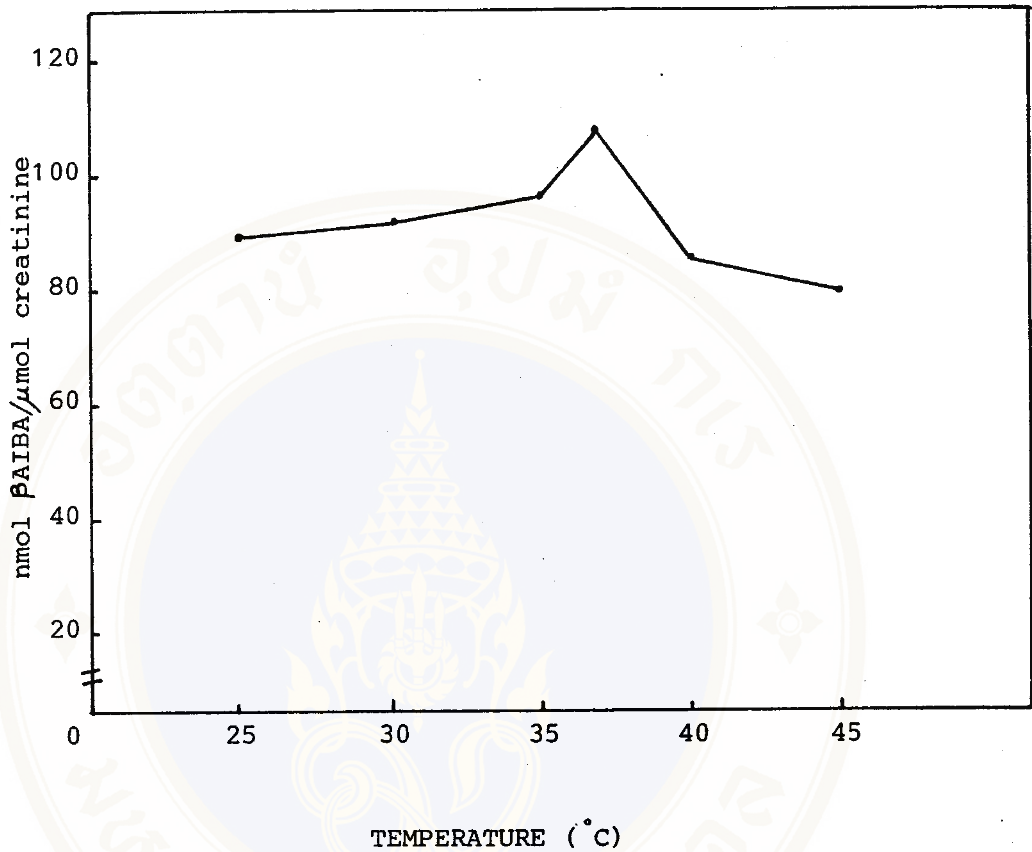

Fig. 3 Effect of incubation temperature on the formation of DNP-βAIBA. The incubation mixture were diluted urine 0.5 ml, buffer 0.5 ml, and dinitrofluorobenzene 0.5 ml. Each point represents the average value for duplicate determinations.

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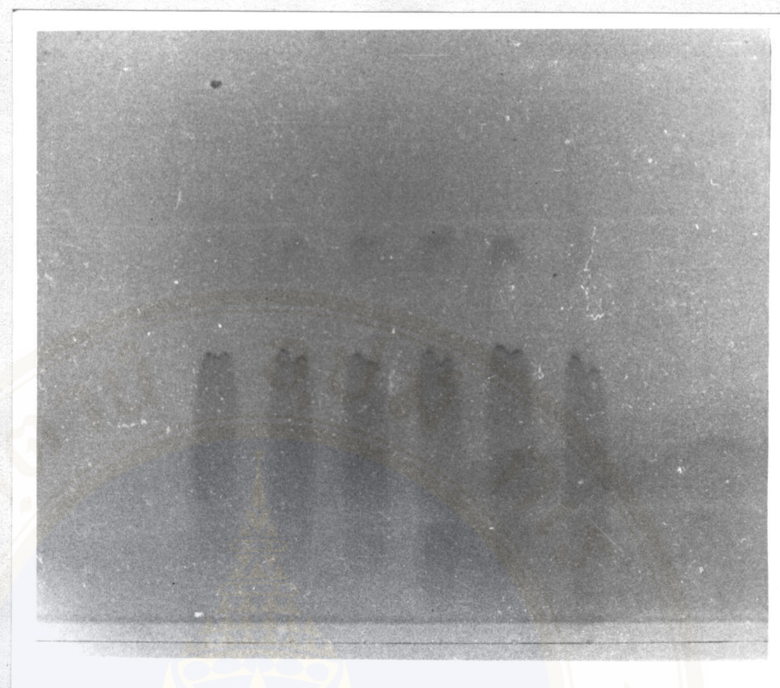

Fig. 4 Separation of DNP-βAIBA by thin layer chromatography (chloroform-pyridine-glacial acetic acid). Each sample corresponded to 0, 5, 10, 15, 20 and 4.73 μg/0.1 ml of standard βAIBA and βAIBA in normal urine, respectively.

A	=	standard βAIBA	0	μg/0.1 ml
B	=	"	5	"
C	=	"	10	"
D	=	"	15	"
E	=	"	20	"
F	=	βAIBA in urine	4.73	μg/0.1 ml

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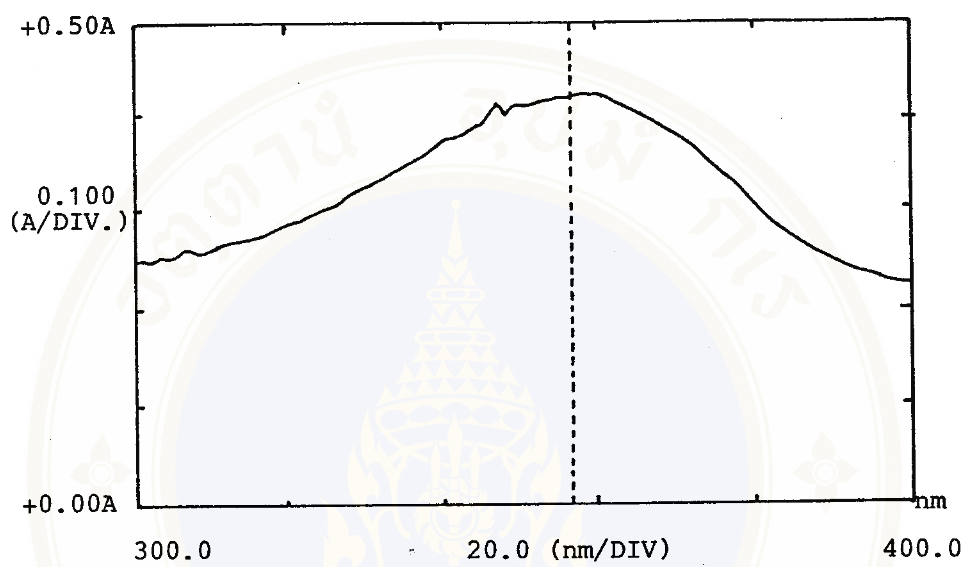

Fig. 5 Absorption spectrum of DNP- βAIBA

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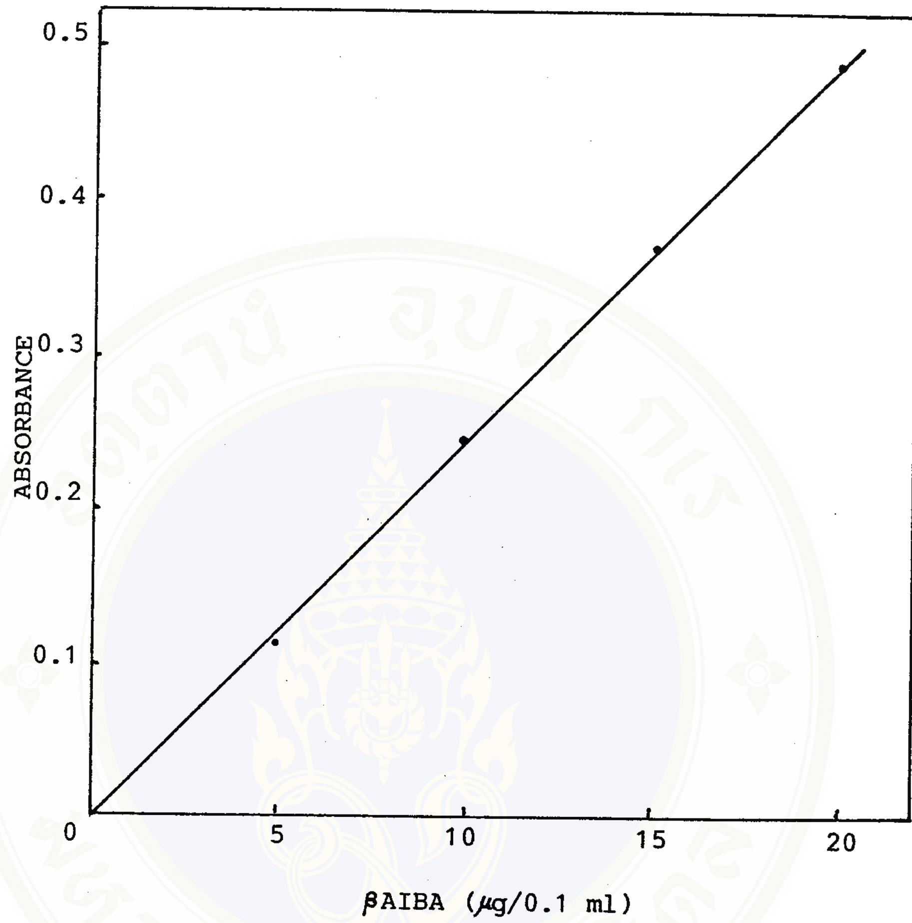

Fig. 6 Effect of increasing concentration of βAIBA absorbance. Each point represents the mean for duplicate incubations.

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The effect of freezing and storage on the concentration of βAIBA in urine is shown in Table 1. In order to determine storage stability of βAIBA in urine, fresh urine samples were mixed well and analyzed for βAIBA immediately upon collection. The remainders of the urine collection were then divided into three aliquots and stored frozen at -20 C for 1, 2 and 3 weeks, respectively. The results showed that after storage for any period of time the amount of βAIBA remained unchange.

Table 2 shows βAIBA levels in urine samples from normal male and females collected at 8 A.M., 10 A.M., 3 P.M., and in the total void in 24 hours. In the x column of the table, present the ratio of the concentration of βAIBA (nmol) to the concentration of creatinine (μmol). It is evident that the ratios in random urine samples are remarkably similar to that in the 24 hour samples. Since the βAIBA excretion levels during the day and 24 hours were comparable, single random urine samples were used in the assays.

Daily variation of βAIBA excretion is shown in Table 3. Urine samples were collected from three normal male and females for three days. Time of day was not specified. The amounts of βAIBA based on creatinine indicate that βAIBA excretion is relatively constant for an individual and the time of the urine collection are not critical for the experiment.

Table 4 show analylical recovery of βAIBA added to urine in the incubation mixture. The average recovery of βAIBA from duplicate determinations was 100% at every concentration of βAIBA added. The results suggest that there was no loss of βAIBA during the extraction.

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Table 5 and 6 show the intra and inter assay precision of the method for normal urine sample. The results obtained show good pre cision (C.V. = 4.61, 4.95%) for intra and inter assay analyses, respectively.

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Table 1. Effect of freezing and storage on the concentration of βAIBA in urine samples from normal male and females.

Sample No.	Disposition of urine samples			
	fresh	frozen at -20°C (week)		
		1	2	3
1	68	67	64	70
2	59	67	56	56
3	64	63	59	57

The number in table represents nmol βAIBA/μmol creatinine from the mean of duplicate determinations.

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Table 2. Excretion of βAIBA by normal male and females

Time	n	βAIBA (nmol/μmol creatinine)			
		$\bar{X}$	S.D.	RSD%	$\bar{x}/\bar{x}_{24}$ [a]
8 A.M.	3	62.0	12.5	20.16	1.01
10 A.M.	3	61.3	11.5	18.76	1.004
3 P.M.	3	64.3	10.5	16.32	1.05
24 hrs.	3	61.0	10.5	17.21	1.00
Total[b]	12	62.15	11.25	18.11	1.016

n = number of sample

$\bar{x}$ = average value

S.D. = standard deviation

RSD = relative standard deviation

a = ratio for the average value of each (random or total) collection to the average value of a 24 hour total collection

b = combination of all data for random and 24 hour samples

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Table 3. Daily variation of the concentration of βAIBA. Urine samples were collected at an unspecified time from each normal subject for 3 days.

Subjects	βAIBA (nmol/μmol creatinine) Days of urine collection		
	1	2	3
1	68	76	62
2	59	47	59
3	64	69	65

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Table 4. The recovery of βAIBA added to normal urine. The creatinine content of the urine was 135 mg/100 ml and the βAIBA content of the sample was initially 40 nmol/μmol creatinine.

μg added to 1 ml of urine	Recovery TLC method	
	μg*	%
5	5.005	100.1
10	10.062	100.62
15	15.182	101.215
20	20.296	101.48

*mean of duplicate determinations

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Table 5. Precision of the method (Intra assay)

	Conc. in urine, nmol/μmol creatinine		
n = 10	$\overline{X}^{a}$	S.D.	C.V.%
βAIBA	116.57	5.38	4.61

a Mean of ten duplicate analyses of a single pooled urine on the same day.

S.D. = standard deviation

C.V. = coefficient variation

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Table 6. Precision of the method (Inter assay)

n = 4	conc. in urine, nmol/μmol creatinine		
	$\bar{x}^a$	S.D.	C.V.%
βAIBA	119.95	5.94	4.95

a Mean of four duplicate analyses of a single pooled urine on different days.

S.D. = standard deviation

C.V. = coefficient variation

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Table 7 shows the amount of βAIBA excretion of normal control adult subjects aged ranging from 17 to 64 years. The level of βAIBA in female urine was slightly higher than that of male urine. The means $\pm$ S.D. of βAIBA obtained from 32 females and 33 males were 80.18 ± 22.98 and 68.02 ± 27.31 nmol/μmol creatinine, respectively.

Figure 7 illustrates the scattergrams of βAIBA excreted from normal males and females.

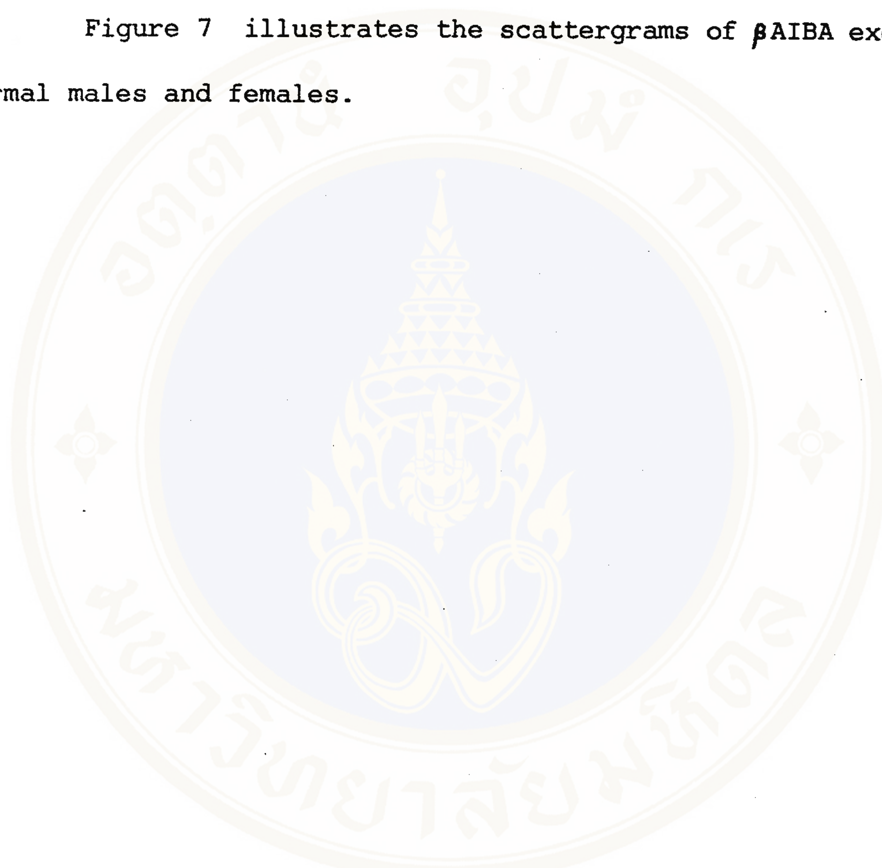

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Table 7. Normal values for βAIBA in normal urine

	Men	Women
n	33	32
$\bar{X}$[a]	68.02	80.18
σ[b]	27.31	22.98
RSD%[c]	40	28

a The values are expressed as nmol βAIBA/μmol creatinine. βAIBA were analyzed in duplicate aliquots of random urine samples.

b Standard deviation

c Relative standard deviation

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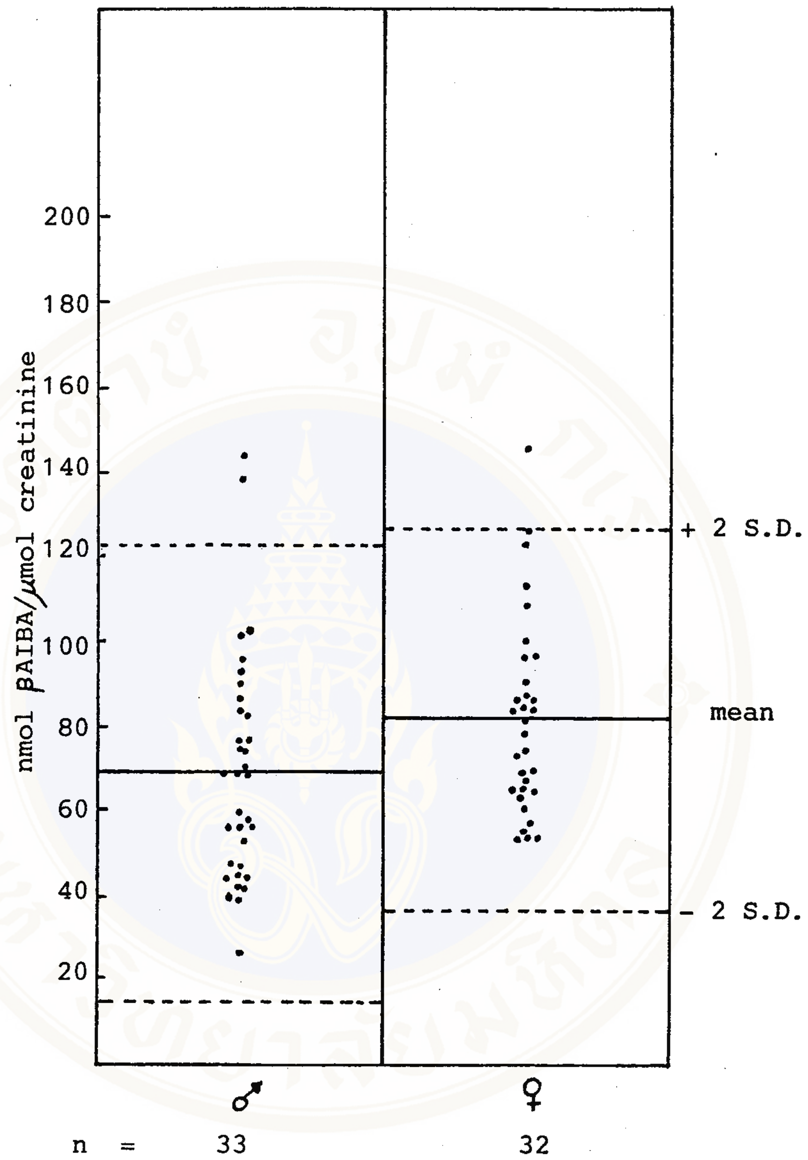

Fig. 7 Scattergram of the βAIBA excretion level in the normal urine of 33 males and 32 females.

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The scattergram of the urinary βAIBA excretion of patients having cancer of various organs is shown in Figure 8. The amounts of βAIBA excreted from cancer patients were ranged from 36 to 955 nmol/μmol creatinine with the mean and standard deviation of 146 and 137 nmol βAIBA/μmol creatinine, respectively (16 males : 154.5 + 218.2 nmol/μmol; 44 females : 143.3 $\pm$ 96.7 nmol/μmol, $\bar{X} \pm$ S.D.). These values are significantly higher than those of 65 normal subjects as analyzed by unpaired-t-test ($p < 0.0005$).

Table 8 illustrates the percentage of patients with cancer of various organs with increased urinary βAIBA excretion. In sixty cancer patients, thirty nine have βAIBA values in the normal range and twenty-one (35%) have significantly high values.

In order to determine the changes of βAIBA excretion in cancer patients after a month of radiotherapy, 15 cancer patients of various organs were studied. Prior to radiotherapy, they excreted above normal levels of βAIBA. The excretion levels of βAIBA from cancer patients before and after radiotherapy were depicted and compared in Fig 9a & 9b.

These patients could be divided into 4 groups ; there were only two in the first group who had βAIBA levels returning to normal ranges after a month of radiotherapy. These were No. 4 and 5 cervical cancer patients stage II whose the βAIBA levels before treatment were 151 and 154 nmol/μmol creatinine, respectively. After radiotherapy, the levels of βAIBA declined to 103 and 70 nmol/μmol creatinine, respectively.

In contrary to the first group, there were five cancer patients whose βAIBA levels increased after a month of radiotherapy. These included cervical cancer patients No. 1 and 6 stage IV and III whose

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βAIBA levels increased from 197 to 404 and 171 to 215 nmol/μmol creatinine, respectively ; patient No. 9 with cancer of floor of mouth, laryngeal cancer patient No. 10 and breast cancer patient No. 14 whose βAIBA levels increased from 179 to 219, 141 to 220 and 226 to 276 nmol/μmol creatinine, respectively.

The third group was three cancer patients whose βAIBA levels after a month of radiotherapy remained as high as before treatment. These were cervical cancer patients stage II No. 2, 3 and cancer patients stage II No. 2, 3 and cancer patient of buccal mucosa No. 8 whose βAIBA levels before and after a month radiotherapy were 176 and 171, 239 and 230, 383 and 382 nmol/μmol creatinine, respectively.

In the last group, there were five cancer patients whose βAIBA levels decreased after a month radiotherapy but the levels remained above normal ranges. These were cervical cancer patient No. 7, patients with breast cancer No. 11, 12, 13 and patient with cancer of bladder No. 15. One exception was patient No. 15, βAIBA levels before and after treatment were very high (955 and 885 nmol/μmol creatinine).

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Fig. 8 Scattergram of the βAIBA excretion level in the urine of 65 normal subjects and 60 cancer patients.

________ = the average value

---------- = 2 standard deviation

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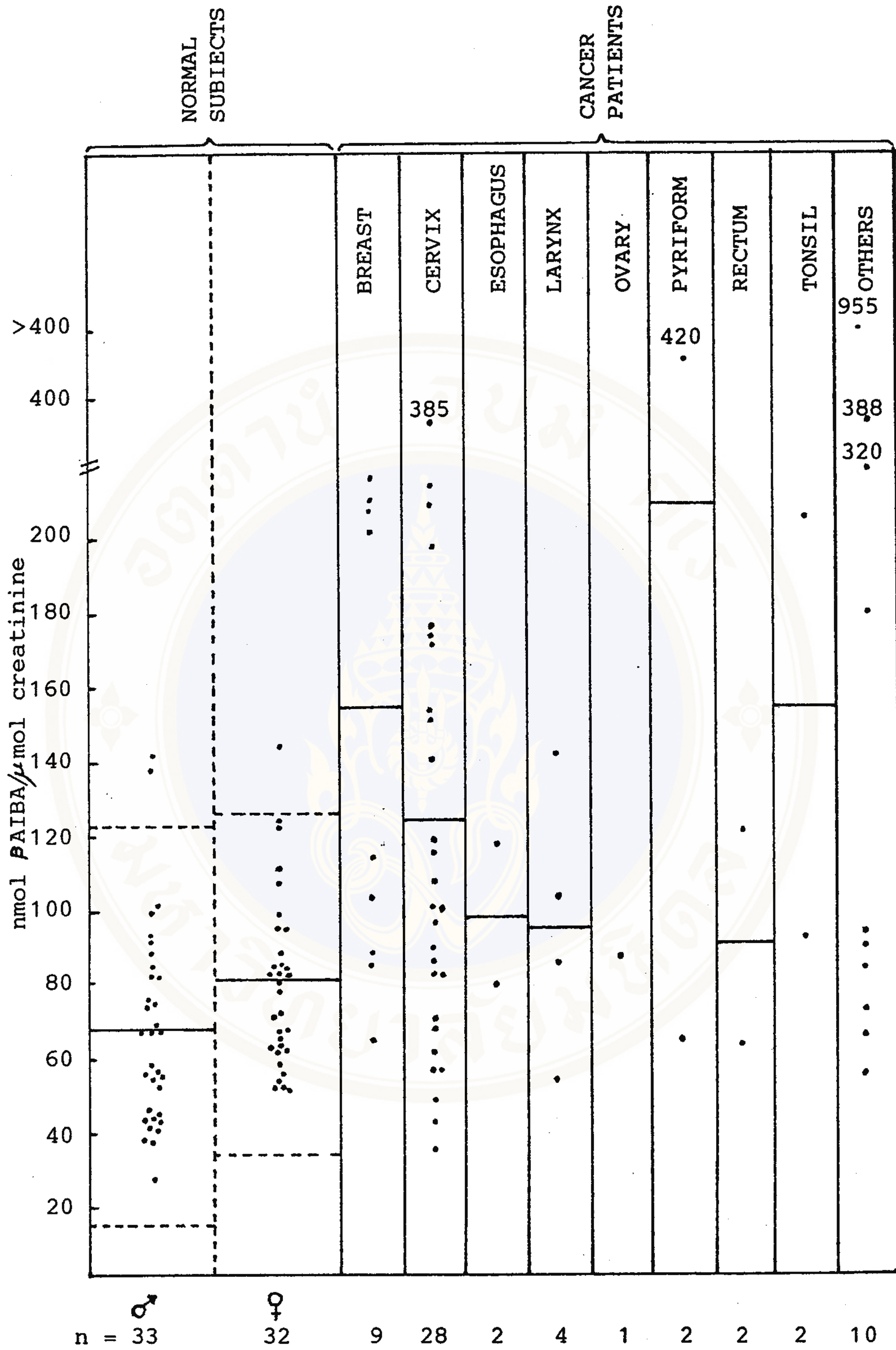

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Table 8. Percentage of patients with cancer of various organs with increased urinary βAIBA excretion

site of cancer	cases analyzed	cases increased βAIBA	%
breast	9	4	44.4
cervix	28	10	35.7
esophagus	2	0	0
larynx	4	1	25.0
ovary	1	0	0
pyriform	2	1	50.0
rectum	2	0	0
tonsil	2	1	50.0
thyroid	1	0	0
bladder	1	1	100.0
nasopharynx	1	0	0
metastatic CA	1	0	0
floor of mouth	1	1	100.0
base of tongue	1	0	0
gum	1	1	100.0
vagina	1	0	0
postcricoid	1	0	0
buccal mucosa	1	1	100.0
Total	60	21	35

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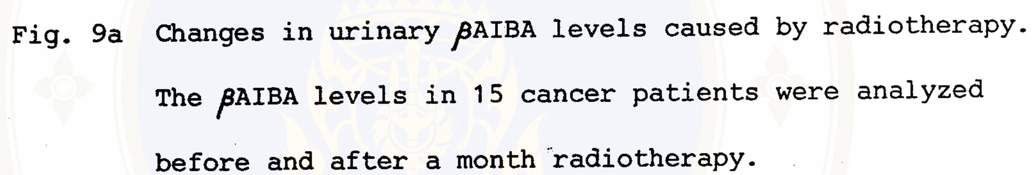

Fig. 9a Changes in urinary βAIBA levels caused by radiotherapy. The βAIBA levels in 15 cancer patients were analyzed before and after a month radiotherapy.

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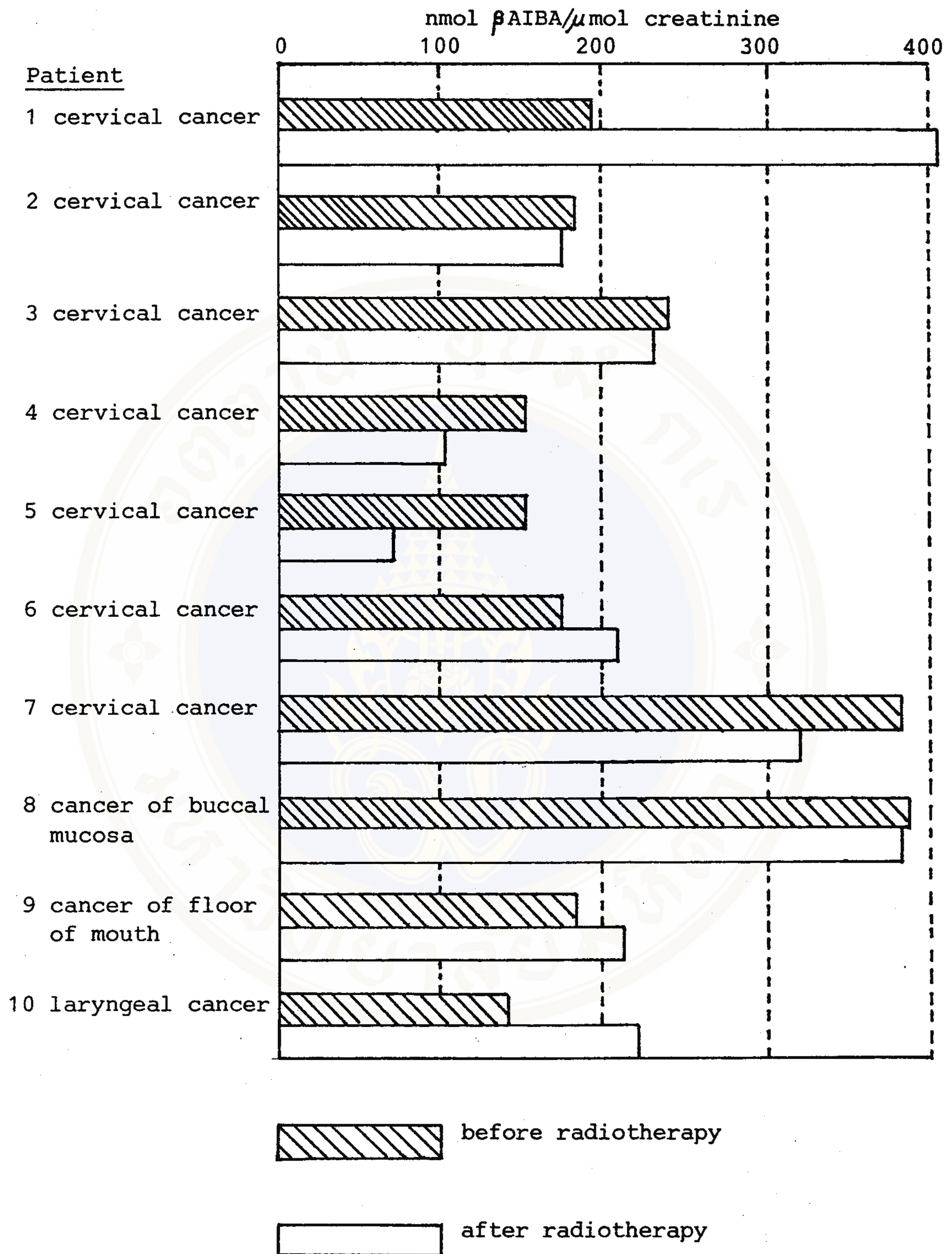

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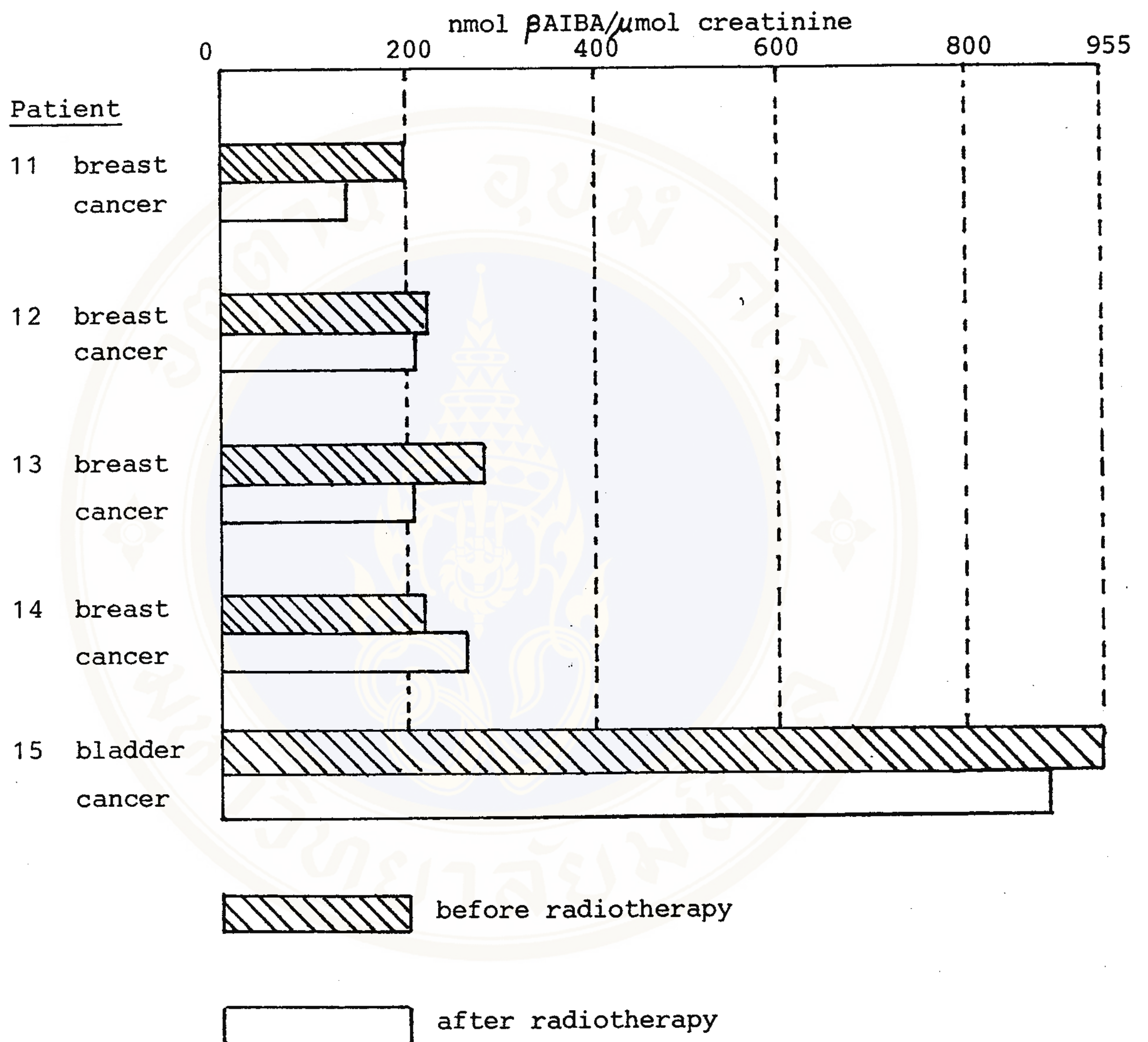

Fig. 9b Changes in urinary βAIBA levels caused by radiotherapy

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CHAPTER IV

DISCUSSION

In order to establish the appropriate assay conditions for measuring urinary βAIBA excretion, the optimum time, temperature and substrate concentration of the incubation procedure were determined. The conditions of 2 h incubation time, 37° C appeared to be adequate conditions of the measurement of urinary βAIBA. These results compare favorably with those optimum conditions used to measure βAIBA by thin layer chromatography (49). After the incubation, the βAIBA was extracted and then separated by thin layer chromatography. The absorption spectrum of DNP-βAIBA was at 355 nm. which was in a good agreement with those reported by Goedde and Brunschede (49).

Urinary βAIBA excretion was not altered by freezing. Urine frozen at a conventional freezer at -20° C for 3 weeks still had the same amount of βAIBA when compared with fresh sample of the same urine.

The assays of beta-aminoisobutyric acid (βAIBA) excretion were originally performed on 24 hour urine (51). To spare the patient the inconvenience of this procedure, this study was done to determine whether random samples of urine could also be replaced the 24 h. sample. Since the previous experiment showed that the βAIBA levels from 24 h. urine among normal subjects had a very narrow range when related to body weight and it was known that the excretion of another product in the urine, creatinine, was also closely related to body mass (44), then this study was undertaken to determine whether the level of beta-aminosobutyric acid excretion could be related to the creatinine level

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of random urine sample. The results showed that the ratio of βAIBA to creatinine in random urine sample was the same as that in total urine excretion in 24 h. (Table 2). These findings suggest that the excretion is constant, not episodic and it is the result of endogenous process.

The normal levels of β-aminoisobutyric acid excretion were determined on volunteers of Occidentals such as American, Europeans and Orientals such as Japanese, Chinese (13,15,18,20). However, for these studies, it seemed important to establish the normal βAIBA levels in Orientals especially Thais because there were evidences about genetic variation in excretion of βAIBA between Occidentals and Orientals (12, 16,19). In addition, for all subjects only single urine specimens were determined, the results were mainly expressed as nmol βAIBA/μmol creatinine. The normal levels of βAIBA excretion expressed as mean values ± standard deviation obtained from these studies were 80.18 ± 22.98 nmol/μmol creatinine for 32 females and 68.02 ± 27.31 nmol/μmol creatinine for 33 males. The results were different from those reported by means of high performance liquid chromatography, which the mean value ± standard deviation were 6.0 ± 3.5 nmol βAIBA/μmol creatinine for 40 males and 24 ± 36 nmol βAIBA/μmol creatinine for 43 females (52). The different results obtained by dual column cation exchange chromotagraphy were the mean values of 4.27 and 5.81 nmol βAIBA/μmol creatinine, with standard deviation values of 1.93 and 4.11 for 125 males and 120 females, respectively (42). In addition, βAIBA levels in urine specimens determined by Hitachi amino acid analyzer were found to vary between a trace and 50 mg per day (38).

The βAIBA excretion in normal females was slightly higher than in normal males. The higher values would be expected in females when

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the expression based on creatinine because the creatinine excretion in females is less than in males. These results are in agreement with those previous reports (42,52). Furthermore, it was also reported that infants and young children excreted high levels of βAIBA as a consequence of low creatinine output (48).

In order to study the frequency of high excretors in normal Thais, the ratio of over 70 nmol βAIBA/μmol creatinine in urine according to Harris (12) was used as a dividing point for classification between high and low excretors. It was apparent that about 40% of Thais in this study were high excretors. This result was comparable to that previously reported by Sutton (17). Some investigators selected the dividing point at the values of about 30 and 55 nmol βAIBA/μmol creatinine for classification between high and low excretors (14,53).

From the family studies suggested that the homozygote for a recessive allele was a high excretor and both the homozygote and heterozygote for a dominant allele were low excretors and the concentration of urinary βAIBA in heterozygous low excretors were higher than those in homozygous low excretors (12,15,17). Furthermore, the concentration of βAIBA in urine of Oriental low excretors may be higher than of the Caucasoid low excretors since frequency of heterozygotes in the low excretor group was higher in the former population than in the latter (15). It was obvious that the activity of human liver enzyme D-β-aminoisobutyrate : pyruvate aminotransferase was low in the high excretors, though not absent. This provided direct support for the hypothesis that the genetic high excretion of D-β-aminoisobutyrate was the result of mutation of this enzyme. Whether this weak activity of the high excretor was the result of a change in

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protein structure by the mutation of a structural gene, or in the amount of enzyme due to the mutation of a regulator gene was unknown (22).

It is known that the concentration of urinary βAIBA is increased in various pathological conditions, especially under conditions in which there is a substantial destruction in tissue cells. In such conditions measurements of βAIBA excretion cannot differentiate genetic high excretors from pathological high excretors who have genetype of the low excretor trait. Therefore, although clinical and histological data were not available, the possibility remains that these two cases are genetic low excretors.

In this study, urinary βAIBA excretion of normal individuals was between 38 and 144 nmol/μmol creatinine. The βAIBA excretion in cancer patients was significantly higher than normal subjects ($p < 0.0005$), and the percentage of patients with increased βAIBA excretion was 35 percent above 144 nmol/μmol creatinine. These results were similar to those previous report (38,40).

The factors responsible for the variations in urinary βAIBA content in some cancer patients according to the type of disease are unknown. Perhaps specific tRNAs with different modifications are preferentially broken down in some tumors.

Although neoplastic tissue invariably had increased tRNA methylase activity in contrast to the normal tissue of the same origin (54), the primary reason for elevation of urinary degradation products of tRNA for patients with cancer was probably the more rapid rate of tumor tRNA turnover in constrast to its normal counterpart (55). The

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high turnover of tRNA had been observed in urine via other products which stemed from tRNA, e.g. pseudouridine and the other methylated purines (40). Suggestive evidence also indicated that the frequency and degree of elevation of individual modified tRNA nucleosides excreted in the urine was related to kinetic growth parameters of the tumor involved. This had been seen in two different malignancies, Burkitt's lymphoma, a tumor with a high proliferation growth fraction and cell death per unit of time, patients with this disease excreted very high βAIBA levels (33,37). In contrary, patients with breast cancer, a tumor with a higher proportion of cells in the Go phase, had a much lower frequency degree of nucleoside elevation (56).

However, the marked decrease of βAIBA excretion as tumors regress after the treatment suggests that the βAIBA excretion may be used in an estimation of the prognosis of cancer patients. In addition, the determination of the urinary βAIBA levels from patients (Fig 9a, 9b) in whom an elevated βAIBA levels remained high after therapeutic procedure such as radiotherapy may be useful in predicting the residual tumors or the progress of the disease.

Radiotherapy might be expected to increase βAIBA excretion, since βAIBA was known to be released in high amounts in men accidentally exposed to large doses of radiation (37). On the other hand, the daily fractionated irradiation to a small part of the body did not lead to an elevated urinary βAIBA excretion (38).

The determination of urinary βAIBA content gave little help in biochemical cancer diagnosis since the elevated βAIBA levels were also found in patients with disease other than cancer such as liver disease,

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lead poisoning, ketoacidosis (57,58,59). However, other data suggested that the urinary βAIBA levels could be used as marker for diagnosis precancerous state in asbestos workers who were at high risk of developing cancer (43).

An intensive study of the relevant case histories as well as the collection of a larger quantity of data will be required before it can be accurately determined whether βAIBA is significantly related to any given type of cancer disease.

Furthermore, increased amount of βAIBA excretion was also found after starvation for about 3 days (60). Hence in assessing the clinical usefulness of βAIBA excretion the physiological status of a patient must be taken into account.

In this study the levels of βAIBA excretion were also followed in 3 patients with choriocarcinoma during chemotherapy for 3 months. Two had remained high levels as their clinical conditions were not improved and their hCG levels remained high. The other had normal level. This βAIBA excretion could be used as valuable indicator for early diagnosis of precancerous condition, especially in women with hydatidiform mole of the uterus in which some instances can become malignant, forming invasive mole that may become choriocarcinoma. Moreover it could be used as a marker during therapy in order to spare the patient of unneeded chemotherapy (47).

The studies reported here were focused on the possible usefulness of this marker as a diagnostic indicator for an early detection of cancer disease or a sensitive indicator of tumor growth and of antitumor response to therapy which may be of value for monitoring

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the course of disease in a patient during treatment. Furthermore, the determination of β-aminoisobutyric acid in conjunction with other modified nucleosides, e.g. pseudouridine may be a valuable mode of monitoring the effectiveness of therapy, especially in conditions with no other objective assessment of remission or relapse is available.

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REFERENCES

1. Armstrong MD, Yates K, Kakimoto Y, Taniguchi K, Kappe T. Excretion of β-aminoisobutyric acid by man. J Biol Chem 1963 ; 238 : 1447-1455.

2. Nielsen HR, Borek E, Sjølin KE, Nyholm K. Dual origin of β-aminoisobutyric acid, a thymine catabolite. Acta Pathol Microbiol Scand 1972 ; 80 : 687-688.

3. Sneider TW, Potter VR. methylation of mammalian DNA : Studies on Novikoff hepatoma cells in tissue culture. J Mol Biol 1969 ; 42 : 271-284.

4. Mandel LW, Borek E. The biosynthesis of methylated bases in transfer RNA. Biochemistry 1963 ; 2 : 555-560.

5. Nielsen HR, Sjølin KE, Nyholm K, Baliga BS, Wong R, Borek E. β-Aminoisobutyric acid, a new probe for the metabolism of DNA and RNA in normal and tumorous tissue. Cancer Res 1974 ; 34 : 1381-1384.

6. Kupiecki FP, Coon MJ. The enzymatic synthesis of β-aminoisobutyrate, a product of valine metabolism, and of β-alanine, a product of β-hydroxypropionate metabolism. J Biol Chem 1957 ; 229 : 743-754.

7. Crumpler HR, Dent CE, Harris H, Westall RG. β-Aminoisobutyric acid (α-Methyl-β-Alanine) : A new amino acid obtained from human urine. Nature 1951 ; 167 : 307-308.

8. Fink k, Henderson RB, Fink RM. Beta-Aminoisobutyric Acid, A possible factor in pyrimidine metabolism. Proc Soc Exp Biol Med (N.Y.). 1951 ; 78 : 135-141.

Copyright by Mahidol University

9. Fink RM, McGaughey C, Cline RE, Fink k. Metabolism of intermediate pyrimidine reduction products in vitro. J Biol Chem 1956 ; 218 : 1-7.

10. Evered DF. The excretion of amino acids by the human. Biochem J 1956 ; 62 : 416-427.

11. Yate K, Armstrong MD. Studies on β-aminoisobutyric acid excretion by familial high excretors. Fed Proc 1962 ; 21 : 268.

12. Harris H. Family studies on the urinary excretion of β-aminoisobutyric acid. Ann Eugen 1953 ; 18 : 43-49.

13. Calchi-Novati C, Ceppellini R, Biancho I, Silvestroni E, Harris H. β-Aminoisobutyric acid excretion in urine. A family study in an Italian population. Ann Eugen 1954 ; 18 : 335-336.

14. Gartler SM. A family study of urinary β-aminoisobutyric acid excretion. Am J Human Gen 1956 ; 8 : 120-126.

15. Yanai J, Kakimoto Y, Tsujio T, Sano I. Genetic study of beta-aminoisobutyric acid excretion by Japanese. Am J Human Gen 1969 ; 21 : 115-127.

16. Grouchy J, Sutton HE. A genetic study of β-aminoisobutyric acid excretion. Am J Human Gen 1957 ; 9 : 76-80.

17. Sutton HE. Beta-aminoisobutyricaciduria. In : The metabolic Basis for Inherited Disease. Edited by Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. 1 st ed. New York : Mc Graw Hill, 1960 ; 792.

18. Sutton HE, Clark PJ. A biochemical study of Chinese and Caucasoids. Am J Phys Anthrop 1955 ; 13 : 53-66.

19. Gartler SM, Firschein IL, Kraus BS. An investigation into the genetics and racial variation of BAIB excretion. Am J Human Gen 1957 ; 9 : 200-207.

Copyright by Mahidol University

20. Gartler SM. A metabolic investigation of urinary β-aminoisobutyric acid excretion in man. Arch Biochem Biophys 1959 ; 80 : 400-409.

21. Kakimoto Y, Taniguchi K, Sano I. D-β-Aminoisobutyrate : pyruvate aminotransferase in mammalian liver and excretion of β-aminoisobutyrate by man. J Biol Chem 1969 ; 244 : 335-340.

22. Taniguchi K, Tsujio T, Kakimoto Y. Deficiency of D-β-aminoisobutyrate : pyruvate aminotransferase in the liver of genetic high excretors of D-β-aminoisobutyrate. Biochim Biophys Acta 1972 ; 279 : 475-480.

23. Borek E. Transfer RNA and its by products as tumor markers. In : Sell s(ed) Cancer markers. The Human Press, Clifton, New Jersey, 1980 : 445.

24. Baguley BC, Stachelin M. Substrate specificity of adeninespecific transfer RNA methylase in normal and leukemic tissues. European J Biochem 1968 ; 6 : 1-7.

25. Gantt R, Evans VT. Comparison of soluble RNA methylase capacity in paired neoplastic and nonneoplastic cell lines in vitro. Cancer Res 1969 ; 29 : 536-541.

26. Hancock RL. Utilization of L-methionine and S-adenosyl-L-methionine for methylation of soluble RNA by mouse liver and hepatoma extracts. Cancer Res 1967 ; 27 : 646-653.

27. Mittelman A, Hall RH, Yolm DS, Grace JT. The in vitro soluble RNA methylase activity of SV 40-induced hamster tumors. Cancer Res 1967 ;. 27 : 1409-1414.

28. Tsutsui E, Srinivasan PR, Borek E. tRNA methylase in tumors of animal and human origin. Proc Natl Acad Sci US 1966 ; 56 : 1003-1009.

Copyright by Mahidol University

29. Borek E, Kerr SJ. Atypical tRNAs and their origin in tumor tissue. Adv Cancer Res 1972 ; 16 : 163-190.

30. Sheid B, Lu T, Nelson JH. Transfer RNA methylase activity in benign human ovarian neoplasms. Cancer Res 1974 ; 34 : 2416-2418.

31. Bergquist PL, Mathews REF. Occurrence and distribution of methylated purines in the ribonucleic acids of subcellular fractions. Biochem J 1962 ; 85 : 305-313.

32. Kuchino Y, Borek E. Tumor specific phenylalanine tRNA contains two supernumerary methylated bases. Nature 1978 ; 271 : 126-129.

33. Waalkes TP, Gehrke CW, Bleyer WA, et al. Potential biologic markers in Burkitt's lymphoma. Cancer Chemother Rep 1975 ; 59 : 721-727.

34. Heirwegh KPM, Ramboer C, Groote JD. Purine and pyrimidine excretion. I. chromatographic separation and identification of purine and pyrimidine metabolites in human urine. Am J of Med 1967 ; 42 : 913-922.

35. Nielsen HR, Nyholm K, Sjølin KE. β-Aminoisobutyric acid in urine from patients with urothelial tumors. Rev Europeenne D'etudes Clin Biol 1971 ; 16 : 444-450.

36. Awapara J, Shullenberger CC. Urinary excretion of β-aminoisobutyric acid after administration of thymine and nitrogen mustard. Clin Clim Acta 1957 ; 2 : 199-204.

37. Waalkes TP, Gehrke CW, Lakings DB, et al. Brief communication : Beta-aminoaciduria of patients with Burkitt's lymphoma. J Natl Cancer Inst 1976 ; 57 : 435-438.

38. Abe M, Takahashi M, Nishidai T, Suyama S. The significance of urinary beta-aminoisobutyric acid in cancer patients. Int J Radiat Biol 1973 ; 24 : 73-79.

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39. Rubini JR, Cronkite EP, Bond VP, Fliedner TM. Urinary excretion of beta aminoisobutyric acid (βAIBA) in irradiated human beings. Proc Soc Exp Biol 1959 ; 100 : 130-133.

40. Speer J, Gehrke CW, Kuo KC, Waalkes TP, Borck E. tRNA breakdown products as markers for cancer. Cancer 1979 ; 44 : 2120-2123.

41. Gehrke CW, Kuo KC, Davis GE, Suits RD, Waalkes TP, Borek E. Quantitative high performance liquid chromatography of nuclesides in biological materials. J Chromatogr 1978 ; 150 : 455-476.

42. Kuo KC, Cole TF, Gehrke CW. Waalkes TP, Borek E. Dual-column, cation exchange chromatographic method for beta-aminoisobutyric acid and beta-alamine in biological samples. Clin Chem 1978 ; 24 : 1373-1380.

43. Fischbein A, Sharma OK, Selikoff IJ, Borek E. Urinary excretion of modified nucleosides in patients with malignant mesothelioma. Cancer Res 1983 ; 43 : 2971-2974.

44. Gehrke CW, Kuo KC, Waalkes TP, Borek E. Patterns of urinary excretion of modified nucleosides. Cancer Res 1979 ; 39 : 1150-1153.

45. Waalkes TP, Abeloft MD, Ettinger DS, et al. Biological markers and small cell carcinoma of the lung. Cancer 1982 ; 50 : 2457-2464.

46. Borek E, Sharma OK, Brewer JI. Urinary nucleic acid breakdown products as markers for trophoblastic diseases. Am J Obstet Gynecol 1983 ; 146 : 906-910.

47. Sharma OK, Waalkes TP, Gehrke CW, Borek E. Applications of urinary nucleosides in cancer diagnosis and cancer management. Cancer Detect Prev 1983 ; 6 : 77-85.

Copyright by Mahidol University

48. Borek E, Sharma OK, Waalkes TP. New application of urinary nucleoside markers. Recent Results Cancer Res 1983 ; 84 : 301-316.

49. Goedde HW, Brunschede H. β-Aminoisobutyric acid : A thin-layer chromatographic method for the quantitative estimation in human urine. Clin Clim Acta 1965 ; 2 : 485-490.

50. Bonsnes RW, Tausoky HH. The colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem 1945 ; 158 : 581-596.

51. Waalkes TP, Gehrke CW, Zuwatt RW, et al. The urinary excretion of nucleosides of ribonucleic acid by patients with advanced cancer. Cancer 1957 ; 36 : 390-398.

52. Buschman FL, Apell G, Sharma OK. High performance liquid chromotographic determination of β-aminoisobutyric acid in the picomole range. J of chromatography 1986 ; 374 : 129-136.

53. Evered DF, Barley JF. Infrequency of urinary excretion of β-aminoisobutyric acid by healthy humans. Clin Clim Acta 1978 ; 84 : 339-346.

54. Borek E. Introduction. Can Res 1971 ; 31 : 596-597.

55. Borek E, Baliga BS, Gehrke CW, et al. High turnover rate of transfer RNA in tumor tissue. Cancer Res 1977 ; 37 : 3326-3366.

56. Toemey DC, Waalkes TP, Gehrke CW. Biological markers in breast carcinoma : clinical correlations with pseudouridine and 1-methylinosine. J Surg Oncol 1980 ; 14 : 267-273.

57. Walshe JM. Disturbances of amino acid metabolism following liver injury. Q J Med 1953 ; 22 : 483-505.

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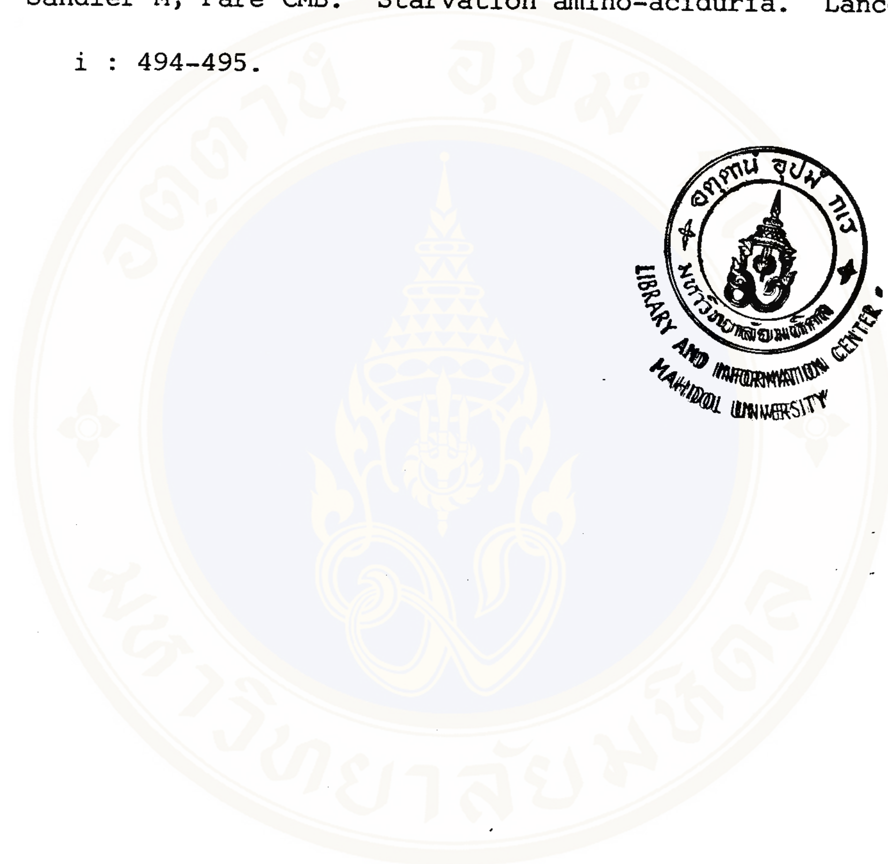
58. Wilson VK, Thompson ML. Dent CE. Amino-aciduria in lead poisoning. Lancet 1953 ; ii : 66-68.

59. Landaas S, Solem E. High excretion of β-aminoisobutyric acid in patients with ketoacidosis. Scand J Clin Lab Invest 1983 ; 42 : 95-97.

60. Sandler M, Pare CMB. Starvation amino-aciduria. Lancet 1954 ; i : 494-495.



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