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Studies on β-aminoisobutyric aciduria of Korean population*

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Introduction

The occurrence of β-aminoisobutryic acid (BAIB) in human urine was first reported by Crumpler et al[1] and Fink et al.[2] in 1951. This identification of BAIB in human urine was followed by the demonstration that thymine from nucleic acid metabolism may serve as its precursor in man and in laboratory animals[3-7]. The excretion of an increased amount by persons suffering from leukemia[2,8] or after irradiation[9], was explained on the assumption that extensive cell destruction results in an increased release of thymine from nuclear material.

In the initial report on the occurrence of BAIB in human urine, Crumpler et al[1] noted that some persons consistently excrete large amounts of BAIB, and suggested that this may be genetically determined characteristics[10-17].

Further studies have confirmed that the characteristic of excreting BAIB is familial and inherited as a recessive traits[14]. Studies on varying ethnic groups have indicated that 6 to 10% Europeans are BAIB excretors, and that a considerably higher proportion occurs in races of the Western Pacific. It is notworthy, however, that there were no BAIB excretors in a group of some 400 healthy persons, largely of Northern European ancestry. On the other hand, approximately one fourth of the persons of Chinese or Japanese ancestry tested, were found to be consistent excretors at the rate of 100μg or more/mg creatinine[18]. It was thus shown that the frequency of high excretors is the lowest (<10%) in Caucasoid population, in contrast with the highest one (about 40%) in Oriental populations and intermediate in Negro people[10,11,12,14,15,18,19,20,22,23]. Oriental populations thus far examined, are Japanese, Chinese (including emigrants to U.S.A.), Thai people, American Indians and Eskimos, except Koreans. As there was no report of the study on BAIB excretion in Koreans, our laboratory has attempted to examine the BAIB exeretion in Korean population and reported very recently its preliminary report[24].

The present study was undertaken in an attempt to extend the former survey by increasing the number of subjects.

Material and Methods

The urine specimens of about 20 ml were obtained at random from healthy persons of both sexes with varying ages. The specimens were usually analyzed on the day of the collection, but occasionally kept at −20°C during 2 or 3 days until analysis. The BAIB concentration in urine was expressed on the basis of creatinine content, owing to the constant excretion of creatinine by individual.

Method of BAIB determination

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The BAIB content in urine was assayed according to Kakimoto et al[17] and the creatinine concentration was determined by the alkaline picrate method. An appropriate amount of urine was passed through a column (0.9cm ×30cm) of Amberlite IR-120, H^+ form, the resin was washed with 10 ml of distilled water, and BAIB was eluted with 6 ml of 2 M pyridine. The eluate was evaporated to dryness *in vacuo*, and the residue was dissolved in 0.1ml of water, and the 10 μl aliquote was subjected to paper electrophoresis. The Whatman 3MM filter paper was used in size of 20cm×40cm.

Two samples were applied in duplicate on a sheet of filter paper along with 10μg of BAIB as standard. The authentic DL-BAIB was the product of Sigma Co., and the standard solution of BAIB was corrected by its cristal water content. The electrophoresis was run at a voltage gradient of 3.000 V/40cm for 30 min, with a buffer consisting of pyridine, acetic acid and water (1:10:189) using CAMAG High Voltage Electrophoresis system. The paper was then dried, immersed in a mixture of 0.2% ninhydrine in acetone and acetic acid (8:2), and dried at 100°C in oven for 10 min. The

BAIB →

Origin →

Fig. 1. High voltage electropherogram of urine BAIB.

Table 1. Daily fluctuation of urine BAIB concentration

Sample No.	μg BAIB/mg creatinine						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
1	4	11	10	10	7	8	11
2	6	10	11	10	5	6	8
3	34	40	32	35	38	30	36
4	52	40	45	48	50	54	42
5	59	65	50	60	52	48	55
6	102	101	98	85	100	93	98

colored band was cut off and eluted with 40 ml of 50% ethanol, and the optical density of the solution was measured at 570 nm using Spectronic 20 spectrophotometer. The blank area adjacent to BAIB band was cut off in similar size and eluted in the same condition for control use.

Results

The determination of BAIB content was made for seven consecutive days on six persons to observe the daily fluctuation of BAIB excretion. The result was shown in Table 1, which indicates a wide variation of individual BAIB concentration, but marked consistency of its daily excretion for same individual. The Figures 2a, 2b and 2c represent the distribution of BAIB excretion of 570 Koreans above 11 years of age including 326 males and 244 females, which showed evident bimodality, though the two modes overlap each other.

It is difficult to establish the dividing line to separate clearly these two modes. Assuming a symmetrical curve in the higher mode, the approximate dividing line could be set at 1.7 (log 50). Thus, the 50μg BAIB per mg creatinine is assumed to be dividing concentration between high and low excretors, as suggested by Kakimoto[17] and Blummberg and Gartler[22].

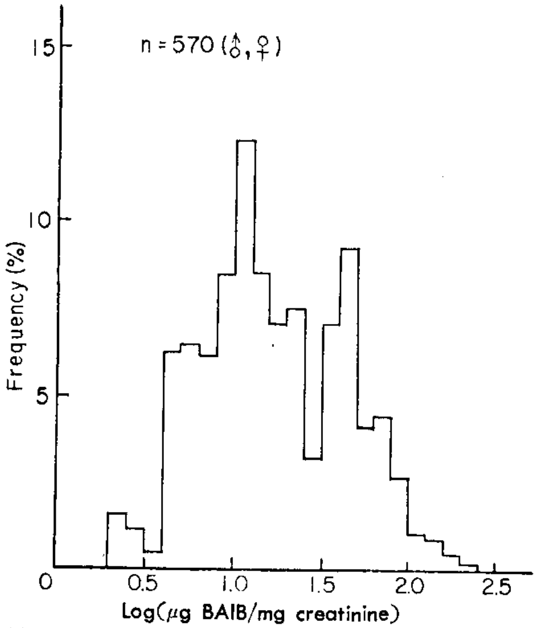

Fig. 2a. Frequency of high excretors of Korean population above 11 years of age.

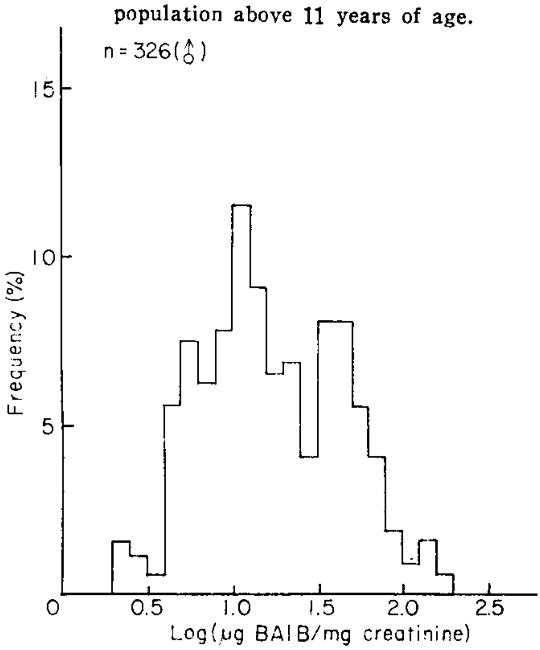

Fig. 2b. Frequency of high excretors of males above 11 years of age.

The data showed that 14.7% of 570 Koreans of both sexes above 11 years of age are high excretors, including the same value of 14.7% in 326 males and in 244 females. The frequency of high excretors of Koreans, therefore, is higher than that of Caucasoid population,

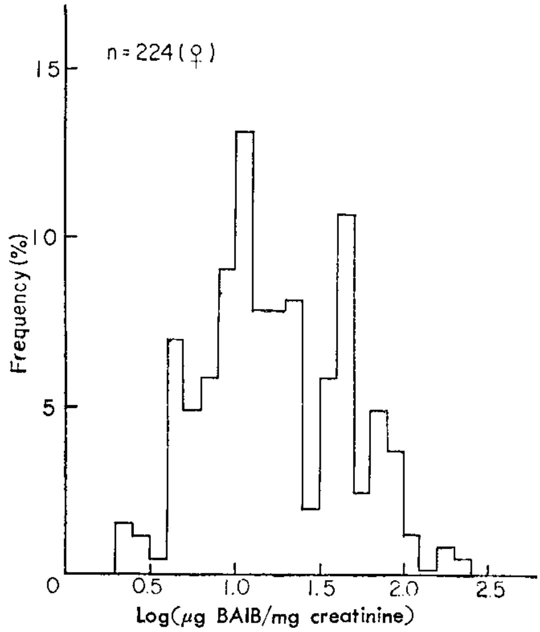

Fig. 2c. Frequency of high excretors of females above 11 years of age.

but considerably lower than that of Japanese one.

The amount of BAIB per unit amount of creatinine has been reported higher in young children[11,12,17]. The BAIB excretion was thus examined for 135 young children of both sexes under 10 years of age.

The average amount of BAIB was higher in the young group under 10 years of age, as illustrated in Figures 2d and 3.

The distribution of their BAIB excretion also exhibits an evident bimodality as shown

Table 2. Correlation of the urinary concentration in pairs of twins

Sample (twin) No.	Sex	Age	μg BAIB/mg creatinine	
1	♀	4	32.0	32.4
2	♀	4	9.0	24.0
3	♀	39	4.0	3.5
4	♀	7	66.0	58.0
5	♂	10	12.6	12.0

Table 3. Family survey of BAIB concentration in urine

Sample No.	Father		Mother		Offspring											
	Age	μg BAIB/mg creatinine	Age	μg BAIB/mg creatinine	Age	Sex	μg BAIB/mg creatinine	Age	Sex	μg BAIB/mg creatinine	Age	Sex	μg BAIB/mg creatinine	Age	Sex	μg BAIB/mg creatinine
1	51	16	46	33	22	M	42	18	F	31	16	F	43	11	M	10
2	46	6	43	8	13	F	3	11	M	10						
3	69	10	61	4	36	M	16	28	M	16	16	M	18	22	F	5
4	36	16	28	41	4	F	51	2	M	74						
5	28	16	27	9	2	M	15	0.5	M	9						
6	30	13	27	49	0.5	F	7									
7	40	10	37	43	14	M	46									
8	34	0	30	77	4	F	32	4	F	32						
9	36	38	34	18	8	F	27	6	M	37	4	F	103	2	F	13
10	36	9	26	17	4	F	46	1	F	60						
11	47	26	46	4	16	M	35	14	M	66						
12	31	1	24	10	1.5	F	132									
13	48	4	44	6	22	F	18	18	F	10	13	M	5	8	M	24
14	35	5	40	8	4	F	9	4	F	18						
15	48	6	37	5	10	F	54	8	F	96	2	M	0	0.3	F	14
16	27	18	28	87	1	F	92									
17	37	6	31	38	7	F	66	7	F	52	4	F	16	2	F	6
18	37	36	34	12	4	M	19	2	F	92						
19	46	18	42	9	17	M	4	10	M	13	10	M	12			
20	27	10	24	7	0.3	F	0									
21	53	45	43	24	5	F	76									

Table 4. Correlation between the extent of low excretion of low excretor parents and the frequency of low excretor offspring

Number of family	BAIB concentration in parents (μg BAIB/mg creatinine)	Number of offspring	
		Low excretor	High excretor
19	Below 49×below 49	34	13
7	Below 14×below 14	15	3
8	Below 14×above 15	9	5
5	Above 15×above 15	7	5

in Figure 2d, and the frequency of high excretors was much higher than that of older age group, reaching to 34.8%. However, if the antimode of this distribution was set at 1.9 (log 79.4), as suggested by Kakimoto[17], the frequency of high excretors in this group approached to 15.7%, corresponding to that of older age group. The concentration of urinary BAIB was expressed on the basis of creatinine, as described above. The higher frequency of BAIB excretion in children group is supposed to involve the factor of lower content of creatinine per body weight in lower age group. The content of BAIB in urine were measured on 5 pairs of twins to examine the hereditary effect on its excretion. The concentrations of BAIB

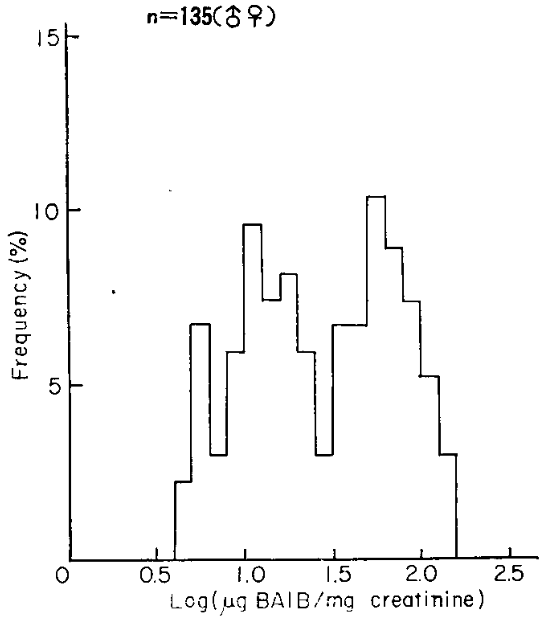

Fig. 2 d. Frequency of high excretors of children below 10 years of age.

were quite similar in each pair of twins examined except one, as shown in Table 2 and Figure 4, suggesting that the BAIB excretion is determined by genetic factors. The result of family survey was presented in Tables 3 and 4.

The 34 low excretors and 13 high excretors were observed among 47 offspring issued from 19 couples of low excretor parents (Tables 3 and 4).

If the additional division of low excretor parents was made by the extent of BAIB concentration, 15 low excretor and 3 high excretor offspring were born from low excretor parents both with BAIB concentration below 14μg, 9 low excretor and 5 high excretor offspring from low excretor parents with its concentration below 14μg and above 15μg, and 7 low excretor and 5 high excretor offspring from parents both with its concentration above 15μg (Table 4). Thus, low excretor parents tend to produce more low excretor offspring than high excretor one, that is, the lower the

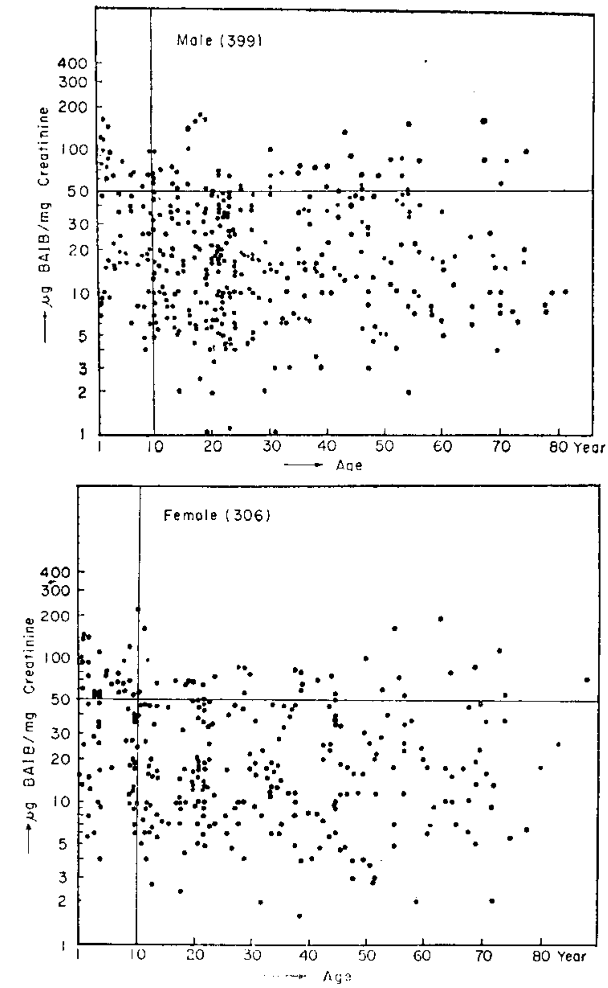

Fig. 3. Age variation of urinary BAIB concentration of Korean population.

BAIB concentration in low excretor parents, the more the low excretor offspring were produced.

Discussion

The BAIB excretion of Korean population varies widely in individuals from 0 to as much as 211 μg/mg creatinine. The daily excretion of BAIB, however, was proved to be nearly constant in same individual by its determination for seven consecutive days on six persons.

It was demonstrated the bimodality of urinary BAIB concentrations in 570 Koreans examined above 11 years of age, though two modes

Table 5. Frequency of high BAIB excretors of Korean population

	Male			Female			Total		
	No. of subjects	No. of excre-tors	Fre-quency (%)	No. of subjects	No. of excre-tors	Fre-quency (%)	No. of subjects	No. of excre-tors	Fre-quency (%)
Frequency of high excretors above 11 years of age	326	48	14.7	244	36	14.7	570	84	14.7
*Frequency of high excretors below 10 years of age	73	22	30.1	62	25	40.3	135	47	34.8
**Frequency of high excretors below 10 years of age	73	10	13.7	62	11	17.7	135	21	15.7

* Putative dividing line was set at 1.7 (log 50)
** Putative dividing line was set at 1.9 (log 79)

Table 6. Frequency of high BAIB excretors of different races

Race	Number of subjects	Frequency of high excretors (%)	References
Japanese	41	41.5	18
〃	246	32	23
〃	1,373	35.8	7
Chinese	33	45	18
Thai people	13	46	18
American (White)	400	0	15
〃	218	10.2	19
〃	120	4	23
〃	255	9.8	12
〃	148	11.5	14
British	345	9.6	10
Italian	711	5.6	11
Negro	75	20	18
〃	38	15	21
〃	285	31.6	21
Indian Apache	110	59.2	21
Alaska Indian	25	56.0	20
Eskimo	120	23.3	20
Marshal Micronesian	188	86.0	22

overlapping considerably. This was the case for both sexes, in 326 males and 244 females. The poor resolution of two modes is partly

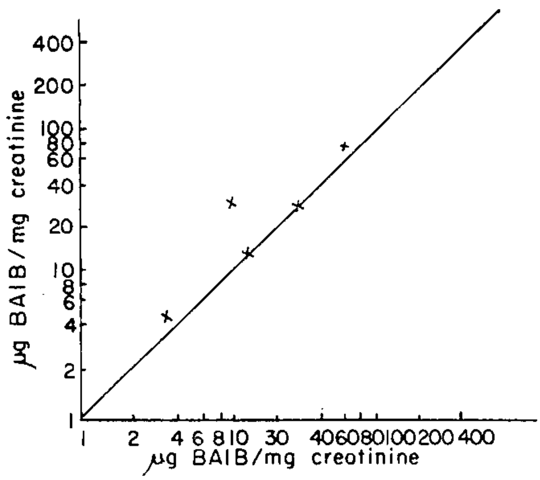

Fig. 4. Correlation of the urinary concentration of BAIB in pairs of twins.

attributed to the fact that the lower mode consists of both the homozygous and heterozygous low excretors and BAIB concentration of heterozygotes is higher than that of homozygous low excretors[17]. Although an accurate separation cannot be made between the high and low excretors, the approximate dividing line can be established at 1.7 (log 50), on the assumption of symmetrical curve in the higher mode, being in agreement with foreign authors[17, 22].

The BAIB concentration above 50μg was thus considered high excretor and that below 49μg low excretor. The high excretors were found 14.7% in 570 Koreans examined above

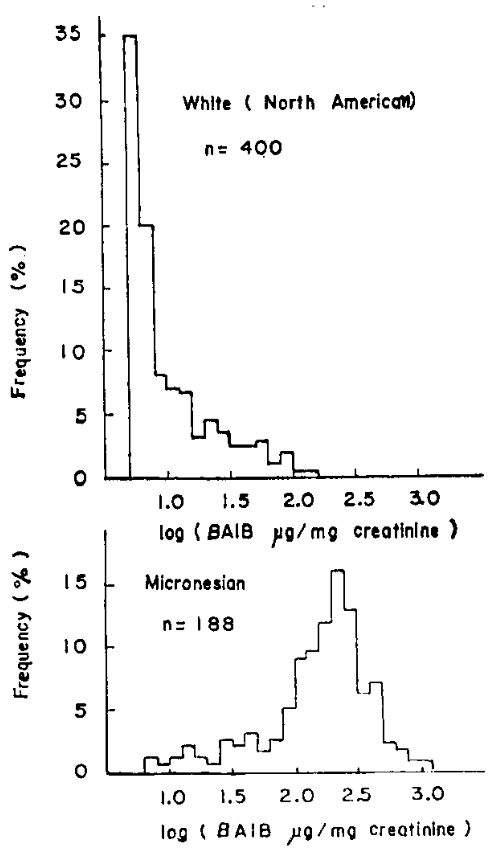

Fig. 5. Distribution of urine BAIB content of North American Whites and Micronesians.

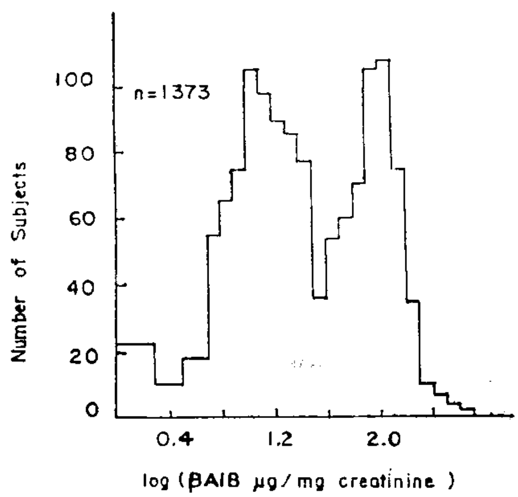

Fig. 6. Distribution of urine BAIB content of Japanese.

11 years of age, regardless of sex, but the same value of 14.7% in 326 males and in 244 females. The frequency of BAIB high excretors in the present study is slightly lower, comparing to 21% in previous report[24] in this laboratory, possibly due to inclusion of children under 10 years of age in former study. The high excretor frequency of Korean people is higher than that of White populations, but lower than other Oriental populations (Tables 5 and 6) (Figures 5 and 6)

The frequency of high BAIB excretors was observed in Oriental populations with very small size of samples except in Japanese people, and it was reported that the frequecy of high excretors is generally above 40% in Chinese, Thai people, Apache and Alaska Indians except 23.3% in Eskimos[18,20,21]. The frequency of high excretors was also examined with large number of samples in Japanese people, indicating 35.8%[17]. Whereas the lowest frequency of high excretors is 0-11.5% in White populations, highest one as high as 86% was observed in Micronesian people, and the intermediate frequency of 15-31.6% in Negro population.

In the present study, the frequency of high excretors was examined with 135 children of both sexes under 10 years of age, indicating 34.8%. The excretion of BAIB per unit content of creatinine has also been reported higher in young children[11,12,17]. Such a higher frequency of high excretors in young children is probably attributed to the lower content of creatinine per body weight in young children, but the possible enhanced catabolism of thymine cannot be excluded in this younger group. If the division of bimodel distribution is set at 1.9 (log 79.4) in this young group, as suggested by Kakimoto[17], the frequency of high excretor approximates to that of older age group, demonstrating 15.7%. As indicated in Table 2 and Figure 4, the concentration of BAIB was quite similar in each pair of 5 twins, except one, suggesting the genetic control of urinary BAIB excretion. Harris[10] first, suggested that the high excretion of BAIB is determined by homozygosity for a recessive allele, but he failed to detect the bimodality pattern of distribution of Caucasoid population examined, Calchi-Novati et al[11] followed the method of Harris and obtained similar conclusion, and Gartler[12] also attempted the family survey without high excretor couples, showing no bimodal distribution.

Gartler et al[13] later examined the families of black Carib population and obtained a bimodal distribution pattern but with considerable overlapping between the two modes, suggesting monogenic autosomal inheritance. Grouchy and Sutton[20] conducted family study, but they also could not demonstrate a bimodal distribution, consequently with no conclusion on heredity. Finally Yanai et al[17] reported the confirmative evidence that the high excretion of BAIB is controled by homozygosity for a recessive allele with a large size of family samples, using relatively accurate method of measurement. Our survey data on 21 families were presented in Table 3. Small size of our family samples and lack of high excretor parent couples impeded us to conduct genetic analysis in special reference to recessive inheritance. As shown in Table 4, 34 low excretor and 13 high excretor offspring were born from 19 low excretor parents, and the lower the BAIB concentration in low excretor couples the more the low excretor offspring were produced. This results also suggest the genetic control of BAIB in urine but it awaits a further study on a larger sample size of family.

The BAIB excreted in urine was identified as D(−)-BAIB[15]. It was already established that the high excretion of BAIB is ascribed to genetic defect in metabolizing D(−)-BAIB[25, 7], but the enzyme responsible for this metabolism, remains to be elucidated. BAIB:α-ketoglutarate aminotransferase was once believed to be an enzyme participating in breakdown of BAIB[26], but it was demonstrated that this enzyme catalyzes the transamination of L(+)-BAIB, in place of D(−)-BAIB[27]. A different enzyme, D(−)-BAIB: pyruvate aminotransferase was found in mammarian liver and proposed to be responsible for BAIB metabolism[28]. It is a possible explanation for the genetic control of BAIB excretion that a dominant allele (T) controls the synthesis of liver D(−)-BAIB-pyruvate aminotransferase but high excretors (tt) lack the ability to form this enzyme, consequently with high excretion of BAIB.

A gene dose effect exists for the dorminant allele, since the concentration of BAIB in urine in heterozygotes (Tt) than in homozygotes (TT) for the dominant allele.

Conclusion

The urinary BAIB concentration of Korean population was assayed using high voltage electrophorebis with Whatman 3MM filter paper, and the BAIB concentration in urine was expressed on the basis of creatinine content. The survey of twins and families was also made concerning the urinary BAIB excretion. The results obtained are as follows.

1) No appreciable daily fluctuation of BAIB concentration was observed for seven consecutive days in the same individual with 6 persons examined.

2) The BAIB concentration varies individually in the extreme from 0 to 211μg/mg creatinine. The evident bimodality was demonstrated in the distribution of its concentration of 570 subjects above 11 years of age, though two modes overlapping, and the same held for

both 326 males and 244 females. Assuming a symmetrical curve in the higher mode, the approximate division line was made at 1.7 (log 50), and thus 50μg/mg creatinine of BAIB was provisionally set as dividing amount between high and low excretors.

3) 570 subjects of both sexes above 11 years of age exhibited high excretors of 14.7% with same value in 326 males and in 244 females. The frequency of high excretors was thus shown to be higher than that of Caucasoid population but lower than that of other Oriental peoples except Eskimo.

4) The BAIB concentration in urine was higher in children group below 10 years of age than that in other group above 11 years of age.

5) The urinary BAIB concentration was quite similar in each pair of 5 twins except one, suggesting genetic control of BAIB excretion.

6) The BAIB excretion was surveyed on 21 families. Offspring of 34 low excretors and 13 high excretors were born from 19 low excretor parents, and the lower the BAIB concentration in low excretor parents, the more the low excretor offspring were produced. This family study also suggests the involvement of hereditary factor in urinary BAIB excretion.

7) Discussion was attempted on the BAIB metabolism.

Acknowledgements

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—國文抄錄—

韓國人의 β-aminoisobutyric acid 尿排泄에 關한 硏究

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正常韓國人 706名을 對象으로 尿中 β-aminoisobutyric acid(BAIB)를 Whatman 3MM 濾紙를 使用하여 高压電氣泳動法으로 測定하고 尿中 BAIB 濃度는 尿 creatinine 量과의 比로 表示하였으며 또 5雙의 twin과 21世帶의 家族에 대하여 尿中 BAIB 排泄量의 血緣關係를 調査하여 大略 다음과 같은 結論을 얻었다.

1) 正常人 6名에 對하여 連 7일동안 每日 1回 採尿하여 BAIB 排泄量을 測定한 結果 各個人의 日差變動에 큰 差異가 없고 個人에 있어 그 排泄量은 거의 一定함을 알 수 있다.

2) 正常韓國人(706名)에 있어서 尿의 BAIB 含量은 最低值 0에서 最高值 μg 211/mg creatinine(以下 mg creatinine을 省略함)에 이르기까지 變動이 甚하다.

3) 11歲以上의 男女 570名에 對한 BAIB 尿 排泄量의 分布圖를 보면 中間에 重複은 되었지만 兩峰性을 나타내고 있음을 確實히 認知할 수 있다. 男(326名) 女(244名) 別로 본 BAIB 排泄量의 分布도 亦是 같은 兩峰性 pattern을 보여 주고 있다. 兩峰의 分岐點을 大略 1.7 (log 50)로 볼 수 있어 BAIB 排泄量이 50μg 以上인 것은 高排泄者, 그 以下인 것을 低排泄者로 區分하였다.

4) 11歲以上의 男女 韓國人 570名中 高排泄者는 14.7%이고 男(326名) 女(244名)에 있어 同一한 14.7%를 보여 주었다. 따라서 韓國人의 高排泄者의 頻度는 白人보다는 높지만 다른 東洋人, 即 日本人, 泰國人 및 中國人等에 比하여 낮고 Eskimo 人과는 비슷하다.

5) 10歲以下의 男女 兒童 136名에 對하여 BAIB 尿排泄量을 調査한 結果 다른 年齡層에 比하여 높은 排泄量을 보았다.

6) 雙童이 5雙의 BAIB 尿排泄量을 調査한 結果 1雙을 除外하고 모두 거의 同一하여 BAIB 排泄量이 遺傳的으로 支配되어 있음을 짐작할 수 있다.

7) 21世帶의 家族에 對한 BAIB 尿排泄量의 血緣關係를 調査하여 본 結果 配偶者가 모두 低排泄者 即 低排泄者兩親에서 出生된 子女 47名中 低排泄者의 子女가 34名(72%)이고 高排泄者의 子女는 13名(28%)이었다. 또 低排泄者의 兩親 中에서도 BAIB 排泄量이 적을수록 低排泄者 子女의 수효가 많아지는 傾向을 보았다.

BAIB 尿排泄이 遺傳的으로 크게 支配를 받고 있음을 示唆함을 알 수 있다.

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