An Investigation into the Genetics and Racial Variation of BAIB Excretion[1]

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INTRODUCTION

THE STUDY OF URINARY β-AMINOISOBUTYRIC acid (BAIB) excretion has progressed to the stage where there is no doubt that most of the variability between individuals in excretion rate of this substance is under genetic control. In fact, all studies to date have been in agreement with the monogenic hypothesis originally proposed by Harris (1953) (high excretors being homozygous for a single recessive gene and low excretors either homozygous or heterozygous for the dominant allele). However, since it was shown that the distribution of the variation in excretion rate of BAIB is continuous (Gartler 1956) and not dimorphic as assumed in earlier studies, it has become essential to ascertain whether this distribution is bimodal, which would be a requisite of a simple genetic hypothesis. Unfortunately most of the work to date on BAIB excretion has been carried out on Caucasoid populations, which are not suitable material for answering the question of bimodality since they contain such a low proportion of high excretors of BAIB. Work at this laboratory has shown that the Apache Indians of Arizona and the Black Caribs of British Honduras have relatively high excretion rates of this substance (Gartler, Firschein and Gidaspow 1956), and consequently it was felt that further work on these two populations would shed light on the problem posed above.

MATERIALS AND METHODS

Single urine specimens were collected from unrelated Apache Indians at the Fort Apache Reservation, Whiteriver, Arizona. Thymol was added to the specimens, after which they were stored in a deep freezer until ready for shipment (under dry ice) to the laboratory in New York. In British Honduras, single urine samples were collected mainly from complete family units (i.e., father, mother, and at least one child), plus a small number of unrelated individuals. Thymol was added to all samples and they were then stored in a refrigerator until shipment under ice to the laboratory in New York.

Creatinine determinations were run on all specimens utilizing the alkaline-picrate method. Aliquots of urine corresponding to various amounts of creatinine were then analyzed by two-dimensional chromatography with phenol and lutidine as solvents and with a 0.2 per cent solution of ninhydrin in acetone as a developer (Gartler 1956). The optical densities of BAIB and glycine were determined on all chromato-

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grams and the BAIB concentration expressed as the ratio of the optical density of BAIB/optical density of glycine. The use of an internal standard such as this permits greater accuracy than can ordinarily be achieved by paper chromatographic techniques. Also, since glycine is a constant and major constituent of the urinary amino acids, it was found that the BAIB concentration expressed in this manner corresponded closely to other methods of reporting its concentration (e.g., mg. BAIB/mg. creatinine).

All specimens were initially analyzed with aliquots of urine equivalent to .010 mg. of creatinine. On subsequent runs, the amount of urine applied was adjusted so as to give optical density readings within the range which we have found to yield a fairly linear response with changes in concentration. No lower limits were set on the amount of urine applied, but we have found that, for most urine specimens, satisfactory quantification cannot be obtained with an amount of urine equivalent to more than .020 mg. creatinine.

Overloading of the chromatogram can lead to serious quantitative errors, particularly in a genetic investigation. Urine specimens with high concentrations of the substance under investigation will be underestimated due to the very limited linear response range of optical readings on chromatograms. Samples with low concentrations will be overestimated due to interference from the numerous minor ninhydrin positive substances in urine which will appear when large amounts of urine are run. These errors will lead to a compression of the distribution and, if severe enough, can obscure the underlying genetic picture.

RESULTS

Bimodality of the distributions.

In figure 1 are given the distributions of the excretion rates of BAIB, reported as a ratio of the *optical density of BAIB/optical density of glycine*, for the Apache and Black Carib populations. As can be seen, both distributions are highly skewed with the long tail in the direction of higher values. In both cases, there is an indication of a dip in the distribution between .2 and .4 and a more detailed examination of this region revealed that these antimodal regions were real. This is better illustrated by plotting the logs of the excretion ratios, which essentially compresses the upper tails of the distributions. For simplicity in plotting, the excretion ratios were multiplied by 100 before taking their logs. In figure 2 such distributions are shown, and it can be seen that the distributions for both the Apache and Black Carib populations are definitely bimodal, though with considerable overlap in both distributions in the antimodal regions. The marked skewness of the original distributions essentially reflects the greater range of expression of high excretors of BAIB, which is what one might expect in the case of a physiologically conditioned genetic variable such as we are dealing with here.

Agreement of family data with bimodality of distribution.

The genetic validity of the bimodality in the preceding distributions depends on the absence of sex and age differences when individuals are classified according to

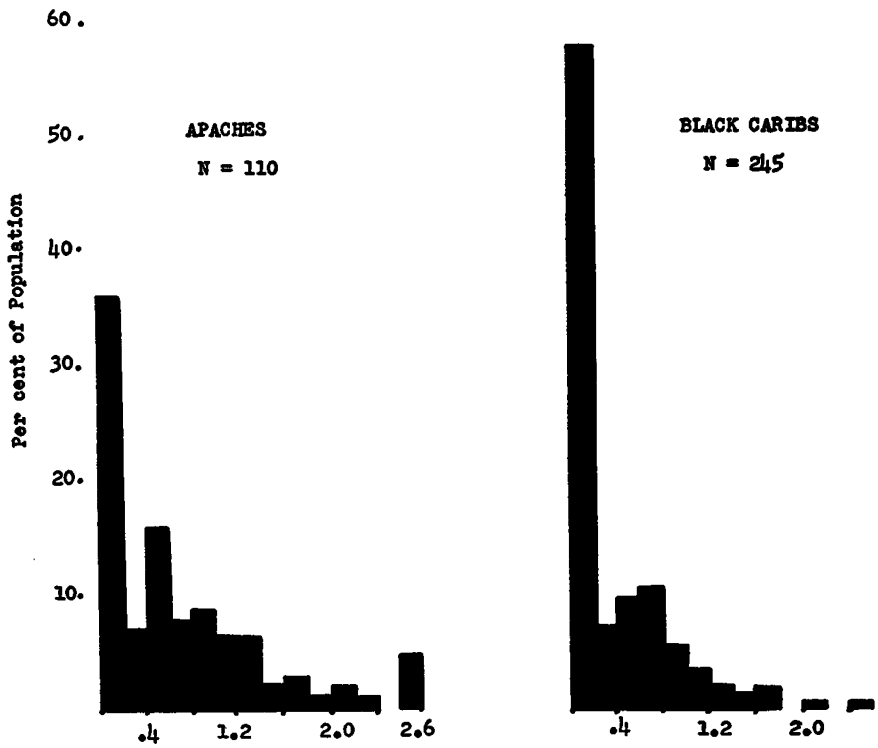

FIG. 1. Frequency distribution of BAIB excretion reported as the optical density BAIB/optical density glycine in the Apache and Black Carib populations.

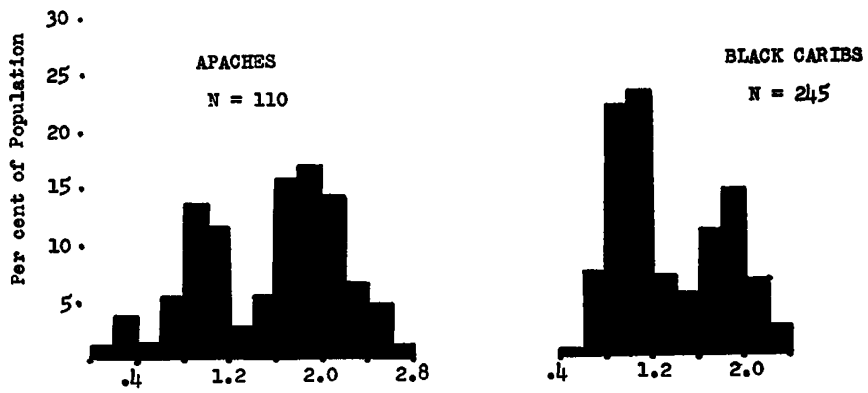

FIG. 2. Frequency distributions of BAIB excretion reported as the log of the optical density BAIB/optical density glycine in the Apache and Black Carib populations.

the observed antimode, and most important, on the agreement of family data with these distributions. These points will now be considered.

Except for the possibility of an increased proportion of high excretors among the very young (Calchi-Novati *et al* 1954; and Gartler, Firschein, and Gidaspow 1956),

no sex or age differences have been reported in any of the major studies on BAIB excretion. Due to the restricted age range in the Apache sample and the incomplete age data in the case of the Black Caribs, only very limited age testing was possible (5–34, and 35 or older for the Apaches; children and parents for the Black Caribs). These data are given in table 1, taking 1.40 on the logarithmic distribution as the dividing point between high and low excretors. As can be seen, there are no significant sex or age differences.

In table 2 the familial data for the Black Caribs are given. There are 5 families which would be classified as matings of high excretor by high excretor using the the dividing line of 1.40 (families 5, 10, 11, 26, and 35). According to genetic hypothesis, such matings should result in only high excretor offspring. Of the 26 offspring produced in these matings there are 24 high and 2 low excretors (families 10 and 35). The values for the 2 low excretors (.70 and .95) cannot be easily explained away by experimental error. However, in view of the known difficulties in collecting accurate family histories from such populations, it would seem premature at this time to consider these cases as real exceptions to the genetic hypothesis.

In table 3 the agreement of data from the remaining Carib families with a mono-factorial hypothesis is tested using 1.40 as the dividing line between high and low excretors. The agreement is good, as indicated by the non-significant chi-square of 2.37. In tables 4 and 5 the family data are further tested for agreement with the mono-factorial hypothesis by examining the distribution of high excretor offspring within segregating families according to the *a priori* method. As can be seen, the deviations from expected are clearly non-significant. The dividing point of 1.40 was initially selected, since it is the mid-point between the two modes. From the preceding results on age and sex testing, and the genetic analyses given in tables 3, 4, and 5, this dividing line of 1.40 appears to have genetic validity. Further support is given by dividing the distributions at 1.30 and 1.50 and carrying out similar analyses to those reported in table 3. The chi-squares obtained, though not significant, are higher (a chi-square of 3.16 for dividing at 1.30 and a chi-square of 4.72 for dividing

TABLE 1. PERCENTAGE OF HIGH EXCRETORS OF BAIB BY AGE AND SEX OF INDIVIDUALS

	Age Group	Sex	No.	High Excretors in per cent
Apaches	5–34	♀	50	50.0
		♂	33	63.7
	35 and over	♀	6	83.4
		♂	21	66.7
	Total	♀	56	53.6
		♂	54	64.8
Black Caribs	children	♀	96	39.6
		♂	83	42.2
	parents	♀	34	38.2
		♂	34	32.4
	Total	♀	130	39.3
		♂	117	39.3

$$\text{High Excretors} = \log \frac{\text{BAIB}}{\text{glycine}} > 1.40$$

TABLE 2. FAMILIAL DISTRIBUTION IN THE BLACK CARIBS OF BAIB EXCRETION $\left(\text{LOG } \frac{\text{OPTICAL DENSITY BAIB}}{\text{OPTICAL DENSITY GLYCINE}}\right)$

Family	Parents		Offspring									
	♂	♀										
1	2.22	1.15	1.96	.95	1.76							
2	1.26	.90	.70	1.04	1.86							
3	2.21	1.11	2.16	1.30	1.96	2.18						
4	.95	1.20	1.23	1.61								
5	1.75	1.41	1.45	1.91	1.87	1.94	2.06					
6	1.11	1.38	1.18	1.04	.70	1.18	.95					
7	1.08	1.08	1.26	1.73	1.65	1.72	1.00	.90	.90			
8	1.56	.95	1.00	1.00	.78	1.08						
9	1.28	.95	1.86	1.00	1.00	1.73	1.61					
10	1.48	1.98	.70	1.67	2.23	2.00	1.48					
11	1.81	2.04	1.83	2.10	2.08	1.86						
20	1.48	.95	1.50	1.91	1.78	1.78						
22	2.10	.90	1.97	1.04	.78	1.04	1.00	1.15				
23	1.15	1.84	1.96	1.26	1.89	1.04	1.70	1.08				
24	1.34	1.23	.78									
25	.70	1.00	1.21	.95	.78	.90						
26	1.84	1.43	1.63	1.88	1.90	1.67	1.83	1.83	1.81			
27	.95	1.00	1.08	.95	.95	.95	.84	.84	1.08	.84		
28	1.40	1.04	1.78	1.00	1.00	1.15	1.70	.95	1.20			
29	1.15	1.65	.90	.95	2.12	1.20						
31	1.04	1.49	1.20	1.04	1.26	1.20	1.08					
32	1.00	2.25	1.23	1.82	.78	1.90	1.08					
33	1.11	1.81	1.08	1.04	1.15	1.08						
34	1.15	1.00	.70	.95	1.00	1.59	1.28					
35	1.69	1.88	.95	1.93	2.06	1.62	1.41					
38	1.00	1.63	.90	1.48	1.65	1.38	1.00	1.38	1.78	1.15		
40	1.26	1.18	.78	.70	.70	.78	.90					
41	.90	1.69	2.03	1.94	2.02	1.21	.60					
45	.90	2.04	2.03	1.08	1.83	1.88	1.04	.95	.84	1.18	1.59	1.96
46	1.15	1.04	1.08	2.31	1.76	1.18						
47	1.00	.84	1.00	1.45	1.08	1.11	1.54					
48	2.10	1.18	1.18	1.11	1.23							

TABLE 3. TEST OF HYPOTHESIS THAT HIGH EXCRETION OF BAIB IS INHERITED AS A MENDELIAN RECESSIVE. TESTED ACCORDING TO FISHER (1939) ON THE BASIS OF A GENE FREQUENCY OF THE RECESSIVE ALLELE (t) OF .59

Mating	Class of Family	No. of Families		x^2	Degrees of Freedom
		Observed	Expected		
T_ × T_	All children T_	5	7.722	2.362	1
	At least one child tt	8	5.278		
T_ × tt	All children T_	4	4.136	.006	1
	At least one child tt	11	10.594		

TABLE 4. ANALYSIS OF HIGH BY LOW EXCRETOR MATINGS WHERE AT LEAST ONE OFFSPRING IS A HIGH EXCRETOR

Family Size	No. of Families	No. of High Excretors Observed	No. of High Excretors Expected	Variance
3	1	2	1.72	0.49
4	3	8	6.40	2.35
5	2	5	5.16	2.16
6	2	4	6.09	2.76
8	1	3	4.02	1.94
10	1	5	5.00	2.49
Totals	10	27	28.39	12.19

TABLE 5. ANALYSIS OF LOW BY LOW EXCRETOR MATINGS WHERE AT LEAST ONE OFFSPRING IS A HIGH EXCRETOR

Family Size	No. of Families	No. of High Excretors Observed	No. of High Excretors Expected	Variance
2	1	1	1.14	0.12
3	1	1	1.30	0.26
4	1	2	1.46	0.42
5	3	6	4.92	1.78
7	2	5	4.04	1.94
Totals	8	15	12.86	4.52

at 1.50) and therefore indicate that the dividing point of 1.40 provides the better fit with the genetic hypothesis.

In this respect, it is of interest to estimate the frequency of misclassifications of high and low excretors using the dividing point of 1.40. By assuming normal distributions for the high and low excretors and calculating the proportion of the distributions extending on either side of the 1.40 line, the percentage of errors can be calculated. This procedure is well illustrated by Penrose (1951), and as applied to this data gives a frequency of misclassification of approximately 7 per cent.

DISCUSSION

The data presented strongly support the hypothesis that the major source of genetic variation underlying BAIB excretion is due to genetic differences at one locus. However, it is also clear from the continuous nature of the population distributions of BAIB excretion, and from the overlap between the modes in these distributions, that other sources of variation contribute to the observed differences between individuals.

Experimental errors and environmental variables are two definite sources of variation, although the exact magnitude of these factors are not known, Of major interest is the question of whether a more complicated genetic system, such as incomplete dominance, multiple alleles, or genetic modifiers might contribute to the continuity and overlapping of the bimodal distribution of BAIB excretion.

The possibility of incomplete dominance can be examined by comparing the mean value of low excretor parents who have had at least one high excretor offspring

(heterozygotes) with the mean value of low excretor parents who have had only low excretor offspring (mainly homozygous low excretors plus some heterozygotes). The mean excretion ratio of the known heterozygotes is 1.06 as compared with a value of 1.10 for that of the mixed group. The difference is not significant and in fact is in the opposite direction from that expected for incomplete dominance.

An examination of the potential contributions of multiple recessive alleles for high excretion and genetic modifiers to the variation in BAIB distribution can be made by carrying out an analysis of variance of only the high excretors from the three mating types producing them: (1) heterozygote X heterozygote, (2) heterozygote X homozygous recessive, and (3) homozygous recessive X homozygous recessive. If either multiple alleles or modifiers are important, then significant *between sibs: within sibs* variance ratios should be obtained in all three instances. If multiple alleles are the major modifying influence, then the three variance ratios should form a decreasing series from mating types 1 to 3, whereas with modifying genes, the variance ratios for the three mating types should not differ from each other. This last statement follows from a consideration of the number of high excretor types that can be produced by the different matings. In the case of multiple alleles each mating of type (1) could produce only one kind of high excretor, (2) could produce up to two kinds, and (3) could produce up to four kinds of high excretors. In the case of genetic modifiers, the number of high excretor types would depend on the number of modifying loci and would be independent of the three mating types. The variance ratios for the three matings are (1) 1.82, (2) 2.82, and (3) .96, only (2) being significant at the 5 percent level. These results would indicate that environmental variables and experimental errors are the major modifying forces involved in the observed bimodal distributions of BAIB excretion.

Although there is considerable overlap between the high and low excretor distributions of BAIB excretion, this genetic variable can still be of some importance in anthropo-genetic investigations. The racial variation indicated thus far (Sutton and Clark 1955; Gartler, Firschein, and Gidaspow 1956) covers a remarkably wide range, from less than 10 percent high excretors in Caucasoids to around 60 percent in Mongoloids, with Negroes somewhat intermediate. Furthermore, with improvement in techniques, it is likely that resolution of the high and low excretors can be much improved.

SUMMARY

Urinary BAIB excretion was studied in an Apache Indian (Arizona) and a Black Carib (British Honduras) population. Both populations exhibited bimodal distributions, though with considerable overlap between the two modes. The family data from the Black Carib population was shown to be in good agreement with a monogenic hypothesis, and the combined results were taken to indicate that the major source of genetic variation underlying BAIB excretion is due to genetic differences at one locus. Possible modifying forces causing overlap and continuity of the BAIB distribution, and the value of the BAIB excretion variable in anthropo-genetic investigations, were discussed.

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