A BIOCHEMICAL STUDY OF CHINESE AND CAUCASOIDS [1]

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The study of racial differences has resulted in the discovery of many measures which may be used to characterize human populations. Although very few of these measures are chemical in nature, all genetically controlled differences are ultimately chemical, since the primary action of genes is chemical. It would seem plausible then to expect to find measurable chemical differences among human populations such as are known to exist among individuals (Sutton and Vandenberg, '53; Berry, '53).

Of the chemical substances which might be studied, the amino acids found in urine would seem to offer good chances of finding population differences. Many studies in addition to the two referred to above have dealt with amino acid excretion, and some of them have shown that rather marked individual differences exist (Harris, '53; Ulrich, Schropp, and Martin, '54). Exploratory studies in this laboratory suggested the existence of differences in amino acid excretion between Chinese and Caucasoids. Accordingly, the following study of a larger group of Chinese was carried out, measuring not only the amino acids but also certain other substances in the urine which have been explored in previous studies of biochemical variability.

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PROCEDURE

The Chinese subjects consisted of ten male and eight female residents of Ann Arbor whose ages varied from 20 to 54 years. All of them had been in the United States for at least two years and were living under essentially Western conditions. Although two of them are native Americans, they are of known Chinese extraction. The Caucasoids consisted of sixteen males and thirteen females, ranging in age from 25 to 66 years. They are mostly of Western European origin, although no attempt was made to trace their ancestry. No related individuals were used in either the Chinese or the Caucasoid sample; each of the groups contained several married couples, however.

Measurements were made on first morning urine samples, of which from one to nine were obtained from each individual, the usual number being three. Creatinine was determined by a method similar to that of Bonsnes and Taussky ('45) and uric acid by the paper chromatographic method of Berry ('51). The amino acids, with the exception of histidine, were determined on two-dimensional paper chromatograms with phenol and lutidine as solvents (Berry, Sutton, Cain, and Berry, '51). Measurement of the intensity of the ninhydrin-produced colored spots was done with the reflectance attachment of a Beckman model B spectrophotometer. Histidine was determined by the one-dimensional paper chromatographic method of Cain and Berry ('51). Other substances measured are as yet largely unidentified and hence are designated by their color reactions with diazotized sulfanilic acid (DSA) or bromocresol green (BCG) and by their Rf values in butanol-acetic acid-water (80:20:20). The conditions for these procedures are described in Berry, Sutton, Cain and Berry ('51). For the BCG chromatograms an aliquot of urine containing 40 μg creatinine was used as the test amount and for the DSA chromatograms an aliquot of urine containing 100 μg was used. A quantitative estimate of these substances was made by measuring the area of the spot with a polar planimeter.

TABLE 1

Individual means of urinary constituents

SUBJECT	CREATININE	SPECIFIC GRAVITY	pH	ALANINE	β-AMINOISO-BUTYRIC ACID	GLUTAMIC ACID	GLUTAMINE [1]	GLYCINE	HISTIDINE	LEUCINE [2]	LYSINE	SERINE	TAURINE	THREONINE	TYROSINE	VALINE	URIC ACID	BCG ACID .28	BCG ACID .90	BCG BASIC .32	DSA ORANGE .85	DSA PURPLE .90
	mg/ml	*(−1, × 1000)*		*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*mmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*µmol/g Cr*	*mg/mg Cr*	*in²/40 µg Cr*	*in²/40 µg Cr*	*in²/40 µg Cr*	*in²/100 µg Cr*	*in²/100 µg Cr*
Caucasoid females																						
571	1.13	12.68	6.47	99	163	0	158	389	1.0	155	98	181	87	101	0	150	0.3	.00	.51	.50	.65	.27
530	1.39	9.35	5.47	500	0	0	650	1850	NV [3]	0	0	1080	400	260	0	0	NV	NV	NV	NV	NV	NV
578	1.58	11.36	5.54	171	67	11	274	683	0.8	238	112	293	214	219	0	87	0.2	.12	.43	.24	.65	.32
562	1.18	11.42	6.38	194	100	56	275	479	1.2	303	83	186	386	58	59	7	0.4	.12	.61	.34	1.02	.31
526	0.56	21.36	6.25	188	0	13	152	266	1.1	0	0	236	0	0	0	0	0.4	.19	.38	.53	.20	.15
576	2.08	13.09	5.72	417	138	0	502	1770	0.8	454	227	505	415	269	237	144	0.4	.39	.33	.06	.57	.29
509	1.03	15.90	6.12	163	33	15	164	421	1.0	55	273	141	112	46	0	23	0.4	.20	.31	.54	.40	.21
504	1.53	13.93	6.07	349	33	0	384	790	1.9	289	349	515	376	131	17	26	0.6	.05	.21	.54	.00	.06
565	1.53	15.57	5.38	69	0	0	69	163	0.4	0	0	90	111	0	0	0	0.4	.36	.21	.00	.99	.13
505	1.07	16.92	5.87	242	113	4	283	876	1.5	282	142	344	198	122	108	92	0.4	.26	.43	.10	.85	.25
120	1.95	13.63	5.87	109	0	0	87	335	0.6	0	200	221	99	0	0	30	0.5	.26	.15	.24	.29	.22
502	1.28	15.44	5.93	263	177	79	244	379	0.8	279	168	326	232	122	71	107	0.4	.24	.36	.31	.60	.29
528	1.89	11.75	6.16	332	117	6	345	1609	1.0	345	283	311	251	262	133	66	0.4	.18	.32	.25	.59	.23
Caucasoid males																						
527	2.05	10.09	5.63	93	0	0	65	116	0.3	0	0	93	126	0	0	0	0.2	.02	.27	.16	.18	.20
962	1.04	14.12	6.15	101	25	30	99	185	0.7	70	0	127	126	0	0	38	0.3	.10	.25	.25	.35	.18
507	1.45	12.68	6.25	120	89	0	198	317	0.8	184	88	121	130	92	29	20	0.3	.08	.26	.12	.55	.28
375	0.41	23.28	7.14	60	0	24	46	44	NV	0	0	NV	0	0	0	0	0.3	.10	.21	.86	.06	.04
531	2.53	11.10	5.68	84	33	0	85	153	0.4	0	0	125	143	0	0	0	0.2	.05	.41	.47	.72	.20
506	1.32	12.70	6.16	430	33	0	450	684	1.0	25	390	548	193	153	17	17	NV	.07	.25	.35	.68	.27
577	1.56	9.76	5.80	277	171	0	314	753	0.7	394	100	249	428	224	275	62	0.4	.41	.20	.00	.37	.22
564	1.97	12.11	5.71	155	0	0	168	350	0.7	0	46	193	166	35	0	0	0.3	.15	.11	.17	.51	.20
819	1.53	14.39	6.26	162	33	23	217	444	0.6	0	0	302	234	69	0	0	0.4	.05	.26	.40	.38	.26
068	1.07	14.70	6.38	156	120	3	147	211	1.0	136	98	143	219	144	8	49	0.3	.00	.31	.35	.50	.29
105	1.97	11.44	6.10	186	134	6	258	477	0.6	225	166	174	245	181	55	76	0.4	.06	.25	.25	.59	.29
532	0.67	15.12	6.00	48	0	0	67	85	NV	0	0	65	202	0	0	0	0.5	.00	.25	.33	.18	.04
501	2.21	14.28	5.68	202	108	0	288	415	0.7	160	148	239	244	90	113	43	0.3	.05	.31	.30	.71	.22
508	1.64	13.11	5.93	156	79	11	225	378	0.7	253	68	174	198	119	87	123	0.4	.06	.40	.33	.54	.32
561	1.09	16.58	6.15	154	0	45	174	361	1.1	0	162	217	308	42	17	0	0.5	.21	.22	.45	.47	.21
503	2.91	8.48	5.30	435	100	0	455	1240	NV	38	0	905	345	0	0	470	NV	NV	NV	NV	NV	NV
Chinese females																						
618	1.91	10.63	5.81	175	589	0	161	374	0.5	119	40	211	581	42	0	105	0.3	.11	.35	.24	.30	.43
749	1.29	19.38	5.38	429	510	0	853	1189	1.6	318	350	1300	96	413	230	89	0.9	.28	.35	.00	.29	.24
549	0.79	21.39	6.49	371	0	0	197	646	2.5	0	318	NV	175	65	144	0	0.5	.08	.26	.59	1.08	.27
671	1.57	14.86	7.27	838	486	27	643	800	2.0	437	239	490	98	1003	202	66	0.5	.00	.41	.25	.56	.38
669	0.76	14.59	5.60	330	0	0	342	794	1.2	488	600	NV	326	0	126	152	0.9	.24	.21	.00	.47	.16
668	0.93	24.64	5.85	138	95	0	142	303	1.6	69	254	186	147	74	72	19	0.8	.00	.00	.08	.08	.18
748	0.88	20.59	5.61	202	NV	0	288	422	0.6	208	169	314	454	0	72	164	0.8	.13	.32	.00	.56	.26
620	0.92	16.30	5.64	285	697	0	359	307	1.1	459	226	165	243	0	177	132	0.5	.20	.19	.00	.42	.37
Chinese males																						
662	1.29	16.58	5.50	503	190	0	447	475	1.2	508	781	NV	367	238	281	109	1.1	.40	.21	.00	.44	.27
672	2.55	10.42	5.37	187	0	0	213	263	0.7	179	375	NV	299	0	0	0	0.8	.19	.12	.00	.24	.22
667	1.61	12.74	5.53	116	198	0	131	123	1.3	0	81	99	130	0	50	0	0.4	.19	.00	.00	.15	.15
665	1.83	12.07	5.70	167	322	0	192	146	0.9	357	313	NV	207	149	0	0	0.4	.03	.23	.06	.51	.30
619	2.49	9.69	5.71	193	224	0	221	243	0.6	153	200	163	309	114	0	0	0.4	.30	.24	.06	.43	.26
664	1.67	12.22	5.22	1400	50	0	1055	1070	1.8	639	1000	NV	379	790	179	164	0.8	.38	.12	.00	.18	.25
670	1.97	10.16	5.78	263	186	0	234	226	0.6	298	496	NV	335	166	0	34	0.5	.15	.13	.00	.32	.27
684	1.17	16.24	6.89	321	198	38	321	259	1.1	0	300	NV	160	126	110	27	0.9	.04	.00	.64	.45	.17
621	1.70	11.76	5.78	180	100	0	227	325	0.6	278	181	177	456	0	0	0	0.6	.20	.24	.00	.52	.46
550	1.98	13.29	6.41	144	289	8	132	179	0.6	0	100	156	205	42	0	0	0.6	.02	.09	.44	.45	.12

[1] Alanine used for standard.
[2] Leucine and isoleucine are not separated by the techniques used. Both may contribute to the spot designated as leucine.
[3] NV = No value.

EXPERIMENTAL RESULTS

The individual means of each measure are presented in table 1, grouped according to race and sex. These individual means were used as the basis for later computations, since the number of samples differed for each individual. It should be noted that all the measures except pH are expressed as creatinine (Cr) ratios, as this has been shown to remove most of the effects of varying dilutions. Table 2 presents the means for each measure broken down according to sex and race.

TABLE 2

Mean values of various measures for Chinese females, Chinese males, Caucasoid females, and Caucasoid males

SUBSTANCE	CHINESE FEMALES	CHINESE MALES	CAUCASOID FEMALES	CAUCASOID MALES
Creatinine	1.13	1.83	1.40	1.59
Specific grav.	1.0178	1.0125	1.0140	1.0134
pH	5.96	5.79	5.94	6.02
Alanine	346	347	238	176
β-Aminoisobutyric acid	340	176	72	58
Glutamic acid	3.4	4.6	14.2	8.9
Glutamine	373	317	276	204
Glycine	604	331	770	388
Histidine	1390	940	1008	715
Leucine	262	241	185	93
Lysine	275	383	149	79
Serine	444	149	341	245
Taurine	265	285	222	207
Threonine	200	163	122	72
Tyrosine	128	62	48	38
Valine	91	33	56	56
Uric acid	0.65	0.65	0.40	0.34
BCG acid .28	0.13	0.19	0.20	0.09
BCG acid .90	0.26	0.14	0.35	0.26
BCG basic .32	0.15	0.12	0.30	0.32
DSA orange .85	0.47	0.37	0.57	0.45
DSA purple .90	0.29	0.25	0.23	0.21

To test the significance of the differences shown in table 2, a two-way analysis of variance for race and sex was performed on each substance using the mean values of the various individuals as replications. Since there were different numbers of individuals in the four categories of race and sex, it was necessary to employ a non-orthogonal analysis as described,

TABLE 3

Values of F for differences between Chinese and Caucasoids, between males and females, and for the interaction between race and sex from factorial analyses of variance for race and sex. The variance between individuals within races and sexes was used as the denominator of the variance ratio in each case.

SUBSTANCE	RACE	SEX	INTERACTION	DEGREES OF FREEDOM FOR ERROR VARIANCE [1]
Creatinine	0.01	7.76 [3]	2.54	43
Specific grav.	2.08	8.65 [3]	5.24 [2]	43
pH	0.01	0.61	0.80	43
Alanine	4.39 [2]	0.21	0.23	43
β-Aminoisobutyric acid	22.60 [3]	4.85 [3]	3.40	42
Glutamic acid	2.19	0.16	0.41	43
Glutamine	3.11	1.15	0.02	43
Glycine	0.84	7.27 [3]	0.20	43
Histidine	5.21 [2]	7.84 [3]	0.34	39
Leucine	5.02 [2]	1.25	0.50	43
Lysine	16.57 [3]	0.13	2.85	43
Serine	0.00	3.88	1.08	34
Taurine	2.50	0.00	0.20	43
Threonine	2.12	0.58	0.01	43
Tyrosine	4.74 [2]	2.55	1.34	43
Valine	0.06	1.34	1.33	43
Uric acid	29.74 [3]	0.31	0.31	40
BCG acid .28	0.38	0.16	5.38 [2]	41
BCG acid .90	11.41 [3]	10.84 [3]	0.26	41
BCG basic .32	8.15 [3]	0.01	0.10	41
DSA orange .85	1.52	2.15	0.01	41
DSA purple .90	2.90	0.95	0.22	41

[1] There is always one degree of freedom in the numerator.
[2] Significant at the 5% level.
[3] Significant at the 1% level.

for example, by Kenney and Keeping ('51, pp. 265–267). The variance ratios obtained are shown in table 3. In testing the significance of the race and sex differences, the within-group variances were used in the denominators of the F ratios even in the two cases in which interaction was significant, since the inferences are intended only for the particular races and sexes represented in the data.

It will be noted that the Chinese excreted significantly more alanine, β-aminoisobutyric acid, lysine, leucine, histidine, tyrosine, and uric acid and significantly less of the substances designated BCG basic .32 and BCG acid .90 than did the Caucasoids. Females were found to have a significantly higher specific gravity than the males. In the females the excretion of β-aminoisobutyric acid, glycine, histidine, and BCG acid .90 was significantly greater and that of creatinine significantly less than in males. For specific gravity and BCG acid .28 the interaction between race and sex was significant.

DISCUSSION

Although for most of the measures the difference between the races is not large, in some cases it is highly significant. The causes of such differences are uncertain, but their possible nature is suggested by previous studies. One possibility, of course, is that they reflect genetic differences between the races. Many of the morphological differences between racial groups are obviously genetic, and it is reasonable to suppose that some of the biochemical differences are hereditary also. On the other hand environmental differences between the two races also offer a possible explanation for the racial differences. One would expect the biochemical properties of an organism to be more influenced by environment than perhaps any other group of measures, since all organisms are nutritionally dependent upon their environment. Very likely the true situation is a combination of these two effects since the chemical phenotype results from the inherited biochemical potential plus the available substrate.

Evidence for the role of genetics in biochemical racial differences is furnished for lysine by the observations that lysinuria occurs as a constitutional trait in the cystine-arginine-lysine amino-aciduria (Dent and Harris, '51) and in the condition involving lysine alone (Berry, Cain and Rogers, '51; Ulrich, Schropp and Martin, '54). In the case of β-aminoisobutyric acid, Harris ('53) has reported that the high excretion of this substance can be explained as resulting from a homozygous recessive genotype. The consistently high excretion of lysine or β-aminoisobutyric acid by only certain individuals and under a variety of environments is strong evidence that heredity is very important in determining the excretion of these substances.

That diet also plays an important role in determining the amino acid excretion levels has been shown in a recent experiment in which twelve subjects were studied on their own varied diets and on a uniform diet (Sutton and Clark, '54). It was found that the amino acids most influenced by the change to a uniform diet were ones which show considerable individuality in excretion patterns, and the individuality did not decrease significantly when the subjects consumed identical foods. Lysine was the amino acid showing the greatest effects of diet.

This study does not indicate the relative importance of heredity and environment in producing the observed differences. Inferences can be drawn, however, from the nature of the data. In the case of β-aminoisobutyric acid, Harris ('53) divided the population into two groups on the basis of whether or not the excretion of this substance exceeded that of alanine as measured by the ninhydrin color intensity. He found that excretors continued to be such under a variety of conditions and that different individuals under apparently similar circumstances might vary widely among themselves in their excretion levels. In the study reported here, an attempt was made to express the amount of the amino acid excreted in a more quantitative manner; hence, the subjects have not been separated into "excretors" and "non-excretors." However, in both the Chinese and the Caucasoids, "high" excretors and "low" excretors occur, the difference being the greater number of "high" excretors in the Chinese group. Among the Chinese there were also several married couples of whom only one partner excreted β-aminoisobutyric acid in appreciable quantities, suggesting that diet is not the principal factor affecting the excretion of this substance.

The arguments which have been applied to β-aminoisobutyric acid can also be applied to the other substances showing racial differences, but with the exception of lysine, the other substances have not been sufficiently studied in such a way as to throw light on the relationship of heredity and environment.

From table 3 it may be observed that for some of the measures the differences between the sexes are also significant. The fact that females tend to excrete less creatinine than do males may account in part for the often higher value in females of those measures which involve creatinine in the denominator. The sex differences in excretion of glycine and BCG acid .90 have been observed previously (Sutton and Clark, '54).

Differences in races such as have been described here provide the biologist with another means of understanding the great variability which exists between human populations. The biochemical variability of human beings is an important field in which considerable progress is being made, but the extent to which this variability contributes to racial differences is for the most part unexplored. The possibility that some of the differences can be reduced to different gene frequencies offers hope that increased power can be given to the genetic methods of classifying both individuals and populations.

SUMMARY

The first morning urine samples from eight female and ten male Chinese and from thirteen female and sixteen male Caucasoids were compared for differences in a number of chemical measures, particularly of amino acids. The Chinese were found to excrete significantly more alanine, β-aminoisobutyric acid, lysine, leucine, histidine, tyrosine, and uric acid

than Caucasoids. They excreted less of the substances designated BCG basic .32 and BCG acid .90. In the analysis for sex differences, females were found to have a slightly higher specific gravity than males but a lower creatinine concentration and to excrete more β-aminoisobutyric acid, glycine, histidine, and the substance designated BCG acid .90. The two-way analysis of variance showed significant interaction between race and sex for specific gravity and BCG acid .28.

The relative importance of heredity and environment in contributing to the observed differences was discusssed.

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