

Effects of age and sex on taste sensitivity to phenylthiocarbamide (PTC) in the Berkeley Guidance sample

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Abstract. Using a modification of the Harris and Kalmus method for ascertaining the taste threshold for phenylthiocarbamide (PTC), data were collected on 424 caucasian individuals from the three generational longitudinal study of the Berkeley Guidance subjects in Berkeley, CA. Analysis of the data indicates the distribution of thresholds is significantly different from that in the London sample of Harris and Kalmus in that the frequency of nontasters is about half that found in London. The difference is not attributed to the modifications made in the testing procedure but to a real difference in gene frequency. Both the PTC taster and nontaster phenotypes display a large amount of variation in expression between individuals. They are differently expressed in males and females, the latter being more sensitive. As people age, the expressions of their genotypes change. Neither age nor sex seem to affect the penetrance of the PTC genes which remains the same within each sex at all ages unless some other environmental factor, such as radiation, impinges. Consideration of these relationships is necessary for the investigation of family relationships, the mechanisms responsible for maintaining this ancient genetic polymorphism and the degree of polytypy present in the species.

Introduction

Fox (1932) demonstrated that people are either tasters or nontasters of the bitter substance phenylthiocarbamide (PTC), a chemical that does not occur in nature. It was soon recognized that tasting and nontasting were not discontinuous characteristics but, rather, general terms to describe taste sensitivities that clustered about two modes of a bimodal and continuous distribution along an axis representing taste sensitivity thresholds. Those individuals whose discrimination thresholds cluster about a low concentration of PTC are designated as tasters and those whose thresholds cluster about a higher concentration are said to be nontasters.

The thresholds were determined by presenting the subjects with serial binary dilutions of a stock PTC solution and recording the lowest concentration chosen as having a distinct taste. This approach was first introduced by Blakeslee and Salmon (1935). Much needed objectivity was added by Harris and Kalmus (1949) who supplemented the presentation of the dilution series with a sorting procedure. In their method, the threshold recorded is the lowest concentration at which the subject can discriminate the PTC solution from water.

The Harris and Kalmus technique is a standardized procedure (Kalmus, 1971), employed throughout the world, and makes possible comparison of data from diverse areas. Review of numerous studies has shown that the proportion of tasters and nontasters varies markedly between races (Barnicot, 1950; Alsbirk and Alsbirk, 1972) and also within races (Sunderland and Cartwright, 1968). In general, European populations have a higher proportion of nontasters than other groups.

In evaluating differences from study to study the question arises of how much of the variation is methodological and how much is biological. Basic to its resolution is

consideration of how closely the investigators have followed the standard method and the effect of any variations that may have been made. Although the use of the Harris and Kalmus technique is widespread, so also is the institution of modifications. The most common among these reported are changes in the solvent, the containers and the number of dilutions presented to the subjects. Little attention has been paid to the effect of these variations.

Mohr (1952) employed the Harris and Kalmus technique but substituted distilled water for tap water and paper cups for glass tumblers in his study of 314 Danes. Distribution of the Danish data is significantly different from the English data of Harris and Kalmus. However, the frequency of nontasters was not significantly different. Mohr concluded that the difference in distribution between the two populations represented technical discrepancies rather than biological variation.

This report presents data from Berkeley, CA that have been obtained using a modified Harris and Kalmus technique. The results differ from those of the London investigators in two ways. First, the sensitivity of the Berkeley participants is slightly less than the London group and second, there are significantly fewer nontasters. Arguments are presented to support the position that the differences cannot be attributed solely to variations in methodology.

A large body of experimental evidence exists to support the hypothesis that the taste dimorphism depends upon a single genetic locus with two major alleles: a dominant taster allele, *T*, and a recessive nontaster allele, *t* (Blakeslee, 1932; Snyder, 1932; Das, 1958; Merton, 1958). Although some evidence that the taster allele is incompletely dominant (Klamus, 1958; Martin, 1975) has been advanced, none was found by Rao and Morton (1977) using complex segregation analysis. These investigators also found no evidence for polygenic variation. The extreme sensitivities found in some populations (Lugg, 1955, 1962; Ibraimov and Merrakhimoo, 1979) have led to a hypothesis of a second taster allele. The possibility of a series of dominant taster and recessive nontaster alleles has not been ruled out.

Environment also affects the taste dimorphism. Morton *et al.* (1981) have presented evidence that environmental factors account for up to 72.4% of observed variation in sensitivity to PTC. Over the five decades that have elapsed since the discovery of the trait, numerous reports have described the effects of various environmental factors on the penetrance and expression of these alleles. In this paper, in addition to addressing the methodology issue, we discuss the distinction between penetrance and expression, offer additional data on the effects of age and sex, and argue the hypothesis that these two factors do not normally affect the penetrance of the alleles but do affect their expression.

Methods

Measurement of PTC sensitivity

A stock solution containing 1.3 g/l (0.13%) of phenylthiourea (1-phenyl-2-thiourea, J.T.Baker Chemical Company, Phillipsburg, NJ, reagent grade) was made up in distilled water rather than in boiled tap water as recommended by Harris and Kalmus. This was done because our subjects resided in various geographical areas where tap water has quite different flavors. Further we wished to avoid possible variation in the solutions

resulting from a reaction of chemicals in the water to PTC. The stock solution was designated solution 1 to agree with Harris and Kalmus. A binary series of dilutions was prepared by using equal parts of the preceding solution and distilled water. In our entire experience in over 700 tests the most dilute Harris and Kalmus solution required was number 15 which contained 0.08 mg/l PTC. Table I shows the solution numbers identical to those of Harris and Kalmus, the reversed numbering system to be discussed below, and corresponding concentrations in mg/l as well as moles per litre. Care was taken that all solutions were at room temperature (70–74°F) at the time of the test since temperature of the solution is known to affect the threshold measurement (Fischer, 1967).

The subjects were being seen for extensive physical, psychological and social evaluation and only 15 min were allotted to genetic investigation. PTC testing was just one of four genetic traits studied and it was essential to minimize the time required for the test. Since we wished to carry out threshold determinations using a full dilution series, we made the following further modifications to the Harris and Kalmus technique: (i) the numbering system was reversed and (ii) a two-out-of-five sorting test was substituted for the four-out-of-eight procedure.

To begin the test, 7 ml of each dilution was placed in disposable transparent 1-oz polypropylene medicine cups numbered with black marking pencil 0 through 14 and arranged from the right to the left side of a grey-topped desk in ascending order. The slightly yellow color of the cups, the small amount of solution in them and the light reflection from the desk top served to mask any perception of slight yellowish color in the most concentrated solutions. The cups were odorless and tasteless and considered a good substitute for the glass tumblers used by Harris and Kalmus and preferable to

Table I. Concentrations of PTC solutions

Solution number (Harris and Kalmus)	Cup number (as presented)	PTC (mg/l)	Moles per liter
1	14	1300.00*	8.54×10^{-3}
2	13	650.00	4.27×10^{-3}
3	12	325.00	2.18×10^{-3}
4	11	162.50	1.07×10^{-3}
5	10	81.25	5.35×10^{-4}
6	9	40.63	2.68×10^{-4}
7	8	20.31	1.34×10^{-4}
8	7	10.16	6.70×10^{-5}
9	6	5.08	3.85×10^{-5}
10	5	2.54	1.93×10^{-5}
11	4	1.27	9.65×10^{-6}
12	3	0.63	4.83×10^{-6}
13	2	0.32	2.42×10^{-6}
14	1	0.16	1.20×10^{-6}
15	1/2 ⁺	0.08	6.00×10^{-7}
	0 [‡]	0.00	0.00

*Stock solution.

⁺Required for one subject out of 457.

[‡]Distilled water.

paraffined paper cups used by some investigators. The cup numbered 0 contained distilled water against which the subject was to compare the PTC solutions. Cup 1 contained solution 14 and cup 14 contained solution 1. This manoeuvre facilitated the explanation that the cups contained increasingly strong solutions. The procedure was much more quickly understood when we explained this arrangement than when we described the reverse arrangement.

Also to conserve time, as mentioned above, a two-out-of-five test was substituted for the four-out-of-eight test of Harris and Kalmus. Several two-out-of-five sets were partially prepared before the subject arrived. Six yellow-brown cardboard trays, each with five cups numbered with black marking pencil 1 through 5, were made up. Using a random number table, the two cups to contain the test solutions were determined for each tray and the other cups filled with 7 ml distilled water. The trays were then covered.

Determination of recognition level

After a brief description of the test, the subject was asked to taste the distilled water by swallowing it and then to taste the contents of each cup in the same manner in ascending order, beginning with cup 1. The subject was directed to stop when he noted a taste he considered different from the distilled water. When this cup was reached the subject was asked to describe the taste. This solution was designated the recognition level and was usually within one or two solutions of the threshold of discrimination. If the subject could taste none of the solutions through to cup 14 (solution 1), he was given a pinch of the powder to place on his tongue. At mouth temperature, concentration of PTC may reach 3.2 g/l (Stephen and Stephen, 1963) so that the range of concentrations to which the tastebuds are exposed is greatly extended.

Since it is primarily the posterior part of the tongue where the bitter taste is perceived, the statement in the Harris and Kalmus paper, 'the subject is given a few cc in a tumbler till . . . he perceives a definite taste,' (1949) was taken to mean the stimulus was swallowed. Recent communication with Professor Harris reveals that the solution was swirled around the mouth and then spat out. Although the amount of PTC ingested is small, swallowing is not now acceptable procedure because of possible toxic effects.

The recognition level having been determined, the subject was given a form to complete if the experimenter was alone or was given another genetic test if an assistant was present. In any case the subject was unable to watch the experimenter prepare the two-out-of-five tests by filling the two remaining empty cups in several trays: usually one at the recognition level and one or two at levels below and above the recognition level.

Determination of discrimination level

To determine the discrimination level, the two-out-of-five sorting test was administered. A set of five cups was arranged in order 1 through 5 from left to right placed on the desk in front of the subject who was told that two cups contained the taste substance and three contained distilled water. The subject was instructed to select the two that had the taste. The first set offered to the subject contained the test solutions at his recognition level.

If the subject was successful in choosing both cups correctly, he was given the set containing the next more dilute solution. Had the subject been unsuccessful he would have been tested with the set containing the next more concentrated solution. The procedure was repeated until the subject failed to choose both test cups correctly. The least dilute solution at which a subject could distinguish the two cups of PTC solution from the three cups containing distilled water was considered his threshold of discrimination. Trials at the same level were not repeated.

When the discrimination level had been determined the subject was again asked to describe the taste sensation. In contrast to the many descriptions given at the recognition level, fewer were given at the threshold level. The most common description was bitter or some similar term such as bad, strong or medicinal.

Throughout the testing, the subject was allowed to proceed at his own pace since in almost every case this allowed at least 8–10 s to elapse before tasting the contents of the next cup. This was probably sufficient time to avoid taste fatigue and to allow development of the taste sensation (Zotterman, 1971). The few individuals who proceeded very rapidly tended to overshoot their thresholds of discrimination while determining their recognition levels. The question of possible refractoriness induced by ingestion of PTC in amounts necessitated by the test has not been settled, and therefore it is not known how much error is ultimately caused by first greatly exceeding the true threshold.

Subjects were asked to refrain from eating, drinking or smoking for at least 15 min prior to beginning the test in an attempt to minimize variability of test results within individuals. None of the subjects wore dental plates, which are claimed to decrease sensitivity to PTC.

The two-out-of-five sorting test carries a probability of chance success of one out of ten while the four-out-of-eight test of Harris and Kalmus has a probability of succeeding by chance alone one time in seventy. That this modification would not have a marked effect on the threshold measurement was suspected since it was our experience that subjects were quick to report when a choice was really a guess. When such difficulty arose, the subject became insecure and apologetic and was generally within a step of his threshold. At even more dilute concentrations, some subjects refused to choose saying it would be 'pure guesswork'. Thus subjects were not forced to choose and the test was scored as a failure. Forced choice is a modification of the original method. Administration of both the four-out-of-eight and the two-out-of-five tests to 22 adult individuals (students and staff) on different days, randomly alternating the sequence of the sorting procedures, showed a mean difference in threshold of less than one dilution step (0.53 steps) with the mean for the two-out-of-five test being at a less concentrated level. There were nine males and 13 females ranging in age from 19 to 72 years among the 22 controls. All were by chance tasters. The mean recognition level obtained using the four-out-of-eight test fell at dilution step 10. There is no truly comparable group in the London data but estimating the taster mean from a subsample similar in age and sex composition yields a sensitivity level of 9.6. Since subjects generally resisted guessing, we suggest that this slight effect could be related to two factors that may produce transient taste fatigue: first, more PTC solution was ingested by individuals during the four-out-of-eight test than during the two-out-of-five test, and second, in

the four-out-of-eight test only five arrangements in 70 avoided adjacent PTC solutions while in the two-out-of-five sorting procedure, six of 10 arrangements contained no adjacent PTC solutions.

We expected therefore that our modification of the Harris and Kalmus procedure would have no significant effect upon the distribution of taste thresholds in the population other than perhaps a slight shift, not exceeding one threshold step, of the entire distribution in the direction of lower concentration.

A total of 30 persons from among the longitudinal study members agreed to be retested approximately 1 year later. As seen in Figure 1, the agreement was generally good, being better for the 12 adults (slope 0.875) than for the 18 children (slope 0.755). Whether the Harris and Kalmus repetition experiments for '49 people' included such a high proportion of children is not known. Among our group of children the greatest

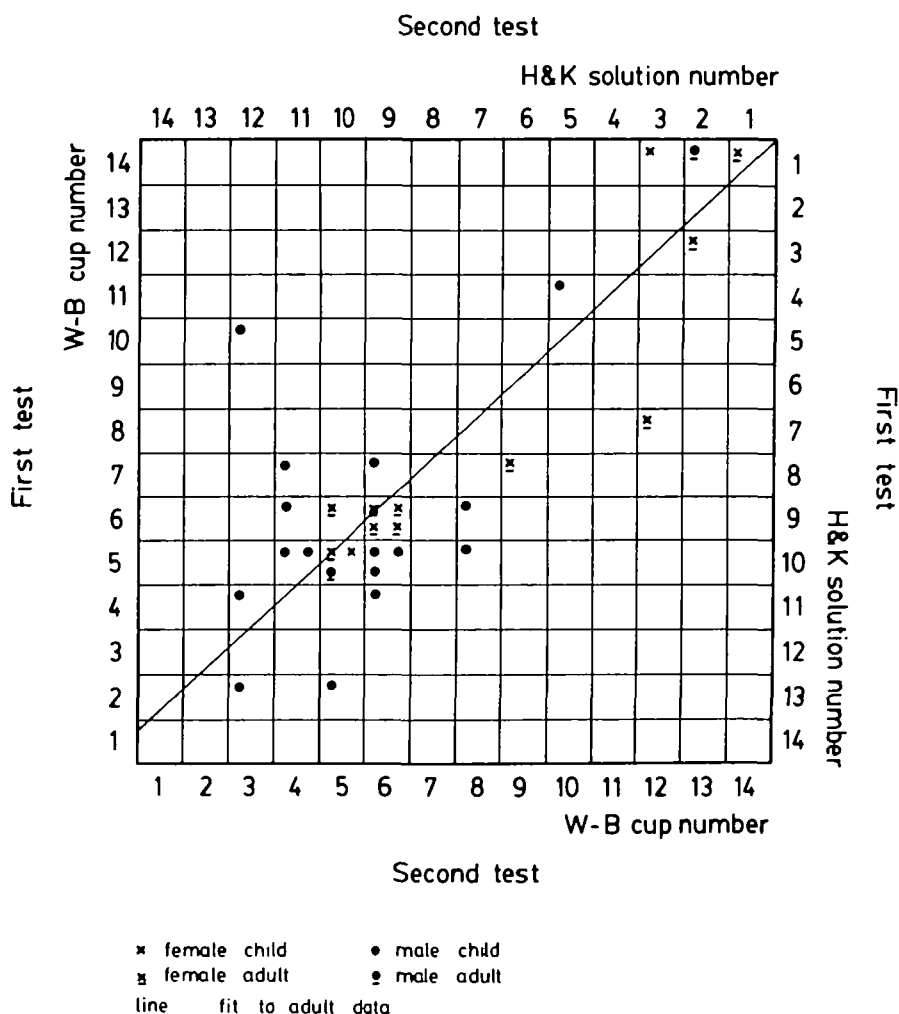


Fig. 1. Results of pretesting 30 persons with the W-B threshold modification.

variability occurred among adolescents aged 12 to 15 years. The average step difference was 2.6 for these children as compared to 0.77 for the combined group of children below age 12 and above age 15 years.

Determination of the antimode

The frequency distribution of sensitivities for all 424 Berkeley participants had the same range (steps 0–14) as the London data. There were two peaks, one at step 10 and one at step 1, with an antimode at step 4. The bimodality is consistent with world data on PTC sensitivity among caucasians. The most striking difference between the Berkeley and London data was the much lowered frequency among Berkeley subjects of insensitive individuals.

When the data were divided into three or four age groups by sex, the bimodality was less apparent because of the decreased frequency of insensitive persons. Nevertheless, bimodality was assumed to underlie each group. In order to classify individuals in each group as tasters and nontasters, the antimode for all groups was determined.

Equal variances of the underlying distributions were not assumed. For this reason and because of the small sample sizes, Pearson's method of moments, a nonic method of asymmetric decomposition of distributions (Pearson, 1894, 1914), was considered an appropriate tool (S.Bieber, personal communication) for generating the sub-distributional means and the point of intersection of the two distributions.

Materials

Using the Harris and Kalmus technique modified as described, 424 individuals over three generations ranging in age from 4 to 78 years were tested. There were 99 index cases (53 women and 46 men), 67 spouses, 15 parents of the index cases and 244 of their offspring. The probands are a subsample from a socioeconomic study, commonly referred to as the Berkeley survey (Macfarlane, 1938). The original sample was derived by selecting every third child born in Berkeley, CA from January 1, 1928 through to June 30, 1929. It contained 454 babies. Of these, 248 children were included in a longitudinal investigation of growth and development known as the Guidance study (Macfarlane, 1938). PTC testing was carried out during the 40-year follow-up of the Guidance group at the Institute of Human Development between June, 1969 and September 1970.

The subjects of this report are all caucasians. The families of over 80% of the index cases and their spouses had their origins in Western Europe before the first World War. For the 99 probands, information on country of origin was gathered in 1928–1929 from their parents, most of whom at that time had living parents. Reported English ancestry accounted for 20% and Irish, Scottish and Welsh together for ~25% of the ethnic makeup. The families were predominantly Protestant. They were economically and intellectually advantaged compared to their birth cohorts (Eichorn *et al.*, 1981).

Results

The taste thresholds grouped in 2-year age intervals are presented in Table II for the 214 males and in Table III for the 210 females. For comparison with the London data

Table II. Taste thresholds for PTC of 214 males classified by age

Age (years)	W-B solution number																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
4-5					3											1	4
6-7				1	5	3			1					1			11
8-9	1	1	1	1	4	2		1	1		1	1		1			15
10-11		1	3	7	2	4	1				2		2				22
12-13		1	1	2	5	7	1	2	1		1			1			22
14-15		1	2	2	2	3	2			1	2	2	1			1	19
16-17			1	3	7	3	2	1					1	2			20
18-19	2		1		2	4	2		1						1		13
20-21					1	1		1									3
22-23										1							1
24-25																	
26-27																	
28-29																	
30-31																	
32-33																	
34-35									1								1
36-37																	
38-39																	
40-41		1	1	8	4	7	6	3	2	1		2		1		2	38
42-43			1	2	1	7	4	2	1					1		1	20
44-45		1			2		3			1		1				1	9
46-47			1	1				2		1							5
48-49					1	1					1						3
50-51																	
52-53																1	1
54-55																	
56-57																	
58-59																	
60-61																	
62-63										1							1
64-65												1					1
66-67									1								1
68-69																	
70-71					1												1
72-73								1									1
74-75																	
76-77														1			1
78-79								1									1
Total	3	6	12	27	40	42	21	14	8	7	7	7	4	8	1	7	214

the data for each sex were first divided into three age groups: children (offspring of index cases) aged 4-23 years, parents (the index cases and their spouses) aged 30 or 35-50 or 53 years, and grandparents (the parents of the index cases) aged 60 years or over. The mean thresholds and standard deviations (in Harris and Kalmus solution numbers) for these groups are shown in Table IV. Figures 2 and 3 present the frequency distributions of taste thresholds of male and female non-elderly adults in Berkeley and

Table III. Taste thresholds for PTC of 210 females classified by age

Age (years)	W-B solution number																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
4-5					3								1				4
6-7			1	1	3	1	1					1	2				10
8-9		1	1		3	1			1		1	1		1			10
10-11		3	4	2	3		2			1							15
12-13		1	2	4	8	5	1	3	1					1			26
14-15	2	1		4	5	4	1	2	3	1			1				24
16-17			1	2	2	4	1		2	1	1		1				15
18-19		1		1	3	1									1		7
20-21							1	1					1				3
22-23																	
24-25																	
26-27																	
28-29																	
30-31						1											1
32-33																	
34-35					1	1											2
36-37				1	1			1			1						4
38-39			1	1	3	6	1			1				1	1		15
40-41		1	3	6	5	11	5	3	3	1		2	2	2	2	1	47
42-43	1	2	1	2	3	2	1		2	1				1	1		17
44-45				1													1
46-47							1										1
48-49																	
50-51																	
52-53																	
54-55																	
56-57																	
58-59																	
60-61																	
62-63					1												1
64-65						1											1
66-67								1									1
68-69				1	1												2
70-71														1			1
72-73				1													1
74-75								1									1
76-77																	
78-79																	
Total	3	10	14	26	45	40	15	11	12	6	3	4	8	8	4	1	210

in London. Table V presents the proportion of tasters and nontasters estimated, as described above, for the offspring and parents by sex.

Comparison of the Berkeley male and female data with the London data

Disregarding the small amount of data available for adults over 60 years of age, it is clear from Table IV that there is very little difference between the threshold means

Table IV. Mean threshold values according to age and sex (thresholds given in Harris and Kalmus numbers)

Year group	N	Mean and SD					
		Males	Females	Both	Males	Females	Both
60–79	7	8	15	5.429 ± 2.936	8.000 ± 3.163	6.800 ± 3.234	
30–53	77	88	165	7.792 ± 3.412	8.034 ± 3.531	7.921 ± 3.468	
4–23	130	114	244	8.523 ± 3.337	8.868 ± 3.082	8.684 ± 3.218	

of the two sexes whether they be adults or children. These means reflect the location of the overall group sensitivities which include both phenotypes and all three postulated genotypes for comparison with the English data. The Berkeley means lie almost one dilution step to the sensitive side of the London means which reflects the lower frequency of insensitive individuals in these data.

Making no assumptions concerning the normality or non-normality of the underlying components of the composite distributions, the Kolmogorov–Smirnov two-sample test (Siegel, 1956) was used to assess the differences between the distributions of the males and females. Neither in adults nor in children was there a statistically significant sex difference ($D = 0.1072$, $P > 0.10$ for adults; $D = 0.0593$, $P > 0.10$ for children).

The female means are indeed consistently higher (lower concentrations of PTC) than the male means. This finding supports those of Harris and Kalmus (1949), Mohr (1952) and Leguebe (1960) in that women seem to be more sensitive to the taste of PTC than men. However, in none of these studies is the sex difference statistically significant.

Influence of age and comparison with London data

A detailed discussion of the influence of age as well as sex on sensitivity to PTC is presented later. First the age findings are compared with those of Harris and Kalmus. In the Berkeley data, the children are more sensitive to PTC than are their parents. This is also true when comparisons are made by sex. As seen in Table IV, the difference between the generations is about seven- or eight-tenths of a dilution step.

The average age of the parental generation is 41.0 years (males 41.9, females 40.3) and of the male and female children, 13.0 years. Calculated from the difference between generations, PTC sensitivity declines at an average rate of 0.027 dilution steps per year or 0.272 per decade. The average age of the entire sample is 25.8 years. The regression coefficient of threshold on age is 0.03 (SE, 0.01). This is a lower value than found by Harris and Kalmus. It is also lower than the 0.05 SD 0.00 decline rate found by Kalmus and Trotter (1962) from retest data on the same individuals after 10–15 years had passed.

If the rate of decline of taste sensitivity is constant from birth to death, the slow rate found in the Berkeley sample requires further scrutiny. Since the number of families is small, the assumption that the offspring genes represent a random sample of the parental gene is tenuous. One reason to reject this simple assumption is the fact that the sibship size does not follow a Poisson distribution. The age effect was therefore examined by comparing individuals of the same genotype assuming a single locus two allele genetic hypothesis. The mean threshold of nontaster (tt) offspring is 2.54 and

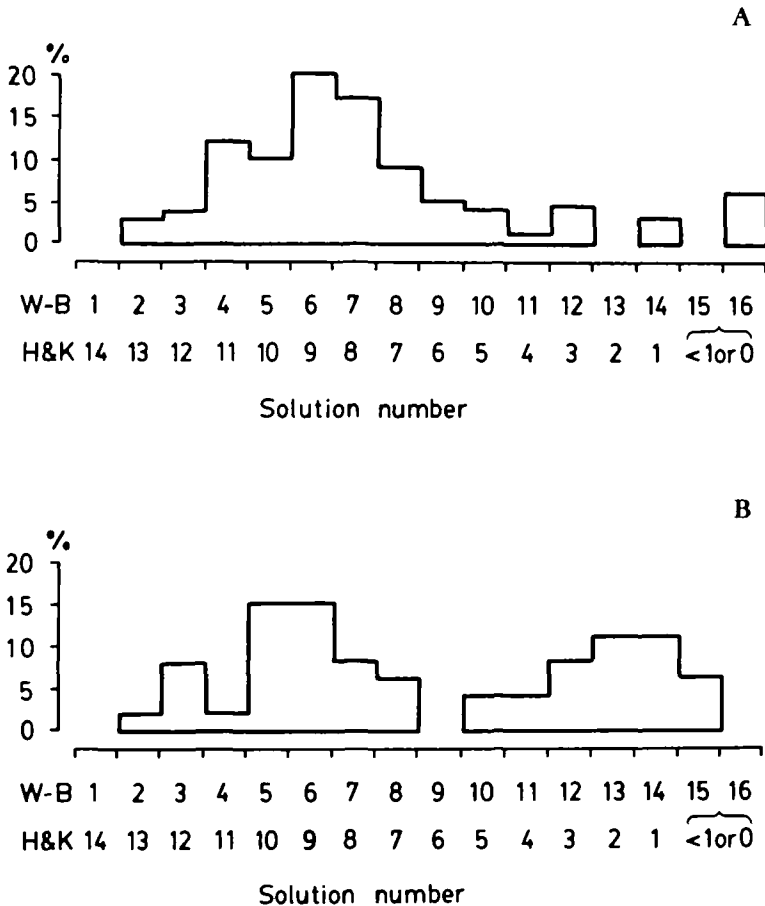


Fig. 2. Distribution of taste thresholds for PTC among males in Berkeley and London data. (a) Seventy-seven Berkeley males between the ages of 35 and 53 years (mean age, 41.883 years). (b) Fifty-two London males between the ages of 35 and 53 years (estimated mean age, 41.827 years).

of nontaster (*tt*) parents 1.37. The mean annual rate of decrease in sensitivity calculated from these data is 0.05 dilution steps, very close to that of the British workers. The mean threshold difference between taster children and taster parents is smaller (0.65) suggesting different proportions of homozygotes and heterozygotes. The findings taken together confirm the general concept of a decline in taster acuity with age and support the findings of other investigators.

Comparison of the Berkeley and London distribution

The distribution of taste thresholds of 77 men and 86 women between the ages of 35 and 53 years are shown in Figures 2 and 3 along with the closest corresponding data plotted from the Harris and Kalmus study.

In both sexes the two sets of data appear bimodally distributed with quite similar locations. The Berkeley distributions lie slightly (0.5 dilution step) to the insensitive

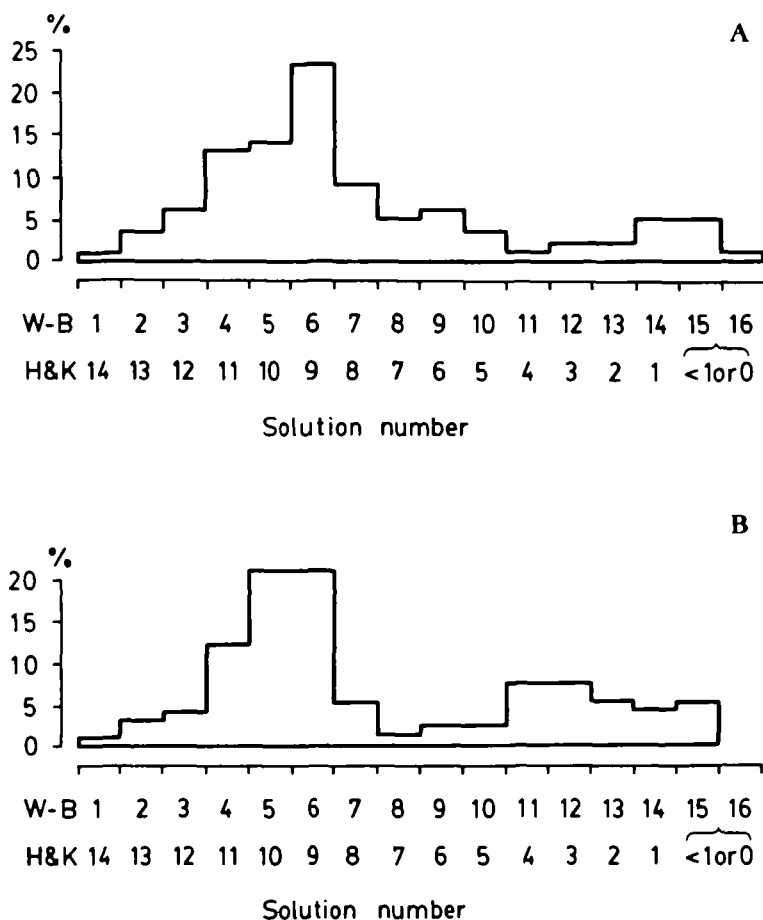


Fig. 3. Distribution of taste thresholds for PTC among females in Berkeley and London data. (a) Eighty-six Berkeley females between the ages of 35 and 53 years (mean age, 40.384 years). (b) One hundred London females between the ages of 20 and 39 years (estimated mean age, 25 years) [redrawn from Harris and Kalmus (1949), Figure 3].

side of the London data. This is opposite to the effect predicted from comparing the two-out-of-five and four-out-of-eight sorting procedures. Although in the female population the shift might be explained as the result of the opposing effects of age and procedure, this is not a satisfactory interpretation since the average ages of the London and the Berkeley males are almost identical and the same shift occurs. In any case, for such small samples as these even a consistent effect would have to be larger than this one to produce acceptably significant differences between the London and Berkeley distributions.

The two independent samples were compared to determine if they could reasonably be accepted as having been drawn from the same population. The Kolmogorov–Smirnov test was used for this purpose. In neither the total male data (441 London males versus 214 Berkeley males: $D = 0.1540$, $P < 0.01$) nor in the 35–53 years old male group

Table V. Proportion of nontasters in two age groups for males and females

Age (years)	Tasters			Nontasters			Total		
	Males	Females	Both	Males	Females	Both	Males	Females	Both
30–53	65	73	138	12 15.58%	15 17.04%	27 16.36%	77	88	165
4–23	109	96	205	21 16.15%	18 13.85%	39 15.98%	130	114	244
Total	174	169	343	33 15.94%	33 16.34%	66 16.14%	207	202	409

(52 London males versus 77 Berkeley males: $D = 0.2609$, $P < 0.05$) could the hypothesis be supported.

For males 10–23 years of age, however, the distributions did not differ significantly (198 London males versus 100 Berkeley males: $D = 0.1034$, $P > 0.10$). This age group is extremely heterogeneous because of large differences in physical and sexual maturity. Their distributions are less clearly bimodal and the variability may have masked differences in the distributions.

For females, the only groups that could be compared were of widely differing ages. The two samples were not distributed in significantly different manners (100 London women versus 88 Berkeley women: $D = 0.1171$, $P > 0.10$).

Comparison of Berkeley and London taster and nontaster frequencies

The point of intersection generated by the method of moments described above is taken to separate tasters from nontasters. The results for comparison with the London data are shown in Table V from which it can be seen that the proportion of nontasters varies little from group to group, ranging from 13.85% among female children to 17.04% among adult women. The differences in numbers of tasters and nontasters among the various age and sex groups were not found to be significant at the 0.01 level using the χ^2 -test.

When these data are compared group by group with those of Harris and Kalmus, however, the differences are highly significant, the proportions in the London data for males ranging from 29 to 34.5% and for females being 32%. χ^2 values and their corresponding probabilities with one degree of freedom comparing the London and Berkeley groups are as follows: males 10–19 years, 4.21, $P < 0.05$; males 20–49 years, 12.16, $P < 0.001$; males 34–53 years, 13.38, $P < 0.001$; all males, 8.17, $P < 0.01$; adult females, 11.00, $P < 0.001$.

The effect of age and sex on the expression and penetrance of taster and nontaster alleles

Figure 4 shows the distributions of discrimination levels for 401 of the 424 participants in the study. The four age groups chosen reflect childhood, adolescence, early adulthood and middle age. Again, bimodality of the underlying data is assumed for each group. Analysis using Pearson's method yielded bimodal distributions in all but one group,

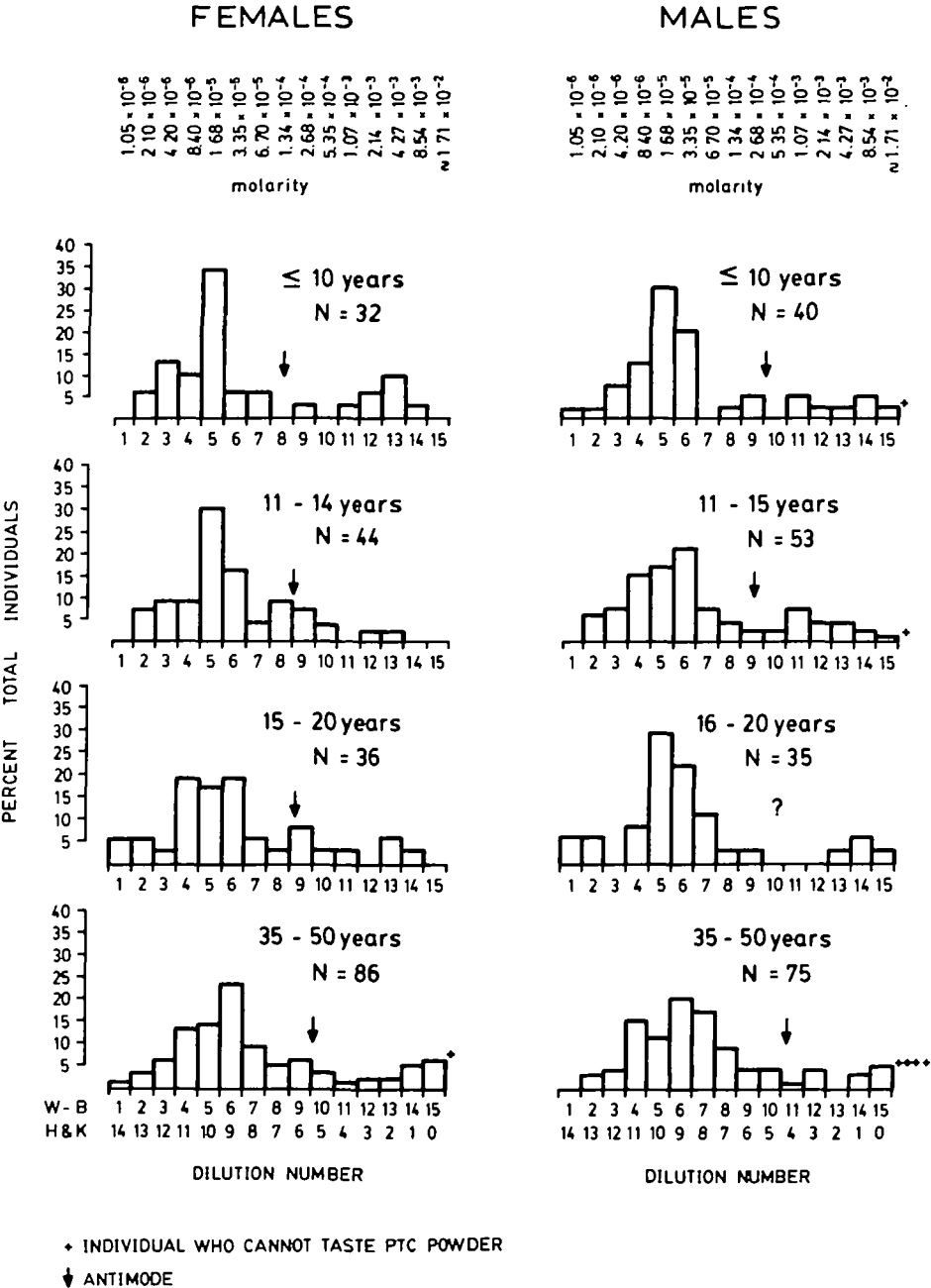


Fig. 4. Distribution of taste thresholds for PTC for four age periods in both sexes.

boys aged 16–20 years, where no solution was possible. The taster and nontaster subgroup means, standard deviations and antimodes are provided in Table VI. The Table shows clearly the increased variance among adolescent nontasters. Only in this group

Table VI. Means and antimodes of threshold distributions of males and females at four age periods

Age (years)	Females			Males		
	Taster mean (SD)	Nontaster mean (SD)	Antimode	Taster mean (SD)	Nontaster mean (SD)	Antimode
10	10.3870 (1.3984)	2.5979 (1.0303)	6.8062	9.9505 (1.6491)	1.8544 (1.6141)	5.4181
10–15 (15)	10.0121 (1.5967)	5.1775 (2.4348)	6.2251	10.0817 (1.5122)	3.6278 (2.2591)	5.8531
14(15)–20	9.9685 (2.0111)	2.9615 (1.4593)	6.1936	—	—	—
35–50	9.4285 (1.8617)	1.4473 (1.4368)	5.3635	9.0442 (1.7989)	1.2628 (1.9056)	4.2728

was the variance for nontasters significantly different from tasters, the probability of the *F*-test falling below 5%.

In Figure 4 the slight increased sensitivity of females is seen in all age groups. Also the shift from childhood to adulthood in the direction of decreased sensitivity is apparent. A fairly constant progression in this direction is seen among the females over the four successive age periods. The pattern is interrupted among the males by a shift of the distribution of adolescent males, ages 11–15 years, to the most sensitive location of the three age groups analysed. These movements of the populations are reflected in Table VI. The means and the antimodes tend to change in the same direction. An exception occurs among the 11- to 14-year-old girls where the proportion of individuals at and around the antimode is increased. This pattern, not seen among adolescent males, may be related to physiological and anatomical heterogeneity in the adolescent girls. Further investigation of this phenomenon is indicated.

The improved sensitivity of males aged 11–15 years over younger aged males parallels that reported by Forrai and Bankovi (1968) for Hungarian schoolboys. The fact that the adolescent females of this study do not show a similar increased sensitivity paralleling that shown in Hungarian girls aged 11–12 years may represent the effect of combining all girls aged 11–14 years because of the small sample size. Analysis of the Hungarian data using the Pearson method suggests a shift of the antimode and the means in opposite directions for girls between the ages of 9 and 13 years and for boys between the ages of 13 and 16 years.

Although a χ^2 -test may not be strictly applicable in this case where the sub-populations are defined from the method of moments and where the age groups are not purely independent, its use did not indicate any significant ($P > 0.05$) variation in the numbers of tasters and nontasters between the various age and sex groups. This has been a consistent finding in investigations where age and sex have been studied regardless of the method used to distinguish tasters and nontasters.

Discussion

Although modification of a procedure is to be avoided, at times it is necessary. In this case, time pressure dictated shortening the Harris and Kalmus technique. This was achieved by renumbering the cups and substituting a two-out-of-five for the original

four-out-of-eight sorting procedure. The use of plastic cups and distilled water as diluent were not expected to have an effect on the results.

To determine what effect substitution of a two-out-of-five test might have, 22 control subjects were tested using both sorting procedures. Regardless of the order in which the tests were given, the two-out-of-five test consistently gave results about one-half step to the sensitive side of the four-out-of-eight test.

The overall data as well as the data grouped by age and sex show the Berkeley samples to be slightly less sensitive than the London samples. This may be the result of ingesting the stimulus but no data are available to determine if this is true.

That the slight decrease in sensitivity could be caused by unsuspected taste pathology is possible. However, the participants all responded negatively to the question, 'Do you have any trouble with your sense of taste?' None wore dental plates, oral hygiene was good, no one suffered from known severe liver disease, active Tbc, Bell's palsey or pernicious anemia. One participant was pregnant. Also there is no reason to believe that in this educated, medically well-cared-for group there was more taste pathology than among the London students, staff and seniors with whom these data are compared.

The major and significant difference between the two sets of data rests in the decrease in frequency of Berkeley nontasters to about half the frequency found in the London data. The conclusion is that although some variability in results may stem from procedure variability, the significant differences in phenotypic frequencies are real. Lawless (1980) compared several methods of testing the bitter taste modality using PTC and prophylthiouracil (PROP). He found no clearly best method and stated that 'the agreement . . . attests to the robust nature of the PTC dimorphism.' He also found that for distinguishing tasters and nontasters PTC was superior to PROP.

Although for an American sample these individuals are very homogeneous as far as race, socioeconomic status, education and intelligence are concerned, they do stem mostly from various Western European areas. Studies in diverse areas of England and Wales show wide differences in the frequency of nontasters. For example, in South Cumberland the frequency of nontasters is 18% while in Tenby, Wales, it is 35% (Sunderland and Cartwright, 1974).

The data reported here are taken to demonstrate that the frequency of nontasters is significantly lower in this Berkeley sample. Confidence that the modifications made to the Harris and Kalms procedure have not seriously affected the measurement of taste sensitivity and phenotypic classification is bolstered by the fact that genetic analysis of families using the classification criteria developed in this analysis has led to an excellent fit of the data to the genetic hypothesis advanced by Rao and Morton (1977). That analysis is communicated in a separate report.

The influence of age and sex on the sensitivity to PTC found in this and other studies shows males to be slightly but not significantly less sensitive than females and that sensitivity decreases with age.

Because of these effects it is necessary to develop age- and sex-specific criteria in populations on whom one wishes to do family studies of taste sensitivity. In the course of developing these criteria it became apparent that the findings were important to the question of expression and penetrance of the PTC alleles.

From extensive data gathered over the years, a consensus has developed that ability

to taste PTC depends on genes with decreased penetrance and variable expression but there is lack of consistency in the use of these terms.

Penetrance of a gene or genotype refers to the relative incidence with which the trait is expressed in a genotype. Theoretically, penetrance can vary from zero in which case the gene or genotype, never evidencing the trait, goes unrecognized, to one in which the gene or genotype, constantly producing the trait, is always recognized. The term penetrance is generally reserved to describe instances falling measurably between the two extremes and the phenomenon is then referred to as incomplete or decreased penetrance.

Expression refers to the variability in the manifestation of the trait associated with either a fully or an incompletely penetrant gene or genotype. The gene may be expressed either as a uniform or as a variable phenotype over all individuals. In the first situation the gene is said to show uniform expression and in the second to show variable expression. Variability, of course, can range from mild to extreme.

Accepting 'tasting' as a dominant trait and 'nontasting' as a recessive trait, consideration of the data presented here has led to the following formalization.

Penetrance refers to the proportion of genotypes correctly classified into taster and nontaster phenotypes. Expression refers to the range of taste sensitivity within the phenotypes. Both the *t* allele and the *T* allele display incomplete penetrance and variable expression.

The penetrance of the *T* allele is incomplete by the proportion of the taster population which lies at the insensitive end of the taster distribution to the insensitive side of the antimode, while the penetrance of the *t* allele is decreased by that proportion of the nontaster population which lies at the sensitive end of the nontaster distribution to the sensitive side of the antimode. The degree of nonpenetrance depends upon the relative means of the taster and nontaster distributions, their variances and their relative proportions. Calculated from *F*-values of *z* scores the penetrance of the taster allele never fell below 97% in any group. For the nontaster allele penetrance exceeded 94% with the exception of the adolescents among whom the variance is significantly increased over taster adolescents. In these heterogeneous groups, penetrance of the nontaster allele is decreased to 66% among the girls and 83% among the boys.

Disregarding the adolescent data, Figure 4 and Table VI strongly suggest that for a population these parameters are fixed for each sex and do not change significantly with age. In other words, the location of the bimodal distribution shifts along the dilution axis with time but the shape of the distribution is not significantly altered and therefore the penetrance of the genes remains fairly stable from birth to death. Disturbances in this relationship at puberty require further investigation. Differential mortality would be expected to produce a change in the pattern, particularly in older age groups.

The discrimination level, or phenotypic expression, within the taster and nontaster groups is clearly variable and is affected by both age and sex. The variability in expression seems fairly stable within any age and sex group, again with the exception of adolescence. The variability could be the result of variation in genetic background, environmental circumstances and chance occurrences, as well as variation in interactions among these three components. A series of multiple taster and nontaster alleles at the single locus could explain a large proportion of the stable variation.

Within-person variability has been extensively documented. Twin studies (Dencker *et al.*, 1959; Martin, 1975) have repeatedly shown variability among monozygotic twin pairs. Repeat examinations of taste thresholds on the same individuals have been carried out in this study and others, and show an average difference of about one dilution step although differences of as much as six, seven or eight steps have been found in a few individuals. Besides the known effect of age on the within-person variability (Kalmus and Trotter, 1962), many other factors have been found to produce effects on individual as well as group acuity levels. Among these are the effects of smoking just prior to testing (Hall and Blakeslee, 1945), mental status (Freire-Maia *et al.*, 1968), ingestion of chemicals (Henkin *et al.*, 1963; Sunderland and Cartwright, 1968), radiation (Kalmus and Farnsworth, 1951; Conger and Wells, 1969), and metabolic disturbances (Kalmus and Trotter, 1962; Sriram *et al.*, 1975).

These rather striking and often transient effects of environmental agents on the expression of the PTC tasting phenotype must also affect the penetrance of the genotypes in some individuals, especially if their thresholds lie in the intermediate range close to the antimode. Longitudinal studies of singletons and of twins would help provide insight into the mechanisms behind both decreased penetrance and variable expression of this trait. It is possible that puberty affects both expression and penetrance and the effect, if any, on penetrance may be transient.

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