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The genetics of tasting in mice

V. Glycine and cycloheximide

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Summary

Glycine tastes both bitter and sweet to mice but there are differences between strains in their ability to detect each taste. With respect to the bitter taste, fifteen strains were classified as tasters and twelve strains as non-tasters. The difference is due to a single gene, *Glb* (glycine bitterness). Cycloheximide tastes bitter to all mice at a concentration of 8 μM, but strain differences in sensitivity to the taste of cycloheximide can be detected at lower concentrations. The BXD RI strains can be classified into two groups with respect to sensitivity to cycloheximide. This is probably due to the segregation of two alleles of a single gene, *Cyx*. A comparison of the distribution in RI strains of alleles of four bitterness-tasting genes shows that the loci are all closely linked and are probably in the order *Cyx–Qui–Rua–Glb*.

1. Introduction

In some preliminary experiments on the ability of mice to taste sweet substances several strains were tested with solutions of glycine. Some strains preferred glycine to water, but other strains showed a strong aversion to drinking the glycine. This aversion came as a surprise because glycine has not been recorded as having any unpleasant taste to humans. On the contrary, it is said to have a very pure sweet taste without any bitter component (Bekesy, 1964). The genetic basis of the aversion to glycine was therefore investigated and the results are presented in this paper.

We have found that cycloheximide, which is known to taste fairly bitter to humans and very bitter to rats (Tobach *et al.* 1974), is also very bitter to mice. There are differences between strains of mice in their sensitivity to the taste of cycloheximide. These differences are sufficiently large to allow a genetic analysis using recombinant inbred (RI) strains. The sensitivities to the bitter taste of glycine and to the taste of cycloheximide are determined by two different genes, but they are treated together here because it was found that both are members of a cluster of bitterness-tasting genes which includes *Qui* and *Rua* (Lush, 1984, 1986*a*).

2. Materials and methods

The strains of mice are those which were used previously (Lush, 1986*a* & 1986*b*) with the addition of the CXS set of recombinant inbred (RI) strains developed by Dr J. Hilgers (The Netherlands Cancer Institute, Amsterdam) from founder strains BALB/cA and STS. The −/By substrains are those used by Dr D. Bailey to found the CXB RI strains. The −/Ty substrains are those used by Dr B. A. Taylor to found the BXD RI strains. The −/Pas substrains came from the Pasteur Institute, Paris. The −/Gr substrains were established at this laboratory by the late Professor Gruneberg.

The taste testing technique has been described in detail (Lush, 1984). The glycine and cycloheximide both came from Sigma and were of the highest purity available. They were dissolved in distilled water and used at the concentrations stated.

3. Results

(i) *Glycine*

All twenty-seven strains were tested with 10 mM glycine as tastant. The results are shown in Table 1 and in Fig. 1A. One group of fifteen strains strongly avoided drinking glycine and had a mean consumption of 6·7%. The other twelve strains formed a group with a mean consumption of 45% and therefore showed only a very slight tendency to avoid the glycine. These two groups will be referred to as 'tasters' and 'non-tasters' respectively. Four taster and four non-taster strains were tested with a range of concentrations in order to obtain a more complete picture of their tasting abilities. They were tested at five concentrations, covering a thousand-fold range, and the results are shown in Fig. 2. The lowest concentration (0·1 mM)

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Table 1. *Consumption of glycine and cycloheximide by mice from twenty-seven strains: each cage contained up to four mice*

	Tastant			
	Glycine (10 mM)		Cycloheximide (1·0 μM)	
Strain	Cages tested	Mean tastant consumed (%)	Cages tested	Mean tastant consumed (%)
TO	6	65	2	19
AU	4	54	2	38
129/Sv	4	53	4	32
STS	2	46	6	28
C57BL/10	2	46	2	42
129/Rr	2	45	3	41
SM	2	45	2	12
C57BL/6By	6	42	5	46
C57L	2	41	2	39
C57BL/6Ty	4	39	8	22
IS/Cam	4	36	4	11
NMRI	4	35	3	13
DBA/1	4	14	4	28
Schneider	4	13	2	54
DBA/2Pas	4	11	6	26
CE	2	8	4	21
A/J	2	7	2	7
CBA/Ca	2	7	2	13
AKR	2	7	3	8
SEA	2	6	2	8
SWR	5	5	4	36
A2G	2	5	3	9
ST/bJ	2	5	4	39
C3H/He	2	4	2	13
DBA/2Ty	2	3	6	29
BALB/cBy	2	3	3	5
BALB/cA	2	2	4	9

appeared to be tasteless to all strains but at 1·0 mM SWR and BALB/c showed some aversion. At 10 mM the distinction between tasters and non-tasters was clear. At higher concentrations this distinction began to be obscured by the increasing attractiveness of glycine to some strains, possibly due to the sweetness of the molecule becoming detectable. The two C57BL substrains showed a strong liking for the higher concentrations of glycine. Strain TO also began to show a preference for glycine as the concentration increased, but the change was more gradual. Strain 129/Sv, which was a non-taster at 10 mM, remained a non-taster at 100 mM. With Schneider and SWR the avoidance of glycine at 10 mM was progressively reduced as the concentration rose to 50 mM and then to 100 mM. Indeed, at 100 mM the Schneider mice had overcome their aversion and showed a slight preference for glycine. BALB/cBy and DBA/2Ty showed no lessening of their avoidance of glycine at the higher concentrations.

The concentration-response curves in Fig. 2 seem to show that the taste of glycine to mice has two components, one unpleasant and the other pleasant (probably sweetness). BALB/cBy and DBA/2 mice can detect the unpleasant taste, C57BL/6 and TO can detect the pleasant taste, Schneider and SWR can detect both tastes, 129/Sv can detect neither taste. It was therefore decided to concentrate on the unpleasant taste revealed by the use of 10 mM glycine and postpone further work on sweetness to a later paper.

The clear separation of the twenty-seven inbred strains into tasters and non-tasters with 10 mM glycine argues for the existence of one gene with a major effect. This hypothesis was tested by a backcross as follows. Four (BALB/cBy × 129/Sv)F_1 mice were tested with 50 mM glycine and were found to be tasters. The concentration of 50 mM was chosen because experience has shown (Lush, 1982) that dominance of one tasting allele is not always complete at low concentrations. Two F_1 males were then backcrossed to 129/Sv females and thirty of their progeny were tested with 50 mM glycine. The results are given in Fig. 1B and show a segregation into nine tasters and thirteen non-tasters. Some of the non-tasters actually seemed to have a preference for glycine, which was unexpected and is not easily explained since neither 129/Sv nor BALB/cBy showed any sign of sensitivity to the sweetness of glycine. Further confirmation of the single-gene hypothesis came from the use of the three sets of RI strains. In each of the RI sets one founder strain was a taster and the other was a non-taster. The taster strains were BALB/cBy, DBA/2Ty and BALB/cA. The non-taster strains were C57BL/6By, C57BL/6Ty and STS. On the single-gene hypothesis the RI strains should segregate into tasters and non-tasters in approximately equal numbers. The results given in Table 2 and in Fig. 1C, D and E show that this expectation was fulfilled. Twenty RI strains were tasters and 18 were non-tasters. The symbol *Glb* (glycine bitterness) with alleles Glb^a (taster) and Glb^b (non-taster) are proposed for this gene.

(ii) *Cycloheximide*

All twenty-seven strains were tested with 1·0 μM cycloheximide as tastant. The results are shown in Table 1 and in Fig. 1F. At this concentration the strains showed wide variation but it was fairly continuous and without any obvious grouping. A survey of the BXD RI strains with 1·0 μM cycloheximide gave the results shown in Fig. 1G. Twelve strains formed a compact group with a mean of 41%. The other nine strains formed a less compact group which showed a greater degree of avoidance and had a mean of 18%. In order to assess more clearly the degree of separateness of these two groups, the concentration-response curves of all the BXD RI strains were obtained from 0·25 to 8·0 μM. These curves are shown in Fig. 3A where it can be seen that

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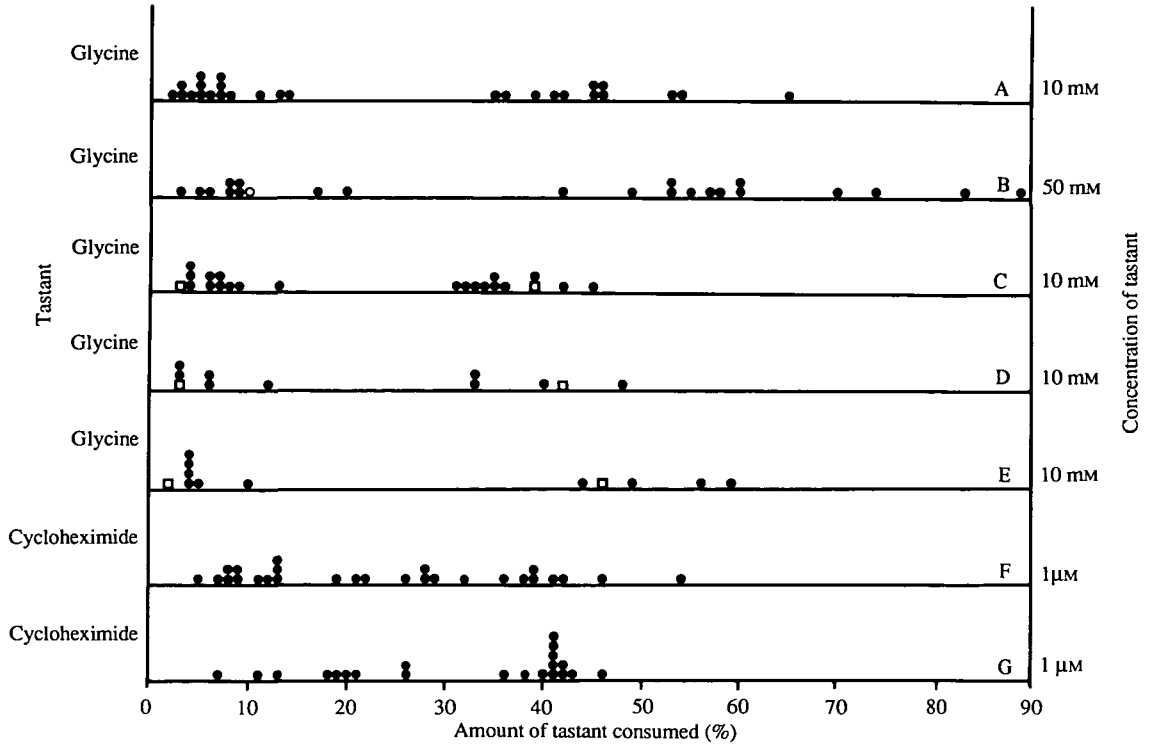

Fig. 1. Consumption of glycine and cycloheximide. Each symbol is the mean value of a strain except in (B), where the filled symbols are the 22 individual progeny from the cross (BALB/cBy × 129/Sv) × 129/Sv, and the empty symbol, ○, is the mean of the two F_1 males used in this cross. (A) and (F) display the data in Table 1 on the consumption by the 27 inbred strains of glycine and cycloheximide respectively. (C–E) display the data in Table 2 on the consumption of glycine by the BXD, CXB and CXS RI strains respectively. In each of these the square symbols are the values (taken from Table 1) for the two founder strains. (G) shows the consumption of 1 μM cycloheximide by the BXD RI strains (see also Fig. 3A), each point being the mean of between two and ten tests.

Table 2. *Consumption of 10 mM glycine by three sets of RI strains*

	Cages tested	Mean tastant consumed (% ± S.E.M.)	SDP		Cages tested	Mean tastant consumed (% ± S.E.M.)	SDP
BXD strains				CXB strains			
1	3	6 ± 0·7	D	D	3	33 ± 4·6	B
2	5	32 ± 6·0	B	E	6	33 ± 3·5	B
5	3	8 ± 4·4	D	G	3	3 ± 1·2	C
6	3	34 ± 5·0	B	H	3	6 ± 5·0	C
8	7	13 ± 2·1	D	I	3	3 ± 2·0	C
11	3	7 ± 2·3	D	J	4	48 ± 1·3	B
12	7	31 ± 3·0	B	K	3	6 ± 0·7	C
15	6	4 ± 1·4	D	P	3	40 ± 2·1	B
16	4	33 ± 4·0	B	Q	10	12 ± 3·0	C
18	4	45 ± 5·6	B				
19	3	7 ± 2·1	D	CXS strains			
22	3	42 ± 2·4	B	1	3	59 ± 6·4	S
24	3	4 ± 1·6	D	2	5	56 ± 3·6	S
25	3	9 ± 3·0	D	3	3	4 ± 0·9	C
27	3	35 ± 7·4	B	5	4	10 ± 4·3	C
28	3	36 ± 2·6	B	7	3	4 ± 1·3	C
29	3	4 ± 0·3	D	8	3	44 ± 13·1	S
30	3	35 ± 5·6	B	9	3	4 ± 1·3	C
31	3	39 ± 5·8	B	10	4	4 ± 0·4	C
32	3	6 ± 2·2	D	12	3	5 ± 1·7	C
				14	3	49 ± 12·7	S

The strain distribution pattern (SDP) symbols are as follows: B = like C57BL, C = like BALB/c, D = like DBA/2, S = like STS.

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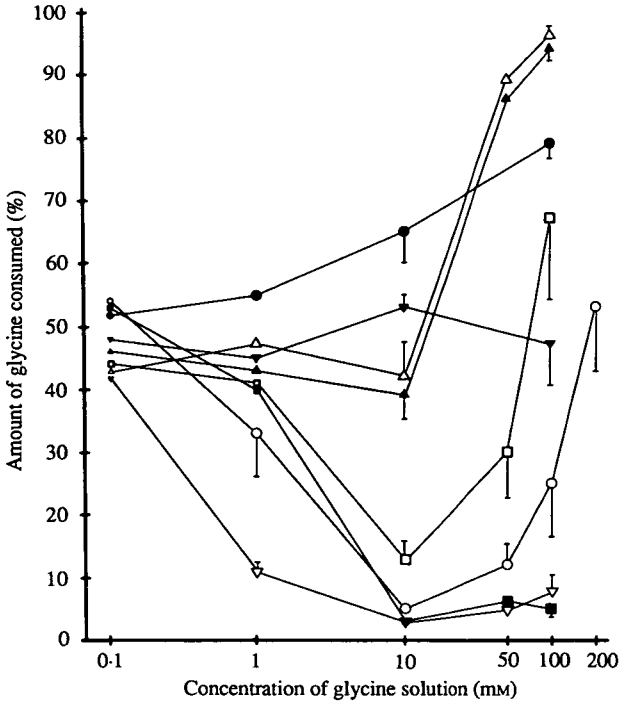

Fig. 2. Concentration–response curves of eight strains tested with glycine. △, C57BL/6By; ▲, C57BL/6Ty; ●, TO; ▼, 129/Sv; □, Schneider; ○, SWR; ■, DBA/2Ty; ▽, BALB/cBy. Each point is the mean of between two and eight tests. The vertical bars are SEMs.

the two groups of curves do remain separate over quite a wide range of concentrations (the reason for the two heavily-drawn curves will be explained later).

Can these two groups be regarded as evidence of segregation of a single gene which has a major effect on cycloheximide tasting? There is one puzzling aspect of the data which must be considered before answering this question. If the two groups do indeed represent the segregation of RI strains which are like C57BL/6Ty from those that are like DBA/2Ty, the concentration–response curves of the two founder strains should differ in the same way as the RI strains. But in fact the two founder strains have concentration–response curves which are rather similar to each other, as shown in Figs 3B and C. One possible explanation of this anomaly is that one of the two founder strains has undergone a mutational change since the RI strains were established, with the result that the original difference between them has been lost. It is difficult to see how such a mutation could be proved without having a detailed knowledge of the gene which has mutated, but circumstantial evidence might be obtained by comparing a number of substrains within C57BL/6 and DBA/2. The argument is as follows. Substrains within C57BL/6 or DBA/2 or BALB/c are nearly always found to be identical in their tasting abilities, as indeed they are in other characteristics. Therefore if, for example, C57BL/6Ty now differs from all other C57BL/6 substrains in its cycloheximide tasting ability, presumably C56BL/6Ty has changed and the other substrains have remained stable. We have been able to test four C57BL/6 substrains, four BALB/c substrains and two DBA/2 substrains and the results are shown in Fig. 3B, C and D. Both DBA/2 substrains have similar curves and the four BALB/c substrains are also very similar to each other, but there is considerable variation between the C57BL/6 substrains. Thus C57BL/6Pas, C57BL/6Gr and C56BL/6By all gave different curves, which is the opposite to what was expected on the basis of the argument outlined above. We will return to the interpretation of the cycloheximide data in the next section.

(iii) *The bitterness-gene cluster*

In the previous paper (Lush, 1986*a*) it was shown that the strain distribution patterns of *Qui* and *Rua* are identical in the nine CXB RI strains and in the 14 BXD RI strains which were then available in this laboratory. This led to the conclusion that the two loci are closely linked. We have now typed seven more BXD strains and the results for all 21 BXD strains are given in Table 3. Only in BXD15 has a crossover occurred between *Qui* and *Rua*. The occurrence of one crossover in 30 RI strains gives an estimate of 0·88 cM (95% confidence limits 0·02–5·80) for the distance between these two loci (Silver, 1985). The SDP for *Glb* in both sets of RI strains is included in Table 3 (with the omission of BXD9 which was no longer available to us) and it can be seen that it differs from *Qui* in BXD15 and BXD8, and from *Rua* only in BXD8. This indicates that *Glb* is closely linked to the other two genes. The probable order is that which requires the fewest crossovers and is *Qui–Rua–Glb*. The estimated distance between *Rua* and *Glb* is 0·91 cM (95% confidence limits 0·02–6·05).

We are now in a position to look again at the cycloheximide data in Fig. 3. Let us first consider the concentration-response curve of DBA/2Ty in Fig. 3C. Although it does not coincide precisely with either of the two groups of BXD strains shown in Fig. 3A, it is closer to the lower group than to the upper group. This lower group comprises BXD1, 2, 5, 9, 11, 19, 24, 29 and 32. If these nine strains are therefore classified as DBA/2-like with respect to a hypothetical gene *Cyx*, it can be seen in Table 3 that nearly all these strains are also DBA/2-like with respect to *Qui*. The only discordant strain is BXD2, which is the strain with the heavily drawn curve in the lower group in Fig. 3A. If the upper group of twelve strains in Fig. 3A is classified as C57BL/6-like, there is again excellent agreement with the classification of the same strains with respect to *Qui*. The only discordant strain is BXD25, which is the strain with the heavily drawn curve in the upper group in Fig. 3A. The cycloheximide data from the two groups of BXD RI strains are therefore exactly what one would expect if the

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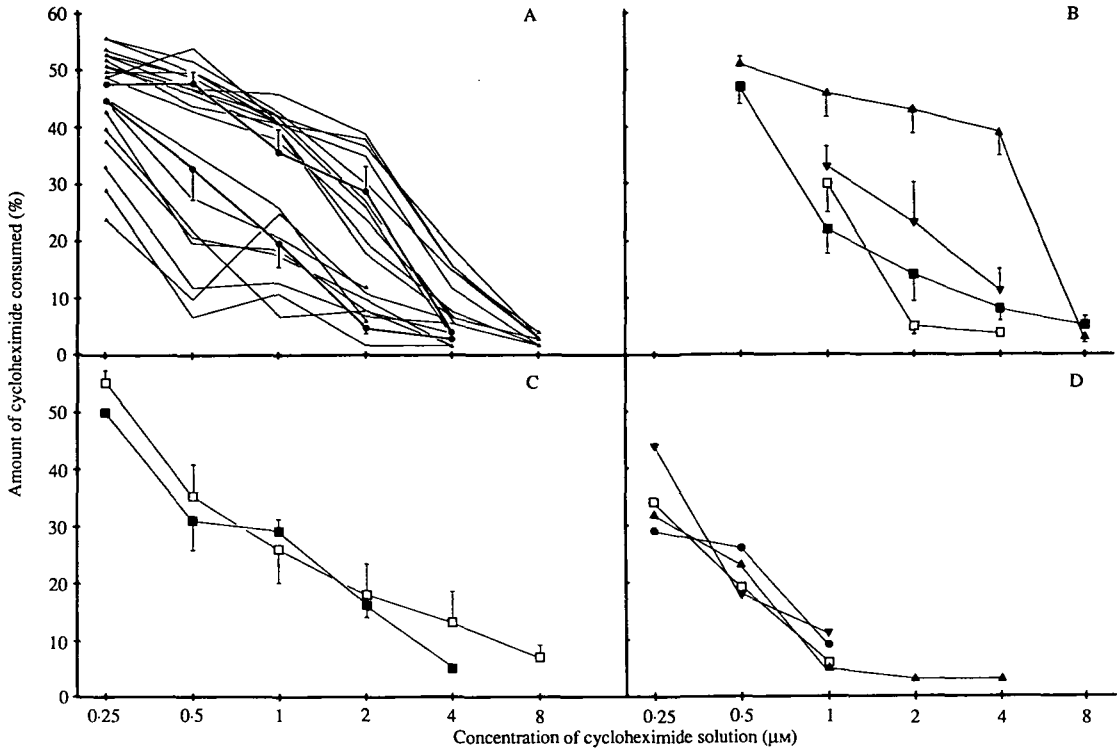

Fig. 3. Concentration–response curves with cycloheximide of (A) BXD RI strains, (B) C57BL/6 substrains, (C) DBA/2 substrains, (D) BALB/c substrains. The substrain symbols are as follows: ▲, By; ▼, Gr; ■, Ty; □, Pas. Each point is the mean of between two and eight tests. Vertical bars are S.E.M.S.

Table 3. *A comparison of the RI strain distribution patterns of the four bitterness-tasting genes. X indicates crossover regions*

	BXD RI strains																				
Gene	1	2	5	6	8	9	11	12	15	16	18	19	22	24	25	27	28	29	30	31	32
Cyx	D	D ×	D	B	B	D	D	B	B	B	B	D	B	D	B ×	B	B	D	B	B	D
Qui	D	B	D	B	B	D	D	B	B ×	B	B	D	B	D	D	B	B	D	B	B	D
Rua	D	B	D	B	B ×	D	D	B	D	B	B	D	B	D	D	B	B	D	B	B	D
Glb	D	B	D	B	D	—	D	B	D	B	B	D	B	D	D	B	B	D	B	B	D

	CXB RI strains								
Gene	D	E	G	H	I	J	K	P	Q
Qui	B	B	C	C	C	B	C	B	C
Rua	B	B	C	C	C	B	C	B	C
Glb	B	B	C	C	C	B	C	B	C

strains were segregating with respect to a gene which has an effect on cycloheximide tasting and which is closely linked to *Qui*, but located on the other side from *Rua* and *Glb*. Any other order of the four genes would require more crossovers. The distance between *Cyx* and *Qui*, calculated from two crossovers in twenty-one RI strains is 2·78 cM (95% confidence limits 0·30–13·95).

4. Discussion

It will be convenient to use the term 'bitterness' to describe the unpleasant taste which taster mice can detect in 10 mM glycine, although we have no direct evidence that this is correct. In previous work (Lush 1981, 1982, 1984, 1986*a*) with substances which are intensely bitter to humans i.e. sucrose acetate, strych-

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nine, quinine and raffinose acetate, it seemed reasonable to assume that when mice show an aversion to these substances they also experience a sensation which can be called bitterness. The situation with glycine is different because it does not taste bitter to humans. It would be unsound to assume that bitterness is the only taste sensation that can cause a mouse to avoid drinking a solution. Nevertheless, the fact that the *Glb* gene is so closely linked to the tasting genes for three bitter substances does support the idea that the whole cluster has arisen from a single, original, bitterness-tasting gene by repeated local duplication and all the present members of the cluster still have the same basic function.

Although the BXD RI data for *Cyx* fit neatly into the pattern that one would expect for a single gene closely linked to the others in the cluster, it must be admitted that we have no convincing explanation for the fact that the RI group which is supposed to be C57BL/6-like in its response to cycloheximide is not very like C57BL/6Ty or any of the other C57BL/6 substrains, as shown in Fig. 3A and B. The close similarity between C57BL/6Ty and C57BL/6By in their response to glycine (see Fig. 2) serves to emphasise the great difference in their responses to cycloheximide. One can only offer the rather unsatisfactory statement that an exceptional amount of variation seems to have been recently introduced into the C57BL/6 substrains in their response to cycloheximide. How this happened is at present unknown.

The bitterness gene cluster probably contains many more genes than the four which have been identified. This conclusion seems unavoidable when one considers that there are hundreds of other chemical substances which are bitter to humans, and probably also to mice. The gene *Soa* may also be a member of the cluster, but a previous attempt to prove this gave an ambiguous result (Lush, 1984). The bitterness gene cluster is closely linked to the *Prp* genes which determine the proline-rich proteins of the saliva (Azen, Lush & Taylor, 1986). The *Prp* genes were assigned to chromosome 8 on the basis of results with mouse × hamster somatic cell hybrids (Azen *et al.* 1984) but new linkage data indicate that they are on chromosome 6 (Azen, personal communication). The homologous human *Prp* genes are known to be on the short arm of chromosome 12 (Mamula *et al.* 1985), and there are several other mouse genes on chromosome 6 which have their human homologues on chromosome 12p (Searle *et al.* 1987).

The very positive reaction of strains such as C57BL/6 to the sweet taste of glycine suggests that the simple behavioural technique which has been so productive in the analysis of the genetics of bitterness in mice may be equally applicable to sweetness. Work has already begun with some intensely sweet substances and the results will be presented in a later paper in this series.

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References

Azen, E. A., Carlson, D. M., Clements, S., Lalley, P. A. & Vanin, E. (1984). Salivary proline-rich protein genes on chromosome 8. *Science* **226**, 967–969.

Azen, E. A., Lush, I. E. & Taylor, B. A. (1986). Close linkage of mouse genes for salivary proline-rich proteins (PRPs) and taste. *Trends in Genetics* **2**, 199–200.

Békésy, G. von (1964). Sweetness produced electrically on the tongue and its relation to taste theories. *Journal of Applied Physiology* **19**, 1105–1113.

Lush, I. E. (1981). The genetics of tasting in mice I. Sucrose octaacetate. *Genetical Research* **38**, 93–95.

Lush, I. E. (1982). The genetics of tasting in mice II. Strychnine. *Chemical Senses* **7**, 93–98.

Lush, I. E. (1984). The genetics of tasting in mice III. Quinine. *Genetical Research* **44**, 151–160.

Lush, I. E. (1986*a*). The genetics of tasting in mice. IV. The acetates of raffinose, galactose and β-lactose. *Genetical Research* **47**, 117–123.

Lush, I. E. (1986*b*). Differences between mouse strains in their consumption of phenylthiourea (PTC). *Heredity* **57**, 319–323.

Mamula, P. W., Heerema, N. A., Palmer, C. G., Lyons, K. M. & Karn, R. C. (1985). Localization of the human salivary protein complex (SPC) to chromosome band 12p13.2. *Cytogenetics and Cell Genetics* **39**, 279–284.

Searle, A. G., Peters, J., Lyon, M. F., Evans, E. P., Edwards, J. H. & Buckle, V. T. (1987). Chromosome maps of man and mouse, III. *Genomics* **1**, 3–18.

Silver, J. (1985). Confidence limits for estimates of gene linkage based on analysis of recombinant inbred strains. *Journal of Heredity* **76**, 436–440.

Tobach, E., Bellin, J. S. & Das, D. K. (1974). Differences in bitter taste perception in three strains of rats. *Behaviour Genetics* **4**, 405–410.

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