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The genetics of tasting in mice

III. Quinine

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SUMMARY

Twenty-nine strains of mice were tested for their ability to taste a 0·8 mM solution of quinine sulphate. There were large strain differences, some strains (tasters) showing a strong aversion to the quinine and other strains (non-tasters) showing very little aversion. It was shown that the difference between strains 129/Sv and A2G is probably due to one gene. By using the CXB RI strains it was also shown that the difference between C57BL/6By and BALB/cBy is probably due to one gene. It is suggested that both differences may be due to the same gene, named *Qui*. In the progeny of a backcross involving both *Soa* and *Qui* there was evidence of an interaction between the genes which cannot be explained satisfactorily. Learning behaviour by the mice influenced their drinking habits, but this did not invalidate the results.

1. INTRODUCTION

In the first two papers of this series (Lush 1981 and 1982) it was shown that the gene *Soa* determines the ability of a mouse to taste sucrose octa-acetate (SOA) and also the sensitivity of a mouse to the taste of strychnine. Both these substances are extremely bitter to humans. Mice homozygous for the allele Soa^a show an aversion to drinking SOA in aqueous solution at concentrations higher than about 1 μM (Warren & Lewis, 1970); they also show an aversion to drinking solutions of strychnine at concentrations higher than about 10 μM. But mice which are homozygous for the allele Soa^b show no aversion to drinking SOA even at the highest concentration tested (1 mM) and show an aversion to strychnine only at concentrations higher than about 100–200 μM.

In the work described here, the ability of mice to taste another very bitter substance, quinine, was studied. The results showed that this ability is determined by a gene which is different from *Soa*. The two genes are probably not linked, but they interact in a way that is at present difficult to understand.

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2. MATERIALS AND METHODS

The laboratories of origin of most of the strains of mice were given in previous papers (Lush, 1981, 1982). The three additional strains were TO and Schneider (from Imperial Cancer Research Fund, London) and A (this laboratory). The quinine hemi-sulphate and sucrose octa-acetate (SOA) came from Sigma Ltd.

The experimental procedure was as previously described except that quinine sulphate was dissolved in distilled water in place of tap water. The SOA was dissolved in a small volume of ethanol and then diluted in distilled water (Lush, 1981). During the experiments the mice were housed in cages identical to those in which they normally lived. The plan of one such cage is shown in Fig. 1. For

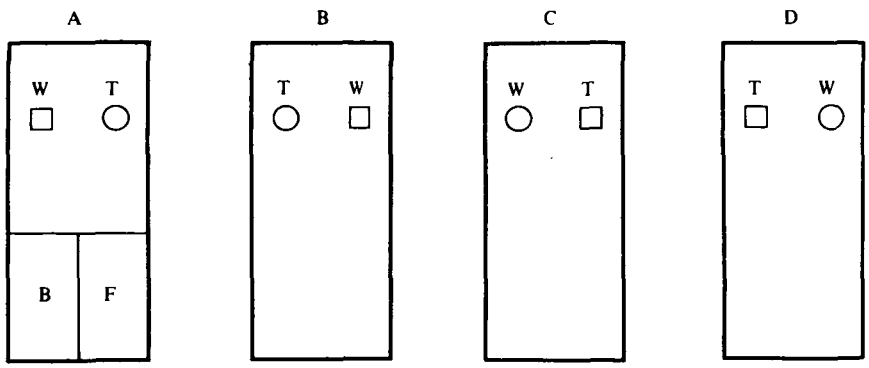

Fig. 1. (A) Plan and dimensions (30 cm × 13 cm) of mouse cage used for tasting experiments, showing the positions of the food hopper, F; the water bottle, B; and the positions of the burettes containing either water, W, or tastant, T. No water bottle was present during experiments. (A–D) The sequence of positions and contents of the two burettes during the 4-day schedule. One burette is represented as a square to distinguish it from the other.

the experiments the water bottle was removed and two open-ended metal drinking spouts connected to burettes were introduced through the top of each cage. One burette contained tastant solution and the other contained either distilled water alone or water and ethanol. All solutions were freshly made up for the first and third day of each experiment. During the normal 4-day schedule the positions and contents of the burette-spout units were rotated as shown in Fig. 1. This procedure was necessary in order to control for any preference shown by the mice to drink from one particular spout or to drink at one particular position. After each day, the volumes of tastant solution and of water consumed were read from the burettes and the amount of tastant solution was calculated as a percentage of total fluid intake on that day. The degree of aversion to the tastant solution was expressed as a mean percentage over the 4 days of the schedule. When mice were re-tested with the same or a different tastant there was a period of at least 7 days between the tests. It is an assumption underlying this work that the intensity of the bitterness perceived by the mice in a tastant solution is reflected in the degree to which they avoid drinking that solution. Thus a mean value of 50 % signified that

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the mouse (or mice) in the cage had shown no aversion to the tastant and therefore probably could not taste it. Both sexes were used since there was no detectable sex difference in tasting ability.

3. RESULTS

(i) *Strain survey*

An initial survey of twenty-nine strains using 0·8 mM quinine as the tastant produced the data shown in Table 1. There was considerable strain variation in the degree of aversion to quinine at this concentration. The strains appeared to

Table 1. *Consumption of 0·8 mM quinine solution by mice from 29 strains. Each cage contained between 2 and 4 mice*

Strain	Cages tested	Mean quinine consumed (%)	Strain	Cages tested	Mean quinine consumed (%)
A2G	2	43	P/J	4	21
DBA/1	2	41	Au	4	19
SEA	2	40	C57BL/6 Past	4	13
A	3	39	TO	4	10
ST	3	36	SM/J	5	9
DBA/2	4	36	CE	4	7
C3H	4	35	Simpson	2	6
NZB	2	34	NMRI	4	6
BALB/cBy	2	31	Schneider	4	5
CBA	4	29	129/Sv	2	5
C57L	4	28	Is/Cam	3	4
BALB/c Past	5	27	C57BL/6By	2	4
C57BL/10	3	27	C57BL/Gr	2	3
AKR	4	27	129/Rr	2	2
			SWR	2	2

cluster at the extremes of the range, and this suggested that one gene of major effect might account for much of the variation. Three strains from each end of the range were tested with several concentrations of quinine in order to produce concentration–response curves. From Fig. 2A it can be seen that A2G, DBA/2 and BALB/cBy are similar in their concentration–response curves, showing some aversion at 0·8 mM and a strong aversion at 1·6 mM. However both 129/Sv and SWR show some aversion even at 0·1 mM and this aversion is complete at 0·4 mM. The C57BL/6By curve occupies an intermediate position.

(ii) *Genetical results*

The differences between the concentration–response curves of BALB/cBy and C57BL/6By was sufficiently large to suggest that the CXB recombinant inbred (RI) strains might be used to test for the existence of a gene of major effect. These strains were developed by Bailey from the founder strains BALB/cBy and C57BL/6By, and their usefulness in mouse genetics is by now well established (E. L. Green, 1981; M. C. Green, 1981).

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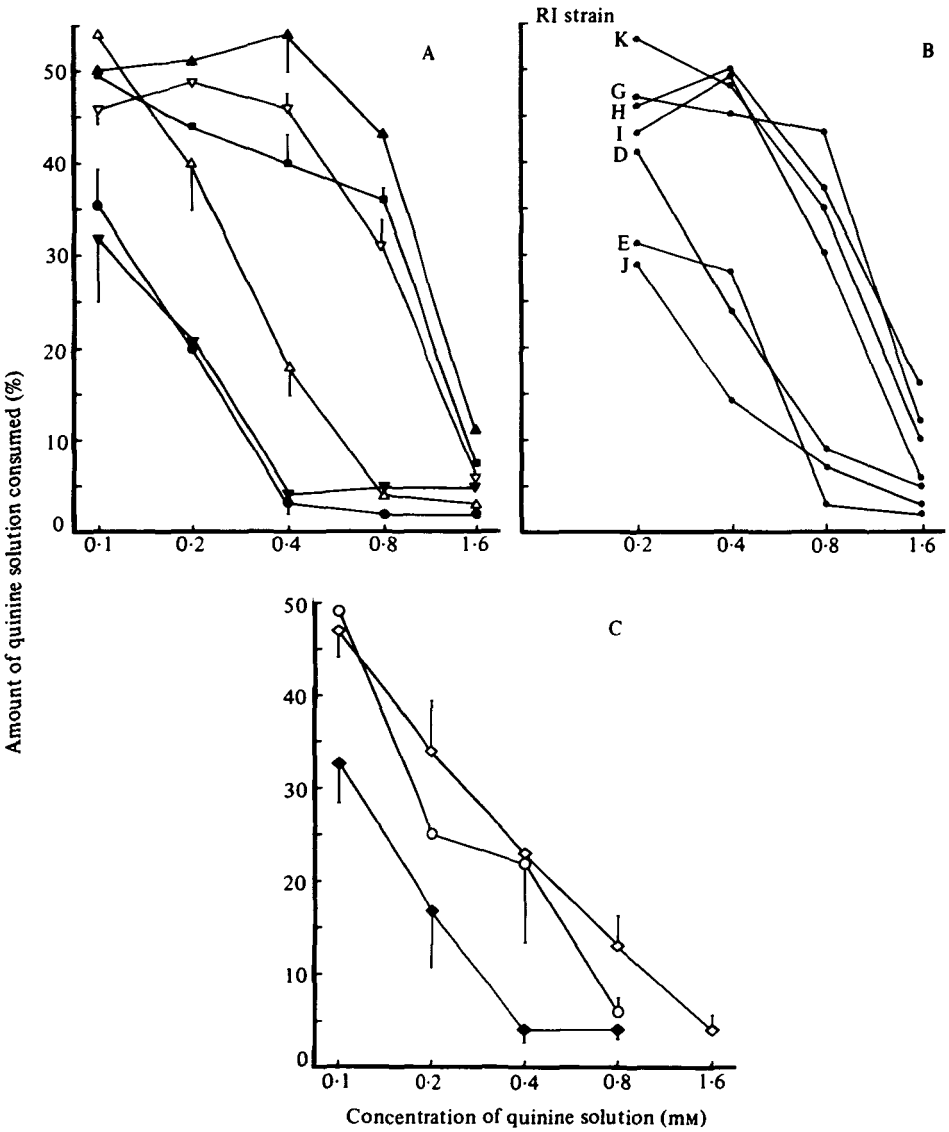

Fig. 2. Concentration–response curves with quinine. (A) six inbred strains: SWR (●), 129/Sv (▼), C57BL/6By (△), BALB/cBy (▽), DBA/2 (■), A2G (▲). (B) Seven CXB RI strains. (C) Three kinds of F_1: SWR × 129/Sv (◆), A2G × 129/Sv (◇), SWR × A2G (○). The female parent is written first in each cross. Each point is the mean of between two and eight experiments. The vertical bars are the S.E.M.S.

The essential point here is that when the founder strains differ with respect to a certain phenotypic characteristic which is determined by a single gene, then each of the RI strains should resemble one or other of the founder strains. The concentration–response curves of the seven CXB RI strains in Fig. 2B show clearly that RI strains K, G, H, and I resemble BALB/cBy, and that RI strains D, E, and J resemble C57/6By. This result supports the hypothesis that a single gene is the cause of the difference between BALB/cBy and C57BL/6By.

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The next step was to see if alleles of the gene would segregate in a backcross. The strains chosen for this purpose were A2G and DBA/2, both of which show a relatively low aversion to quinine and will therefore be referred to as non-tasters, and 129/Sv and SWR which both show a high degree of aversion to quinine and will be referred to as tasters. These strains were chosen so that the two phenotypes expected in the backcross progeny should be as different as possible and therefore easily distinguishable. The concentration–response curves of three different kinds of F_1 mice are shown in Fig. 2C. The curve of the (SWR × 129/Sv) F_1 mice was identical to those of their parental strains, both of which were tasters. The curves of the (A2G × 129/Sv) F_1 and the (SWR × A2G) F_1 mice were intermediate between their parental curves but at 0·8 mM quinine the difference between the F_1 and the non-taster phenotypes was sufficiently large to suggest that the progeny of a backcross between them would show a clear segregation.

The hypothesis to be tested was that there is a major gene, *Qui*, which determines sensitivity to the taste of quinine. Within this hypothesis there were three possibilities to be considered. One possibility was that *Qui* is the same gene as that which determines sensitivity to SOA. Since the two characters are not correlated in any way in a wide selection of strains (SWR is the only strain which can taste SOA) this seemed unlikely to be true, but was a theoretical possibility. The second possibility was that *Qui* and *Soa* are different genes but are linked. A third possibility was that there is some kind of interaction at the phenotypic level such that the ability to taste one substance either increases or decreases the ability to taste the other. This idea was based on the assumption that bitter receptors are not completely specific. Thus although there may be a quinine receptor which is different from the SOA receptor, nevertheless the SOA receptor might to some extent be sensitive to quinine. Therefore the sensitivity of strain SWR to quinine might partly, or wholly, be due to the fact that it possesses an uniquely sensitive variant of the SOA receptor. Four backcrosses were made. The various kinds of mice used in these backcrosses, together with their tasting status with respect to quinine and SOA, are shown in Table 2.

In backcross A, (A2G × 129/Sv) F_1 males were mated with A2G females and 35 of their progeny were tested with quinine. Each progeny mouse was re-tested with quinine and the mean of the two tests was used for each mouse. The same progeny were subsequently tested with SOA. The results are shown in Fig. 3A. With respect to quinine the progeny fell into two groups. The 17 tasters had a mean of $14{\cdot}1 \pm 1{\cdot}4$, and the 18 non-tasters had a mean of $43{\cdot}7 \pm 1{\cdot}4$. Thus segregation has taken place in this backcross, and one gene can account for the difference between 129/Sv and A2G. Since the F_1 mice were males the gene must be autosomal. With respect to SOA the two groups have means of $44{\cdot}1 \pm 1{\cdot}8$ and $46{\cdot}2 \pm 2{\cdot}0$ respectively. This difference is clearly not significant, which merely confirms that variation in quinine sensitivity has no effect on sensitivity to SOA in SOA non-tasters.

In backcross B, (SWR × 129/Sv) F_1 males mated with 129/Sv females and the 29 progeny tested with quinine and subsequently with SOA. The results are shown in Fig. 3B. With respect to SOA there is clear segregation into 15 tasters and 14 non-tasters. With respect to quinine the two groups had means of $11{\cdot}4 \pm 1{\cdot}6$ and $9{\cdot}7 \pm 1{\cdot}0$ respectively. This difference is clearly not significant. There is

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therefore no evidence from this cross that variation in SOA sensitivity has any effect on sensitivity to quinine among quinine tasters.

The results of backcrosses A and B support the hypothesis that *Qui* is a separate gene from *Soa* since each can segregate in the absence of segregation of the other. To prove conclusively that they are two different genes it is necessary to demonstrate recombination between them. Backcross C was therefore made between (SWR × A2G) F_1 females and DBA/2 males and the 61 progeny were tested twice with quinine and then with SOA. The results are shown in Fig. 3C.

Table 2. *Summary of the four backcrosses to show the taster (T), non-taster (N) or heterozygous (N/T) status of the mice used*

	Strains used		Quinine status		SOA status	
Backcross	F_1	P	F_1	P	F_1	P
A	(A2G × 129/Sv)	A2G	N/T	N	N	N
B	(SWR × 129/Sv)	129/Sv	T	T	N/T	N
C	(SWR × A2G)	DBA/2	N/T	N	N/T	N
D	(SWR × A2G)	A2G	N/T	N	N/T	N

In order to make it easier to discuss the data the diagram is divided into four quadrants by a cross at the 30 % value for both tastants. If *Qui* and *Soa* are the same gene then no recombination could take place and the backcross progeny should occur in equal numbers in quadrants 2 and 3. If *Qui* and *Soa* are different and unlinked genes, then segregation should be independent and the progeny should be found in equal numbers in all four quadrants. However, if *Qui* and *Soa* are different but linked genes, then most of the progeny will be of the parental phenotypes (quadrants 2 and 3) but some progeny should be of the recombinant phenotypes (quadrants 1 and 4), unless of course the linkage is so tight that no recombination has taken place in the sample of progeny analysed.

In fact the data conformed to none of these predictions. Twenty-nine progeny fell within quadrant 2, 16 within quadrant 3 and 16 within quadrant 4. With respect to the *Soa* segregation the backcross progeny comprised 32 tasters and 29 non-tasters, which is a good approximation to the expected 1 : 1 ratio. With respect to the *Qui* segregation the progeny comprised 16 tasters and 45 non-tasters, which is clearly very different from the expected 1 : 1 ratio. To check whether this peculiar segregation was in some way caused by the use of strain DBA/2, backcross D was made between (SWR × A2G) F_1 females and A2G males. Only 14 progeny were obtained but they conformed to the pattern obtained with backcross C in that none of them appeared in quadrant 1. The data from backcrosses C and D are summarized in Table 3.

(iii) *Behavioural effects on burette preference*

It was noticed with several strains that when mice were tested with concentrations of quinine lower than those which they could taste, the mice tended to show a regularity in their burette preference. Throughout the 4 days of the test they tended to drink from whichever burette was on the side of the cage where the

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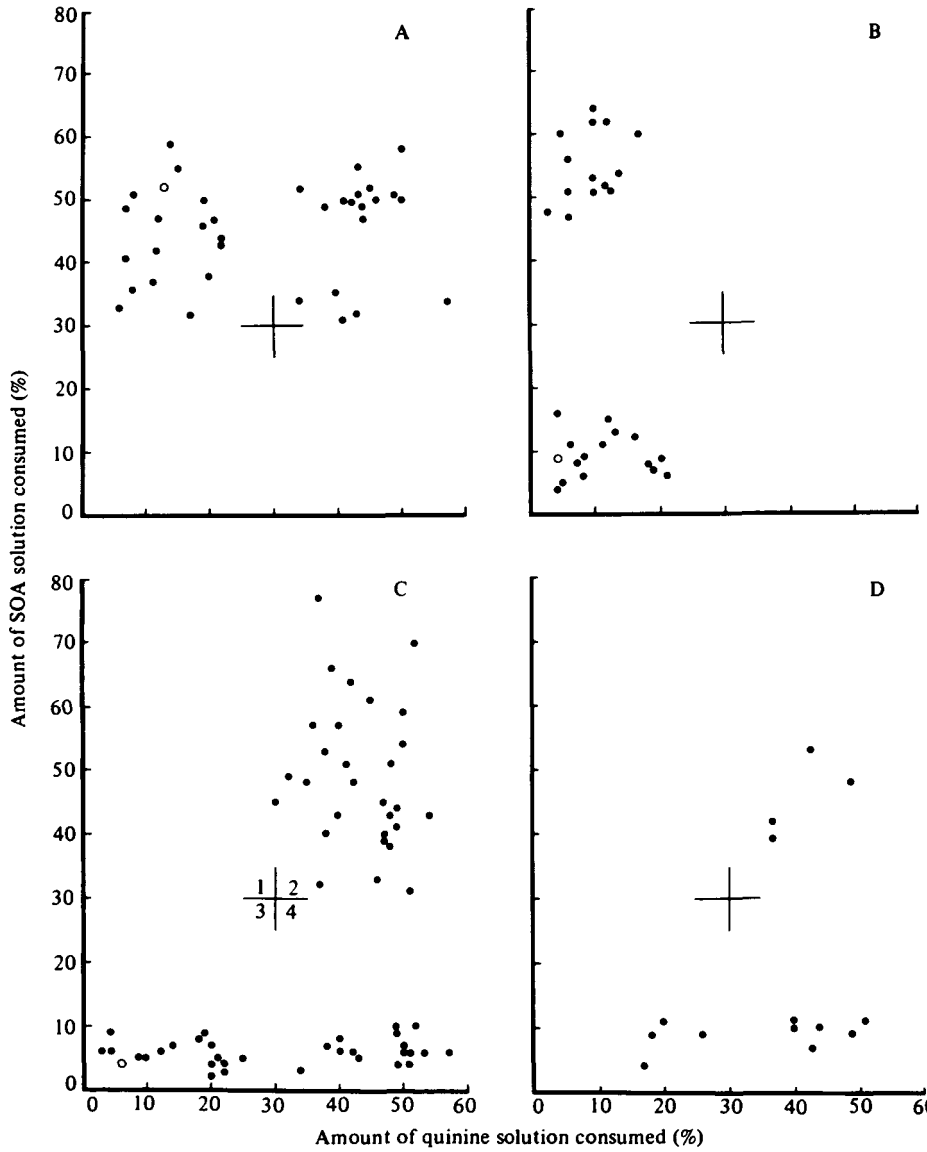

Fig. 3. Quinine (0·8 mM) and SOA (1·0 mM) consumption by the progeny of four backcrosses. Each filled symbol is one mouse. The unfilled symbols are the values for the type of F_1 used in each cross (see Fig. 2C). The cross in each diagram divides the data at the 30% value for both tastants. (A) A2G × 129/Sv), (B) 129/Sv × (SWR × 129/Sv), (C) (SWR × A2G) × DBA/2, (D) (SWR × A2G) × A2G.

drinking bottle was normally located. In order to see how long this preference might persist, six cages of BALB/cBy mice were tested for a period of 10 days. The normal 4-day schedule was first carried out, this was then repeated and then the first 2 days of the schedule were repeated again. The results obtained with BALB/cBy using 0·4 mM quinine are shown in Fig. 4. On the first day the burette which contained the control solution was on the side of the cage where the drinking bottle

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was normally located (see Fig. 1). This was reflected in the less than 50% consumption of tastant on the first day. The burettes were reversed on the second day but the mice still preferred to drink on the same side of the cage. This led to a greater than 50% consumption of tastant on the second day. The alternating low and high values continued throughout the 10 days of the experiment with no

Table 3. *The numbers of mice of each phenotype obtained from backcrosses C and D*

	Quinine		
SOA	Taster	Non-taster	Total
Non-taster	0	33	33
Taster	20	22	42
Total	20	55	75

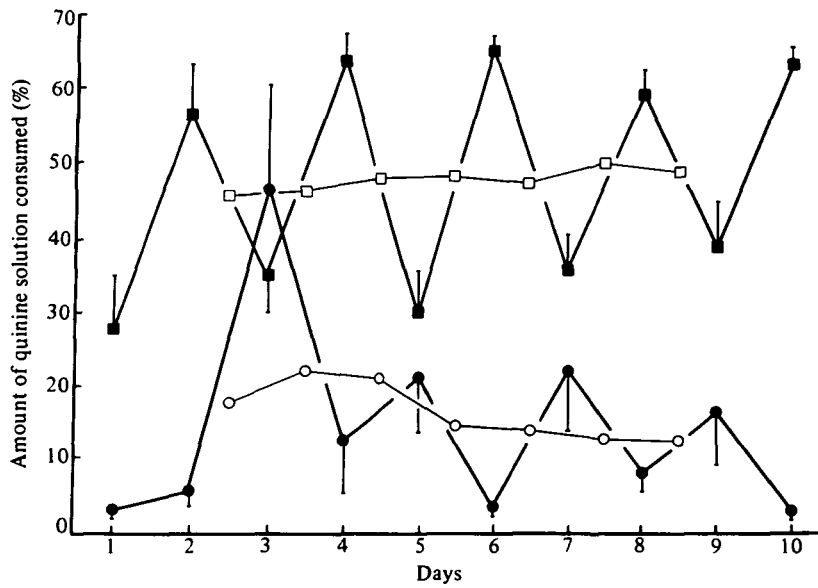

Fig. 4. Daily consumption of 0·4 mM quinine by BALB/cBy (■) and C57BL/6By (●) mice. The open symbols are the running means of each successive 4-day period. The vertical bars are the S.E.M.S.

sign that the mice were losing their preference for drinking on one side of the cage. In Fig. 4 the open square symbols are the means for each successive period of 4 days in the BALB/cBy experiment, i.e. days 1–4, 2–5, 3–6, etc. It can be seen that these running means rise slightly during the experiment, nevertheless the mean of days 1–4 is a good estimate of the overall response of BALB/cBy mice to this concentration of quinine. Since these mice were drinking about 50% tastant it is assumed that they could not taste it. It was therefore not surprising to find that the same pattern of burette preference occurred with BALB/c mice when both burettes contained only water.

A second pattern of burette preference was often seen in several strains when

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mice were tested with a concentration of quinine which produced a degree of aversion which was less than complete aversion. In this pattern the mice showed a strong aversion to quinine on the first 2 days followed by a preference for quinine on the third day followed by a rather variable degree of aversion to quinine on the fourth day. C57BL/6By mice tested with 0·4 mM quinine often behaved in this way. The mean values of six experiments with C57BL/6By are shown in Fig. 4. The large standard errors on the third and fourth days reflect the fact that the above pattern of behaviour only occurred in four of the six experiments, and in those to a variable degree. However, when it did occur it seemed to start a sequence of up and down variation in the daily values which persisted throughout the rest of the 10 days, as can be seen in Fig. 4. Inspection of the running means shows that, in spite of the curious behaviour of these mice, the mean of days 1–4 is a good estimate of the overall degree of aversion of C57BL/6By mice to 0·4 mM quinine.

4. DISCUSSION

Strains A2G, DBA/2 and BALB/cBy are similar in their ability to taste quinine. The difference between these non-taster strains and the taster strain 129/Sv is determined by a single gene, as shown by the results of the backcross in Fig. 3A. The difference between the non-taster strains and C57BL/By is also probably determined by a single gene, as shown by the results with the CXB RI strains. The data presented here do not prove that the same gene is responsible in both cases, however the most economical hypothesis is that there is one gene with three alleles, Qui^a (in 129/Sv), Qui^b (in C57BL/By) and Qui^c (in A2G, DBA/2 and BALB/cBy).

In backcrosses A and B segregation at *Qui* was demonstrated in the absence of segregation at *Soa* and *vice versa* (Figs. 3A and B). This is good evidence that *Qui* and *Soa* are separate genes. However, the double backcross, which was intended to put this matter beyond doubt, gave a result which is difficult to interpret. The results to be expected if the two genes are linked or unlinked were discussed in the Results section. The data do not appear to support the hypothesis of linkage since all the 'crossover' mice in backcrosses C and D were in quadrant 4, whereas one would have expected approximately equal numbers in quadrants 1 and 4. However if the two genes are unlinked, it seems as though all the progeny which should have appeared in quadrant 1 have been moved across to join those in quadrant 2, thus distorting the *Qui* segregation ratio but leaving the *Soa* segregation unchanged. Mice of the same genotype in backcross A appeared, as expected, in quadrant 1; but for some reason they did not appear in quadrant 1 in backcrosses C and D.

The explanation of this curious distortion will probably come with further knowledge of the mode of action of the two genes. Both genes may determine the characteristics of receptors in the taste cells of the tongue, and one receptor may interfere with the functioning of another. But there are other possible sites of action, such as synaptic connections in the neural pathway from the tongue to the brain. Shingai & Beidler (personal communication) applied solutions of SOA to the tongues of mice and measured the neural response in the lingual branch of their glossopharyngeal nerves. They found a strong response in SWR mice and virtually

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no response in LP/J, BDP/J and DBA/2J mice. This result clearly shows that the site of action of the *Soa* gene is in the tongue.

It is possible to construct highly speculative models of mechanisms which might produce the observed distortion, but at present this would be premature. Work is in progress to find variation in the tasting abilities of mice with respect to other bitter substances and some more examples of interaction between genes for tasting ability may be found in the course of this work. Detailed studies are also being made with a variety of acetylated sugars to define more closely the nature of the receptor whose function is determined by the *Soa* gene. An account of these studies will be in the next paper in this series.

The behavioural effects on burette preference might also repay further investigation. The behaviour of the BALB/c mice showed a degree of preference for one drinking position which was remarkably consistent for as long as 10 days. The fact that the preferred position was on the same side of the cage as the normal water bottle appears to explain why this one was preferred to the other, but they were both more than 10 cm distant from the normal position of the water bottle spout. It seems curious that although a mouse roams around its cage so much it remains a creature of habit when it comes to take a drink. The behaviour of the C57BL/6 mice with 0·4 mM quinine was even more surprising. Their aversion to the quinine on the first and second days of the test may have accustomed them to drinking from one spout (the square one in Fig. 1) to such a degree that when the solutions were reversed with respect to the burettes (for the third day of the test) some of the mice failed to re-adjust their behaviour. The result was that in two experiments the mice actually drank more than 80 % quinine on the third day. Although most of them re-adjusted their behaviour on the fourth day, a slight tendency to prefer to drink on the side of the cage *opposite* to the water bottle seemed to persist for the rest of the experiment. This is seen in the slight oscillation in the data which is out of phase with the much greater oscillation in the BALB/c data.

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