ABSENCE OF OTOLITHS IN THE MOUSE: AN EFFECT OF THE PALLID MUTANT

By MARY F. LYON*
Institute of Animal Genetics, Edinburgh

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Introduction

The recessive mutant *pallid*, **pa**, of the mouse (*Mus musculus* L.) was first reported by Roberts (1931), who described the pink eyes and pale coat colour which the mutant produces. Lyon (1951) noticed that some pallid mice also showed defects in the postural reflexes.

These behaviour defects were of two main types. The first type was detectable when mice of 2–3 days or more were held up by the tail. A normal mouse then flexes its spine and neck dorsally and stretches its fore-limbs forwards. Defective mice flexed the spine and neck ventrally and stretched the limbs backwards, i.e. they failed to respond normally to change of position.

The second type of defect appeared during the third week of life. Some pallid mice which had hitherto seemed normal then developed an asymmetrical posture and walked about with the head constantly tilted to one side.

A further group of pallid mice then remained which showed normal behaviour.

The anatomical basis of these postural defects was absence of one or more otoliths (Lyon, 1951). A normal mouse possesses two otoliths in each ear, one in the sacculus and one in the utriculus. Those pallid mice which failed to respond to position change always lacked both otoliths from both ears and those with a normal response always possessed at least one otolith. Animals with asymmetrical posture showed asymmetry of the otolith defect, and tilted the affected side of the head upwards. Those with completely normal otoliths had normal behaviour.

The present paper describes investigations into the inheritance of absence of otoliths. First, evidence is presented that this defect may justifiably be considered as an effect of the pallid gene itself, rather than of some other gene linked to **pa**, and secondly, some of the factors influencing the manifestation of otolith defect in pallid animals are examined.

Materials and methods

The work was carried out in two parts, at Cambridge and Edinburgh. The Cambridge stock was divided into four substrains, with a common ancestor but separated from each other by outcrosses and differing in frequency of otolith defect. The Edinburgh stock was not subdivided and was not closely inbred. In both stocks the matings made were either between pallid homozygotes or were backcrosses to them of heterozygotes.

Classification of the young as 'normal' or 'affected' was based on the behaviour and not on examination of the otoliths. Animals were examined for response to position change at 5 days old, and for head-tilting by at least two observations in the third week of life.

* Member of the Medical Research Council's scientific staff.

ABSENCE OF OTOLITHS AS AN EFFECT OF THE PALLID GENE

As absence of otoliths was not discovered until the pallid gene had been known and used in various laboratories for a number of years, this character was at first thought to have arisen through the mutation of a new gene distinct from **pa**.

If this were so, recombination between absence of otoliths and pallid should be detectable. To test this the young were examined of backcrosses of **papa** homozygotes lacking otoliths to heterozygotes with normal otoliths. The recombination classes would then be the pallid normal and non-pallid affected types. Previous matings between pallid homozygotes, however, had shown that the breeding behaviour of normal pallids did not necessarily differ from that of affected ones. In other words, normal overlapping of absence of otoliths occurs in pallid mice. In these circumstances only the occurrence of affected non-pallids could be accepted as evidence of recombination. In order to maximize the chance of detecting such animals the stock chosen for this test was a part of the Cambridge stock in which normal overlapping was rare. The results were as follows:

Pallid		Non-pallid	
Normal	Affected	Normal	Affected
7	227	282	—

No affected non-pallid mice occurred among a total of 516. Moreover, several hundred more backcross young have since been examined for other purposes without the detection of a single non-pallid mouse lacking otoliths. It thus appears impossible to obtain this otolith defect in mice not homozygous for the gene causing pallid coats.

This does not, of course, prove that the two effects are due to the same gene, since the same results might be given by two very closely linked genes. From the point of view of physiological genetics it is interesting to attempt to decide which is the more probable hypothesis. If stocks of pallid mice existed in which absence of otoliths never occurred, this would suggest that two distinct loci were concerned. However, all stocks of pallids so far critically examined have included some animals with abnormal behaviour. In addition, Castle (1941) and Keeler (1947) reported respectively that some pallid mice were 'jumpers and turned back somersaults' and that they showed 'head-weaving'. Although these terms are at variance with Lyon's description of animals lacking otoliths it seems more reasonable to suppose that the three authors were observing the same character than that pallid mice in the three laboratories exhibited three different defects. Since lack of otoliths is so widespread among pallid animals it seems likely that it was present in the original stock, i.e. that absence of otoliths and pallid coats were the result of the same mutation.

It is therefore assumed, at least as a working hypothesis, that absence of otoliths is an effect of the pallid gene.

FACTORS AFFECTING PENETRANCE OF OTOLITH DEFECT

In contrast to its action on coat and eye colour, the pallid gene shows incomplete manifestation in its effect on the otoliths. The remainder of this study consists of a search for variations in manifestation of otolith defect among pallid mice and of attempts to isolate some of the factors responsible for this variation.

The influences acting on a character inherited in this way may be initially and broadly classified as genetic or environmental. The genetic factors may again be divided into

minor unidentifiable 'modifiers' or 'genetic background' and other major genes segregating. The environment may be broken down into very many components, both physical and biological, and these may again be classified according to the stage of development at which they act. In this case, since presence or absence of otoliths is already established by the time of birth, only the pre-natal environment need be considered. With this restriction there are still many potentially important factors, ranging, for example, from season of year to position of the individual in its mother's uterus. Many of these items, however, do not lend themselves readily to analysis, and only litter-size, litter-order and maternal age, and birth weight will be considered here.

(1) *Effect of environmental factors on penetrance*

All the matings made in this part of the work were between **papa** homozygotes, so that only pallid young were produced. Two distinct stocks were used: one substrain of the Cambridge stock showing about 40 % penetrance and a part of the Edinburgh stock showing about 50 %. Strains with a higher penetrance were available but not used since, by the lack of variation within them, they could supply very little information. Table 1 presents in detail the material on which this section is based. Litter-size, maternal age in lunar months, and the number of normal and affected young recorded are given for each litter. On inspection it appeared that the penetrance of absence of otoliths varied in different litters of the same parents. One litter from a certain pair would consist almost entirely of affected and the next almost entirely of normal animals, and this occurred more frequently than would have been expected if the litters had been part of a homogeneous binomial distribution. This suggested there were environmental factors which caused litter-mates to tend to resemble one another with respect to absence of otoliths. This led to the investigation of the effect of such factors as litter-size and litter-order.

(*a*) *Litter size*

The data of Table 1 on the relation of penetrance to litter-size are summarized in Table 2. A fairly steady increase in the frequency of defect with increasing litter-size is obvious on inspection.

In order to test the statistical significance of this effect a method given by Holt (1948) was used. In studying the effect of maternal age on the manifestation of polydactyly in mice this author used a χ^2 test calculated as follows:

'Let a be the maternal age in 28-day units,

$A = S(a)$ for all polydactylous young, Np in number,

$A^1 = S(a)$ for all normal young, Nq in number,

Then $$\chi^2 = \frac{(qA - pA^1)^2}{pqS(a-\bar{a})^2}$$ for one degree of freedom.'

This is in effect a method for testing the significance of any difference in the mean maternal ages of normal and polydactylous young. In the present investigation it will be used to test the significance of any difference in mean litter-size of normal and affected young. The method has the advantage that it is possible to make the test within pairs. In heterogeneous stocks there may be genetic differences between pairs in penetrance

Table 1. *The material used for determining the effects of environmental factors on otolith defect*

N = normal; A = affected; L = litter-size; M = maternal age expressed in lunar months to the nearest month.

Pair no.	Litters: First				Second				Third				Fourth				Fifth				Sixth				Seventh				Eighth				Ninth				Tenth			
	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M	L	N	A	M
Cambridge stock																																								
*1	6	1	3	4	5	2	.	5	3	2	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*2	4	.	.	2	4	2	1	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	9	.	4	2	3	1	2	4	8	5	.	6	9	3	1	6	?	.	.	7	1	.	.	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	8	1	6	2	3	.	.	3	8	6	2	4	8	7	1	4	10	.	1	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	5	1	4	3	3	3	.	4	3	3	.	5	8	1	7	7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	5	2	3	3	4	1	1	4	6	.	.	5	2	2	.	6	4	4	.	6	5	2	3	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*7	2	1	.	2	?	.	.	3	4	.	2	4	4	.	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	7	.	2	2	6	4	.	3	6	2	4	4	8	3	4	6	3	.	.	7	8	6	.	8	2	.	.	9	7	.	.	10	9	7	2	12	.	.	.	.	.
9	6	1	4	3	?	.	.	4	4	2	.	5	6	4	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	5	3	2	2	5	4	1	3	5	4	1	4	2	.	.	7	6	4	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*11	5	5	.	3	5	.	.	4	4	4	.	5	4	4	.	6	4	.	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*12	8	7	.	3	6	.	.	4	6	5	.	6	5	5	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	6	.	5	2	4	.	.	3	4	4	.	4	7	2	5	6	8	.	8	7	6	.	4	9	8	1	7	9	5	.	.	10	.	.	.	.	.	.	.	.	.
14	6	3	3	2	8	4	4	3	6	2	4	5	7	3	1	6	3	3	.	7	3	3	.	9	2	2	.	10	3	3	.	11	2	2	.	13	5	4	1	14
15	5	.	4	3	5	2	2	4	4	.	.	5	?	.	.	6	8	3	4	7	7	5	2	9	5	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.
16	6	3	3	3	?	.	.	5	4	3	.	6	4	2	2	7	7	4	3	8	9	.	.	9	?	.	.	10	2	2	.	10	3	3	.	11	5	.	1	12
17	3	.	1	3	2	2	.	4	?	.	.	4	3	3	.	5	3	2	.	8	1	.	.	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*18	2	1	1	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*19	9	1	8	2	2	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*20	4	.	.	2	3	.	3	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Edinburgh stock																																								
1	2	2	.	2	5	2	2	3	4	.	.	4	6	.	6	5	9	6	2	6	4	3	.	8	4	4	.	9	6	2	4	10	6	.	.	11	.	.	.	.	.
2	7	1	6	2	5	5	.	3	6	5	1	4	10	6	4	5	7	1	6	6	9	.	.	7	9	4	5	8	.	.	.	.	.	.	.	.	.	.	.	.	.
3	8	3	5	2	6	6	.	3	7	4	2	4	9	1	7	5	9	3	6	6	9	2	7	6	6	.	.	7	9	2	6	8	?	.	.	9	6	.	.	10
4	4	.	4	3	8	.	7	4	10	1	9	5	12	1	10	6	11	.	.	8	7	.	7	9	6	.	6	10	5	.	3	12	.	.	.	.	.	.	.	.	.
5	8	5	2	3	5	4	1	3	9	4	2	4	5	5	.	5	4	3	1	6	2	.	2	7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	7	2	5	2	9	4	5	3	10	1	9	4	10	2	7	5	9	6	3	6	8	6	2	7	5	5	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.
7	7	2	5	2	9	1	8	3	10	2	7	5	12	3	6	6	12	3	6	7	8	2	6	8	11	.	.	9	8	2	6	11	7	3	4	12	.	.	.	.	.
8	6	4	2	2	4	1	3	4	4	4	.	5	7	6	1	5	10	8	1	6	11	.	.	8	11	3	6	9	11	1	5	11	.	.	.	.	.	.	.	.	.
9	8	6	2	2	7	3	4	4	6	6	.	5	3	2	1	6	6	6	.	7	7	.	.	8	6	.	6	11	.	.	.	.	.	.	.	.	.	.	.	.	.
10	9	1	5	2	8	6	2	3	10	7	3	4	10	7	2	5	10	7	2	6	6	.	.	7	8	5	3	8	2	.	.	9	.	.	.	.	.	.	.	.	.
11	8	.	7	2	5	4	1	3	12	7	4	4	11	2	6	5	13	2	11	7	7	.	6	8	10	6	4	9	.	.	.	.	.	.	.	.	.	.	.	.	.
12	8	3	4	2	11	2	8	4	9	.	.	5	12	.	9	5	5	.	.	6	11	4	5	7	?	.	.	9	?	.	.	10	10	1	8	11	.	.	.	.	.
13	9	3	.	2	9	4	3	5	8	4	2	6	9	3	5	7	9	6	3	9	7	6	.	10	4	3	.	11	.	.	.	.	.	.	.	.	.	.	.	.	.
14	9	.	.	3	10	6	3	4	6	.	.	5	6	3	3	6	9	.	.	7	8	.	.	8	7	3	1	9	6	1	3	11	6	.	.	12	.	.	.	.	.
15	8	6	2	3	11	.	2	4	11	.	.	5	4	3	1	6	5	.	.	7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16	10	2	8	3	11	3	8	3	11	.	.	5	9	.	.	7	12	4	6	8	10	.	10	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
17	8	.	.	2	4	3	1	3	8	7	1	4	5	5	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
*18	6	.	.	2	7	6	.	3	11	6	3	4		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

of defect and if the pairs by chance also showed differences in mean litter-size there might be an apparent correlation of litter-size with penetrance in the stock, but it would be eliminated if the test were performed, as it is here, within pairs. The young of pairs marked with an asterisk have been excluded from this test, the marked pairs being those which yielded only a very small amount of information (less than three classified litters)

Table 2. *Relation of penetrance of otolith defect to litter-size*

	Cambridge stock				Edinburgh stock			
	Pallid young				Pallid young			
Litter-size	N	A	Total	Penetrance (%)	N	A	Total	Penetrance (%)
2	12	1	13	8				
3	26	6	32	19	28	13	41	32
4	26	6	32	19				
5	34	22	56	39	30	7	37	19
6	29	30	59	51	33	31	64	48
7	14	13	27	48	37	47	84	56
8	44	43	87	49	55	51	106	48
9	11	16	27	59	50	67	117	57
10	—	—	—	—	56	77	133	58
11	—	—	—	—	21	43	64	67
12	—	—	—	—	20	52	72	72

Table 3. *Significance test of litter-size effect within pairs*

	Cambridge stock 12 pairs			Edinburgh stock 17 pairs		
	χ^2	D.F.	*P*	χ^2	D.F.	*P*
Total	40·33	12	—	41·66	17	—
Deviation	15·24	1	<0·001	7·25	1	0·01–0·001
Heterogeneity	25·09	11	0·01–0·001	34·41	16	0·01–0·001

or, in the case of pairs 11 and 12 of the Cambridge stock, no information at all since all their young fell into the same category (normal). The results obtained are shown in Table 3. The deviation χ^2's are clearly significant for both stocks, showing that the increase in penetrance is a real effect of increasing litter-size. The heterogeneity χ^2's are also significant, but this merely indicates that other factors in addition to litter-size were operative.

The relation between penetrance and litter-size is roughly linear. In the Edinburgh stock the smallest litters have a higher penetrance than those of size 5, but there is no means of deciding whether or not this is a real effect. In all other respects the agreement between the very distantly related Cambridge and Edinburgh stocks seems remarkably close both in the penetrance at any litter-size and in the amount of change. This amount of change itself seems remarkable for its size, the proportion affected ranging from 8 to 60 % in the Cambridge and from 30 to 70 % in the Edinburgh stocks.

In order to find the form of the relation of penetrance to litter-size a regression line was plotted. Since penetrance was expressed as a percentage the probit of penetrance was taken as ordinate. The logarithm of litter-size was chosen as abscissa, since a plot by eye suggested that this transformation would result in a better fitting line. This means that penetrance was being related to proportionate changes in litter-size rather than to arithmetic ones. The method of calculation was that given by Finney (1947) and the results are shown in Table 4. In both stocks the regression χ^2's are highly significant,

and the remainder χ^2's non-significant, so that statistically the data are well fitted by a linear regression of the probit of penetrance on the logarithm of litter-size. The values of b for the two stocks differ by less than their standard error, confirming that the agreement between the two stocks is close.

Table 4. *Regression of probit of penetrance of otolith defect on the logarithm of litter-size*

	Cambridge stock			Edinburgh stock		
	χ^2	D.F.	P	χ^2	D.F.	P
Total	28·41	7	—	41·97	8	—
Regression	25·79	1	<0·001	31·15	1	<0·001
Remainder	2·62	6	0·9–0·8	10·82	7	0·2–0·1
	$b=2{\cdot}46$ $V_b=0{\cdot}235$			$b=2{\cdot}20$ $V_b=0{\cdot}156$		

(*b*) *Litter-order and maternal age*

In the Cambridge data there is a remarkable disparity between the frequency of defect in first litters and that in all subsequent litters. If the asterisked pairs are again excluded, the remaining pairs all have litters classified up to or beyond the fourth. Consideration of the first four litters only of these pairs (Table 5) thus reduces the risk of spurious results due to genetic differences between pairs which produced different numbers of litters. The effect still remains. The weighted mean litter-sizes, calculated by weighting each litter according to the number of classified young, show that the effect cannot be accounted for by variations in litter-size.

Table 5. *Relation of penetrance of otolith defect to litter-order. Cambridge stock*

Litter no.	N	A	Total	Penetrance (%)	Litter size
I	14	41	55	75	6·11
II	21	10	31	32	5·26
III	31	11	42	26	5·86
IV	30	21	51	41	6·70

A change in litter-order involves a change in maternal age, maternal parity and paternal age. Paternal age was not investigated, but an attempt was made to evaluate the relative importance of maternal age and maternal parity in producing this effect. Accordingly trios of sisters (A, B and C) were mated as follows:

(A) Mated to pallid brother at 3–4 weeks. At least two litters to be obtained (A_1, A_2).

(B) Mated to homozygous non-pallid male at 3–4 weeks and remated to pallid brother after one litter, one litter by the pallid male being obtained (B).

(C) Mated to pallid brother when A had produced one litter (C).

The following comparisons were then obtainable:

Comparison	Variable	Constant
A_1–C	Age	Parity and number of pallid young previously borne
B–A_2	No. pallids borne	Age and parity
C–B	Parity	Age and number of pallids borne

An effect of the number of pallid young previously borne, if detectable, would indicate that some reaction between mother and foetus was involved. The results (which are not included in Table 1) are shown in Table 6, together with the heterogeneity χ^2's calculated

for each of the appropriate comparisons. (Yates's correction has been applied to the C–B and B–A_2 comparisons.) Of the three experimental comparisons that for age (between A_1 and C) is clearly the important one. There is an obvious difference in the proportions of affected animals in the two types of litter. It is in the direction expected and was consistent when individual litters from sisters were compared. Moreover, the mean litter-sizes of A_1 and C litters show very little difference, being 5·83 and 6·31

Table 6. *Investigation of the relative importance of maternal age and parity*

Comparison			N	A	Total	Litter size	χ^2	P
Age	A_1–C	A_1	14	22	36	5·83		
		C	29	10	39	6·31		
		Total	43	32	75		9·60	0·01–0·001
Parity	C–B	C	29	10	39	6·31		
		B	22	3	25	4·60		
		Total	51	13	64		1·01	0·5–0·3
No. papa borne	B–A_2	B	22	3	25	4·60		
		A_2	12	7	19	5·95		
		Total	34	10	44		2·51	0·2–0·1

respectively, so that the results are not likely to have been disturbed by litter-size. Such differences as exist in the other comparisons may well be due to litter-size differences, since B litters had a considerably lower mean litter-size than all the other groups. The conclusion reached was therefore that maternal age rather than maternal parity or number of pallid young previously borne was the factor concerned in the change of penetrance with litter-order. Only a small number of mice were bred, however, and small effects of parity and number of pallids borne are not excluded.

The data were then retabulated according to maternal age in an attempt to find the form of the relation between age and penetrance. The Edinburgh data were also examined, asterisked pairs again being excluded (Table 7).

Table 7. *Relation of penetrance of absence of otoliths to maternal age*

(Maternal age is expressed in lunar months to the nearest whole month.)

Maternal age	Cambridge stock					Edinburgh stock				
	N	A	Total	Penetrance (%)	Litter size	N	A	Total	Penetrance (%)	Litter size
2	7	22	29	76	6·79	27	41	68	60	7·47
3	19	24	43	56	5·86	51	44	95	46	7·68
4	32	13	45	29	5·31	47	49	96	51	9·01
5	10	4	14	29	4·43	51	61	112	55	8·82
6	31	12	43	28	6·40	52	51	103	50	9·06
7	9	21	30	70	6·97	24	31	55	56	10·04
8	18	6	24	25	6·00	25	32	57	56	8·42
9	9	13	22	59	6·64	22	21	43	49	8·67
10	23	4	27	15	5·22	8	20	28	71	7·64
11						11	35	46	76	7·74
Total	158	119	277			318	385	703		

In the Cambridge stock, apart from the initial decrease in penetrance between 2 and 4 months, there were large fluctuations associated with fluctuations in mean litter-size. In the Edinburgh stock, however, the relation of penetrance to maternal age gave

a smooth curve. The penetrance fell as the mothers aged from 2 to 3 months, remained unchanged until the females had reached 10 months and then rose again markedly. Changes in litter-size did not seem responsible, since the mean litter-size also showed a smooth curve in its relation to maternal age, but this curve was in the wrong sense to have been the cause of the change of penetrance with age. Correction for litter-size would thus enhance the apparent age effect.

Since the numbers of young in the end age classes were rather small, there seemed some doubt about the significance of their divergence from the intermediate classes. In order to test this statistically a second-order regression of penetrance on maternal age was calculated by the method of partial regression, each observation being weighted according to the number of young classified. The sums of squares removed by the linear and the quadratic components of the regression are both significant while the remainder term is non-significant (Table 8). Thus these observations differ from a straight line,

Table 8. *Second-order regression of penetrance of otolith defect on maternal age. Edinburgh stock*

	Sum of squares	D.F.	Mean square
Overall	1,742,000	702	—
Total between groups	42,590	9	—
Linear regression	13,607	1	13,607
Linear + quadratic	27,269	—	—
Quadratic	13,662	1	13,662
Remainder	15,321	7	2,189
Within groups	1,699,410	693	2,452

$t_L = \sqrt{\frac{13607}{2452}}$ $t_Q = \sqrt{\frac{13662}{2452}}$

$= 2{\cdot}36$ $= 2{\cdot}36$

P is 0·02–0·01 P is 0·02–0·01

$b_L = -6{\cdot}31$ $b_Q = +0{\cdot}635$

and the increases of penetrance at the ends of the age range must be considered statistically real. Biologically this implies that in the Edinburgh stock there was an optimum period for the normal development of the otoliths in the young, namely, when the mother was in middle life. Similarly in the Cambridge stock, young females produced considerably more affected young than mature females, but there was no rise in penetrance among the oldest mothers. Whether this difference was due to insufficient data or to a real stock difference it is not possible to say.

(c) *Birth weight*

The effects of litter-size and maternal age suggested that more animals with otolith defect were born in those litters where birth weight was low. Therefore in thirty-eight litters of the Edinburgh stock the birth weights were recorded. The mean birth weight of ninety-three affected pallids was 1·38 g. and that of seventy-five normal pallids was 1·42 g., so that affected pallids were just significantly lighter than normals ($t = 2{\cdot}0$, $P < 0{\cdot}05$), a difference such as would be expected as a result of the litter-size effect. Birth weights were also compared within the twenty litters in which both affected and normal mice occurred. Here the difference in mean weight was very small (0·002 g.), and affected mice were heavier than normals. Thus there is no evidence that there is

a critical weight below which all animals are affected, but it is not possible from these limited data to decide whether the lower birth weight of young born in large litters is of importance in causing the high penetrance of these litters.

(2) *Effect of genetic factors on penetrance*

(*a*) *Genetic background*

In a genetically heterogeneous stock one may look for effects of the genetic background by comparing the offspring of normal and affected parents. This was done for the Edinburgh stock, but no significant effects were found.

The frequencies of absence of otoliths in the four substrains of the Cambridge stock are shown in Table 9. Line D, which provided the material for the study of environmental effects, obviously has a much lower penetrance than the remaining three lines.

Table 9. *Penetrance and expressivity of otolith defect in the Cambridge stock*

Grade 1 = unilaterally affected. Grade 2 = bilaterally affected.

			Pallid young						
				Grade affected					
Line	No. of pairs	Litter-size	Normal	1	2	?	Total	Penetrance (%)	Expressivity (%)
A	7	5·97	1	—	36	—	37	97·3	100·0
B	7	7·57	1	10	88	—	99	99·0	89·8
C	8	8·51	11	19	113	—	143	92·3	85·6
D	20	5·98	196	51	63	23	333	41·1	55·3

Differences in mean litter-size are also apparent but these do not seem to be the sole cause of the penetrance differences, since between lines A and D there is no litter-size difference, and in lines B and C those pairs which had litter-sizes as low as line D still showed a considerably higher frequency of young lacking otoliths. The interpretation must therefore be that the different genetic backgrounds of the substrains have affected the frequencies of defective ears.

(*b*) *Maternal genotype*

In view of the existence of the environmental effects already demonstrated, it is possible that genetic differences exert their effects on absence of otoliths indirectly by producing differences in the intra-uterine environment, rather than directly by altering the susceptibility of the young. A genetic difference whose possible effects on the intra-uterine environment may easily be examined is that of the maternal genotype at the **pa** locus. Such an effect exists in the case of the mouse mutant *fused*, **Fu** (Reed, 1937), where in reciprocal crosses the offspring of fused and of normal mothers show different frequencies of defect.

In the Edinburgh stock seventeen backcross matings had been made, in ten of which the mothers were non-pallid and in the remaining seven pallid. In the offspring of the first group 71 % of 123 pallids lacked otoliths and of the second group 74 % of 89 pallids. There was clearly no evidence that the pallid gene in a mother significantly affected the penetrance of otolith defect in her young.

(c) *Major mutants*

Other major factors segregating in the stocks used for this work included *spotting*, **s**, *brown*, **b**, a diluting factor which may have been either *dilute*, **d**, or *leaden*, **ln**, and sex. Of these only **b** showed any effect, namely, that a higher proportion of brown pallid mice lacked otoliths than among non-brown pallids (Table 10). As a statistical test the single-factor segregations of brown have been analysed. The heterogeneity is due to an excess of brown among the affected and a deficiency among the normal mice. It is not possible to say whether this is an effect of **b** itself or of some closely linked factor. It is unjustifiable at present to make any deductions from the fact that both **b** and **pa** affect pigmentation.

Table 10. *Segregation of brown among normal and affected pallids of Edinburgh stock*

			+	b	Total	χ^2
Intercrosses for **b**	N		193	51	244	2·19
(Expectation 3 : 1)	A		173	77	250	4·49
		Total	366	128	494	0·22
Backcrosses for **b**	N		40	34	74	0·49
(Expectation 1 : 1)	A		44	76	120	8·53
		Total	84	110	194	3·48

	χ^2	D.F.	P
Total	15·70	4	0·01–0·001
Deviation	3·70	2	0·30–0·20
Heterogeneity	12·00	2	0·01–0·001

A further effect of **b** is to produce an average increase of 3–4 % in the adult body weight (Green, 1931*b*; Castle, Gates, Reed & Law, 1936). This is of interest in view of the possibility that changes in birth weight affect the frequency of absence of otoliths. Green (1931*a*), however, found that **b** had no effect on the weight at birth. Moreover, Castle (1941) stated that when **b** was combined with **pa** it *decreased* adult body weight, but he gave no data on birth weights. Thus there is no clear evidence that **b** affects the birth weight in pallid mice, and this possible explanation of its effect on absence of otoliths must be discarded.

FACTORS AFFECTING EXPRESSION OF ABSENCE OF OTOLITHS

In studying the expression of absence of otoliths anatomically it was possible to recognize up to four grades for each ear; the granules might be fewer or completely absent, and the utriculus alone or both sacculus and utriculus might be affected. The present study was based on the behaviour, however, and in this only two main types could be recognized, corresponding to bilateral complete absence of otoliths and unilateral or incomplete defect. Thus the behaviour is a poor indication of the anatomical grade. However, the bilateral complete type, which fails to respond to change of position, is undoubtedly more severely affected than the incomplete type with asymmetrical posture, and this distinction was used as the basis for a search for variations in expression.

In the Edinburgh stock the direction of head-tilt, and consequently the side lacking otoliths, was noted for the unilaterally affected mice (see Table 11). Among these animals 107 were defective on the left side only and 104 on the right only. Thus absence

of otoliths is somewhat unusual among abnormalities with variable expression in that neither side of the body is favoured. This table also showed that the two ears of an individual were not independent in development but were correlated to some extent; a by no means unusual result. If the two ears were affected independently the frequency of bilaterally defective animals would be in proportion to those unilaterally affected; actually there was a large excess of the bilaterally affected class.

Table 11. *Relative frequency of otolith defect in the left and right ears. Edinburgh stock*

			Right ear		
			N	A	Total
Left ear	N		331	104	435
	A		107	156	263
		Total	438	260	698

$\chi^2 = 87{\cdot}9$. P is very small.

The effects on expression of those factors which altered penetrance were also examined, taking as a measure the proportion of abnormal animals which were bilaterally affected. The animals of Table 1 were used in demonstrating the effects of litter-size and maternal age on penetrance. Examination of the grade of defect of these same animals failed to reveal any effects of these factors on expression. It is possible, however, that small effects passed undetected since the data were limited.

The genetic background of the stocks, on the other hand, had a clear effect on expressivity. Table 9 shows that in lines B and C of the Cambridge stock a much higher proportion of affected animals showed the bilateral type of defect than in line D, an effect which parallels the difference in penetrance between these lines.

Discussion

Since absence of otoliths has been shown, to a reasonable degree of certainty, to be an effect of the pallid gene of the mouse, this gene must be considered a pleiotropic one. Discussion of the possible links between pigmentation and the development of otoliths may more suitably be left until the completion of embryological studies which are still in progress. It may be said, however, that for no other pleiotropic mouse mutant has a connecting link between pigment and the other defects been discovered, and at present it seems unlikely that investigations with pallid will be any more successful.

Pallid is also a gene of which one effect shows incomplete and the others complete penetrance. Many mouse mutants are already known to behave similarly, but some interesting points have emerged from a study of the factors influencing manifestation of absence of otoliths. Penetrance was affected both by environmental factors, including litter-size and maternal age, and by the genetic background. No attempt was made to partition the variance quantitatively. Superficially, however, the environmental effects appeared strong, especially since neither of the stocks used was closely inbred and genetic differences might have been expected to mask the importance of environmental effects.

Similar effects of litter-size and maternal age have been found for other mouse mutants. Reed (1936), studying harelip with cleft palate, found that penetrance increased with increasing litter-size and decreased sharply as the mothers aged from 2 to 3 months.

Decreases of penetrance with increasing maternal age were also reported for polydactyly (Holt, 1948) and an external ear anomaly (Kobozieff & Pomriaskinsky-Kobozieff, 1949); increasing litter-size resulted in decreased frequency of the external ear anomaly and of pigtail (Crew & Auerbach, 1941). Normal variations in presacral vertebra number (Green, 1941) and in size and presence of third molars (Grüneberg, 1951) are also affected by the intra-uterine environment. In all these cases penetrance decreases as maternal age increases, absence of otoliths being the only defect in which a terminal rise of penetrance has been reported. The effects of litter-size show no such general similarity. Increasing litter-size may enhance or diminish the frequency of particular defects.

The mechanisms by which litter-size and maternal age alter penetrance are unknown. For absence of otoliths the penetrance is lowest, and hence the conditions for normal development are optimal, when the number of foetuses is small and the mother is in middle life. Conditions are unfavourable when litters are large and the mothers are young or old. These unfavourable conditions appear to be those in which the supply of materials by the mother to the individual foetuses is not as good as possible. That this is so in large litters and in young females follows from the well-known fact that the birth weights of the young are low in these cases. Whether the birth weights are also low in the progeny of old mothers is not so certain. Bluhm (1929), working with mice, found birth weight to depend on maternal weight rather than age. King (1935), however, found in the rat that birth weight was related to maternal age and decreased when the females were over 18 months.

It may thus be that the effects of these unfavourable conditions are brought about simply by a general retardation of growth. It is also possible that changes in concentration of specific substances in the maternal blood are responsible. This remains an open question at present, since little work has been done on the experimental modification of frequency of defect in genetically abnormal mammals. Interesting results were obtained by Steiniger (1940), however, from the injection of anterior pituitary extract into pregnant mice of a stock producing harelip young. The frequency of harelip was increased from 10 to 17 %. Fraser & Fainstat (1951) found that cleft palate without harelip could be produced by the injection of cortisone into pregnant mice, and that the susceptibility differed between different strains. It is interesting that strain A, a susceptible strain, is known to produce cleft palate with harelip spontaneously (Law, 1948). Fraser & Fainstat found, however, that the dose of cortisone required to produce cleft palate was so severe as almost to cause foetal death, so that there is no definite evidence that physiological changes in concentration could affect the penetrance of genetic defects.

SUMMARY

The absence of otoliths found in mice homozygous for the *pallid* gene, **pa**, could not be separated genetically from this gene and is very probably caused by it. Unlike the effects of **pa** in diluting coat and eye colour, however, the effect on the otoliths shows incomplete penetrance and variable expression. Penetrance was strongly affected by environmental factors common to litter-mates, including litter-size and litter-order. Increasing litter-size produced an increase of penetrance. The litter-order effect was due to maternal age rather than parity; penetrance was high in the progeny of young and old females and lower at intermediate ages. Differences in the genetic background affected

both penetrance and expression in the same sense. In pallid mice homozygous for *brown*, **b**, the penetrance of otolith defect was enhanced. Among unilaterally affected mice the two sides of the body were affected equally often.

REFERENCES

Bluhm, A. (1929). *Roux Arch. EntwMech. Organ.* **116**, 348–81.
Castle, W. E. (1941). *Genetics*, **26**, 177–91.
Castle, W. E., Gates, W. H., Reed, S. C. & Law, L. W. (1936). *Genetics*, **21**, 310–23.
Crew, F. A. E. & Auerbach, C. (1941). *J. Genet.* **41**, 267–74.
Finney, D. J. (1947). *Probit Analysis.* Cambridge University Press.
Fraser, F. C. & Fainstat, T. D. (1951). *Pediatrics*, **8**, 527–33.
Green, C. V. (1931*a*). *J. Exp. Zool.* **58**, 247–58.
Green, C. V. (1931*b*). *J. Exp. Zool.* **59**, 213–45.
Green, E. L. (1941). *Genetics*, **26**, 192–222.
Grüneberg, H. (1951). *Proc. Roy. Soc.* B, **138**, 437–51.
Holt, S. B. (1948). *Ann. Eugen., Lond.*, **14**, 144–57.
Keeler, C. E. (1947). *J. Hered.* **38**, 294–8.
King, H. D. (1935). *Anat. Rec.* **63**, 335–54.
Kobozieff, N. & Pomriaskinsky-Kobozieff, N. A. (1949). *Proc. VIIIth Int. Congr. Genetics*, p. 609.
Law, L. W. (1948). *J. Hered.* **39**, 300–8.
Lyon, M. F. (1951). *J. Physiol.* **114**, 410–18.
Reed, S. C. (1936). *Genetics*, **21**, 339–60.
Reed, S. C. (1937). *Genetics*, **22**, 1–13.
Roberts, E. (1931). *Science*, **74**, 569.
Steiniger, F. (1940). *Z. ges. Anat.* 1. *Z. KonstLehre*, **24**, 1–12.