GENETICAL STUDIES ON THE SKELETON OF THE MOUSE
XI. THE INFLUENCE OF DIET ON VARIATION WITHIN PURE LINES

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(With Four Text-figures)

The extensive skeletal polymorphism discovered by Grüneberg (1950) in the A/Gr and C57BL/Gr inbred strains, among others, has since been analysed in such a way as to reveal the main causes of variation (Searle, 1954*a*). There is marked genetic differentiation between sublines; within sublines sex dimorphism is frequent. Most of the non-genetic variation is due to intangible factors acting independently on individuals, but in fourteen out of twenty-one variants there is some evidence for maternal effects. Sometimes these were revealed by trends with maternal age and/or parity, with litter-size or with length of gestation; sometimes by significant correlations between litter-mates for a particular character, due to factors acting on the litter as a whole. Usually only about 10% or less of the total variability is due to such maternal factors, but the figure is 35% for cranial dystopia of the processus spinosus of the second thoracic vertebra.

The intra-uterine environment is thus by no means constant; its own variation is reflected in the behaviour of these skeletal characters. In these experiments the external environment was kept constant as far as possible and the changes causing variation seemed to be almost entirely intrinsic, although there were signs of a seasonal effect. It therefore seemed of interest to find out how far extrinsic changes could alter the frequency of these variants via the maternal physiology. The following experiments were performed in order to throw light on this point: (*a*) adding 2-thiouracil to the drinking water of C57BL mice, (*b*) radically changing the diet of C57BL mice, (*c*) making a slight change in the diet of A strain mice.

These treatments, especially the second, did indeed change the pattern of variation considerably, thereby raising a number of new problems which will be discussed later. Details of the experimental results will now be given.

The action of 2-thiouracil on C57BL/Gr mice

Thiouracil depresses thyroid activity and thus slows down growth and metabolism. Dickie & Woolley (1948) found that if various JAX inbred mouse strains were given a 1% (saturated) solution of 2-thiouracil in their drinking water for from 3 to 8 months weight usually fell and the females were unable to nurse their litters. But in the C57BL strain there was no weight loss and litters were successfully reared. Smith & Gardner (1949) found that the increase in thyroid-gland weight on thiouracil feeding was small in the C57BL as compared with the CBA strain. So the former seems fairly resistant, but because of its high skeletal variability it was decided to use this pure line, to find out if thiouracil did nevertheless have some effect on the maternal physiology.

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Diet 86 of the Rowett Research Institute was used as food in cube form. Month-old mice were set up in pairs within a few days of each other and given drinking water ad lib., either without thiouracil, with 0·05% thiouracil or with 0·1% thiouracil. All the pairs were from the same subline, mostly very closely related; as far as possible litters were split up evenly between the three treatments. The first five litters from each pair were killed at 6 weeks and their skeletons prepared by the papain method. To swell the numbers of controls, which were small because of poor reproduction, parents were added to the material later. But they were not included in the calculations when there was any possibility that frequency of the skeletal character might alter with age, as seems to happen with foramina transversaria imperfecta (f.t.i.), owing to bone growth closing slight gaps.

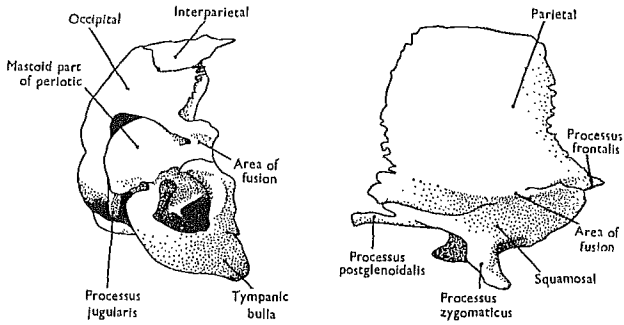

Fig. 1. Lateral views of C57BL skull abnormalities. Left, fusion of occipital and periotic bone of auditory capsule; right, fusion of parietal and squamosal bones. Camera lucida drawings.

Mice were weighed at birth and death and classified for the following variants: dyssymphysis of atlas and axis, atlas-axis fusion, other cervical fusions, f.t.i. of CIII–CVI, absence of the tuberculum anterius, poor development of the processus spinosus of ThII with possible shift on to ThI (all described by Grüneberg, 1950), size of lower third molars, dystopia of the sacrum, dyssymphysis of ThI (Searle, 1954*a*), interfrontals, parted frontals (Truslove, 1952), fusion of occipital to periotic bone, fusion of squamosal and parietal bones. These last two characters, shown in Fig. 1, have not been described previously. In the first, the lateral wing of the large occipital bone becomes fused with a process from the periotic, not normally present. So the whole auditory capsule remains in position in papain preparations, instead of dropping out as is usual. The second anomaly involves a partial fusion between the squamosal and parietal, both membrane bones. Both characters seem to be clear-cut examples of 'quasi-continuous' variation (Grüneberg, 1952*a*); neither are commoner in older mice, so the fusions are certainly not just age effects. The orientations in which teeth were measured in this and later experiments are shown in Fig. 2; for upper and lower molars the bucco-lingual diameter of the crown was taken, for the left lower first molar it was the cranio-caudal length. The third molar to be measured was picked at random with respect to whether left or right; in the A strain experiment, however, both were measured wherever possible and the mean taken.

No significant differences were found between controls and treated mice in birth weight or in the mean litter size, which was 5·6 for all litters combined. The mean death weight

however, is significantly lower in each sex of the treated animals than in the controls (Table 1). Comparing the weights of controls and those on 0·1% thiouracil, for males $t=4·21$ and $n=20$; for females $t=4·91$ and $n=36$; for both $P<0·001$. So in this substrain of C57BL there is some failure to maintain normal weight on thiouracil.

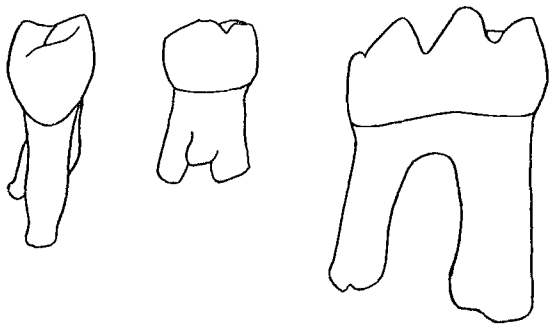

Fig. 2. Orientations of teeth for measurement of crown size. Left, lower third molar; centre, upper third molar; right, left lower first molar.

Table 1. *The effect of thiouracil on 6-week weight in grams of* C57BL *mice*

	Controls	0·05% thiouracil	0·1% thiouracil
Males	20·37	15·96	14·78
Females	16·94	13·82	12·96

Table 2. *Thiouracil treatment and foramina transversaria imperfecta*

	Male foramina			Female foramina		
% thiouracil	F.t.i.	Total	% f.t.i.	F.t.i.	Total	% f.t.i.
0	93	208	44·7	92	208	44·2
0·05	48	167	28·7	76	208	36·5
0·1	49	136	36·0	85	191	44·5
	$\chi^2_2=8·12$, $P=0·02$			$\chi^2_2=3·45$, $P=0·18$		

Table 3. *Thiouracil treatment and the processus spinosus of* ThII

% thiouracil	P.s. − or +	P.s. + + or + + +	Total	% − or +
0	13	59	72	18·1
0·05	4	44	48	8·3
0·1	1	43	44	2·3

Combining last two rows, $\chi^2_1=5·35$ and $P=0·02$.

There were few significant differences between treatments in the frequencies of variants. Table 2 shows that there is significant heterogeneity with respect to f.t.i. But the lowest frequency is found with the intermediate treatment, which throws some doubt on the real origin of the heterogeneity. There is a significant rise in the frequency of well-developed spinous processes (+ + or + + +) on ThII with thiouracil treatment (Table 3). With retardation alone one might expect the opposite trend, so this seems to be a real effect of thiouracil on the maternal physiology.

Lower third molar size has been shown by Grüneberg (1951) to be highly sensitive to maternal influences in the CBA strain, in which these teeth may be absent altogether. Thiouracil treatment leads to a significant fall in their mean size in the C57BL strain,

from 0·632 mm. for controls to 0·609 mm. for mice on 0·1 % thiouracil ($t = 5{\cdot}75$, $n = 94$ and $P < 0{\cdot}001$). Distributions are shown in Table 4 and Fig. 3. As will be shown later, third molar size is not fully determined at birth; post-natal factors connected with the mother's milk seem to be important.

These results show that thiouracil does have some slight effect on the frequency of variants, through the post-natal and possibly the pre-natal maternal physiology; it also causes some failure to maintain normal growth after weaning.

Table 4. *Thiouracil treatment and size of lower third molars in mm./100*

% thiouracil	53	54	55	56	57	58	59	60	61	62	63	64	65	Total
0	—	—	—	—	—	—	1	1	8	8	16	14	4	52
0·05	—	—	—	1	—	3	1	12	6	6	10	6	1	46
0·1	1	—	—	3	3	1	3	7	5	12	8	1	—	44

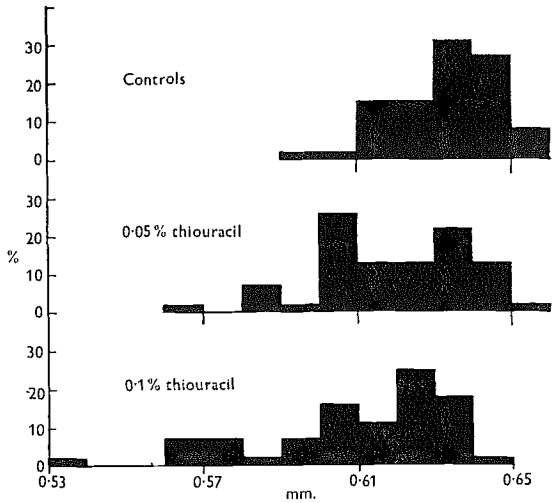

Fig. 3. Grouped percentage distributions of lower third molar size in control and thiouracil treated C57BL mice.

The effect of a radical change of diet on C57BL/Gr mice

Offspring of mice kept since weaning on a handful of oats every alternate day, with a small quantity of hay about once a week, were compared with closely related controls on diet 86. These controls were the same as in the thiouracil experiment; except where there was a significant or near-significant lack of homogeneity for a particular character the thiouracil-treated mice were also included as controls, so as to increase the numbers available for analysis. The oats diet is low in protein, since Heathcote (1950) found that dry oat kernels only contain between 1·8 and 3·4 % nitrogen (which equals 10–20 % protein), and the poor quality hay can have provided very little, though most of it was eaten. The dry weight of diet 86, however, is 23 % protein.

The mice on oats and hay did manage to breed, but litter sizes were low, with a mean of only 4·5. Offspring were very retarded: the mean weight of males when killed at 60 days was only 12 g., compared with over 20 on a normal diet. The same marked reduction in litter size and poor growth were observed by Young (1953) in the C57BL/Fa

strain fed on crushed oats, as compared with a normal diet; in the R III strain, however, litter size did not change.

Birth weights are also significantly lower on the poorer diet (Table 5), so the dietary change has clearly affected the offspring's pre-natal as well as post-natal environment. This was also borne out by some striking changes in the frequencies of particular skeletal variants.

Table 5. *The effect of an oats diet on birth-weight in strain* C57 BL/Gr

Litter-size	Mean birth weights in g. Diet 86	Oats	Difference in means	t	n	P
3–5	1·419	1·326	0·093	2·14	74	0·05
6–8	1·301	1·215	0·086	3·55	119	0·01

Table 6. *Frequencies of* C57 BL *variants on oats and on a normal diet*

Character	Diet 86 Affected	Total	%	Oats Affected	Total	%	χ^2_1	P
Interfrontal	58	165	36·5	40	70	57·1	8·27	0·004*
Parted frontals	2	56	3·6	14	70	20·0	6·17	0·015*
Squamosal-parietal fusion	26	165	15·8	1	70	1·4	8·56	0·004*
Occipital-periotic fusion	13	165	7·9	3	70	4·3	0·51	0·50
Dyssymphysis of atlas and axis	85	165	51·5	36	70	51·4	0·0001	0·99
Foramina transv. imperfecta	185	416	44·5	174	504	34·5	9·49	0·002*
Missing tuberculum anterius	52	165	31·5	10	69	14·5	7·22	0·007*
Dyssymphysis of Th I	16	165	9·7	36	69	52·2	50·8	0·000*
Dyssymphysis of Th II	0	164	0·0	11	67	16·4	—	0·000*
P.s. of Th II small or absent	13	72	18·1	4	67	6·0	3·66	0·06
Cervical fusions other than atlas-axis	6	165	3·6	9	70	12·9	6·99	0·008*
Size of lower third molars	0·632 mm.			0·596 mm., $t=9·75$, $n=115$				0·000*

* Significant.

Nine out of twelve anomalies studied show significant changes in frequency on oats. Those apparently unaffected are the fusion of occipital and periotic, dyssymphysis of atlas and axis, and variation in the processus spinosus of Th II. Table 6 summarizes the results, showing that the frequencies of some variants, such as parted frontals and dyssymphyses of Th I and Th II, greatly increase, but those of squamosal-parietal fusion, missing tuberculum anterius etc. actually decrease. One of the most interesting changes in frequency is that for dyssymphysis of Th II (illustrated by Searle, 1954*b*), in which the usually prominent spinous process is centrally split, usually completely, but sometimes only proximally. This anomaly, absent from the controls, is present in one-sixth of the mice on oats. It was also found in one out of 338 males and six out of 397 females in a distant branch of the same C57 BL/Gr substrain (Searle, 1954*b*), which shows its normal great rarity in this line. But Dr Grüneberg informs me that in a different C57 BL substrain, namely, C57 BL–*b*, kept in the United States, eight out of a sample of ten mice examined by him showed exactly the same anomaly, which has so far not been found in any other pure line.

Table 6 also shows that there is a highly significant reduction in the mean size of third molar teeth on oats, similar to but more pronounced than the reduction with thiouracil treatment.

THE EFFECT OF A SLIGHT CHANGE OF DIET ON MICE OF STRAIN A

The diets used, both in cube form, were no. 1 of the Rowett Research Institute, with 7% skim milk, and no. 86, without skim milk but with more fish-meal, yeast and cod liver oil than diet 1. Actual compositions are:

	Diet 1	Diet 86
Moisture	15·2	14·3
Soluble carbohydrate	51·6	53·4
Protein	20·3	20·0
Fat	4·4	3·8
Fibre	4·3	3·3
Ash	4·2	5·2

Diet 86 is reputed to give better reproduction than diet 1, which needs supplements to bring it up to the same level.

Mice from a number of closely related litters, all on diet 1, were mated up at 4 weeks, half the pairs (some from each litter) being placed on diet 86. The first four litters from each pair were killed at 4 weeks and skeletal preparations made. Some mice, however, were kept back to provide material for a second set of matings, put up about 6 months after the first to give some evidence on seasonal changes.

Table 7. *Litter size with parity in* A *strain mice on diets* 1 *and* 86; *main experiment*

	Diet 1			Diet 86		
Litter	No. born	No. of litters	Mean	No. born	No. of litters	Mean
a	145	22	6·6	109	18	6·1
b	91	21	4·3	103	18	5·6
c	69	13	5·3	117	17	6·9
d	49	11	4·5	102	14	7·3
Total	354	67	5·28	431	67	6·43

Table 8. *Effect of diet and season on mean third molar size in mm./*100 *of* A *strain mice*

Experiment	Lower m_3			Upper m_3		
	Diet 1	Diet 86	Difference	Diet 1	Diet 86	Difference
Main	53·22	55·13	1·91±0·38*	59·21	62·68	3·47±0·47*
Second	55·07	56·02	0·95±0·44*	62·03	63·72	1·69±0·70*
Difference	1·85±0·41*	0·89±0·41*		2·82±0·60*	1·04±0·60	

* Significant.

Diet 86 did indeed seem to be slightly superior to diet 1, increasing both litter size (Table 7) and mean birth weight. For diet 1, the regression coefficient of birth weight against litter size is $Y = 1{\cdot}265 - 0{\cdot}02\ (x - 6{\cdot}49)$; for diet 86 it is $Y = 1{\cdot}304 - 0{\cdot}02\ (x - 7{\cdot}55)$, giving on the average a difference of 0·06 g. for each size of litter, which is highly significant.

No significant changes were found in the frequency of dyssymphysis posterior of the axis (Grüneberg, 1950), absence of the processus spinosus of Th II, lumbo-sacral variation, nor in the incidence of harelip, which was 7·7%. In the main experiment, however, there is a significant increase in upper and lower third molar size on diet 86 (Table 8), but there is little change in the crown size of lower first molars, with a mean of 1·495 mm. In the smaller second experiment, set up to test seasonal effects, third molars from diet 86 are

again larger, but the difference in means is only just over twice its standard error (Table 8). It is interesting to note that the sizes of both upper and lower third molars on both diets have risen in the second experiment, significantly so in three out of the four categories, which makes it very probable that some seasonal factor is operating.

In the A strain, as in CBA, lower third molars are sometimes absent altogether (Searle, 1954*a*), especially in early litters from young mothers. Teeth in later litters are also significantly larger (Searle, 1954*c*), which indicates that, as in the CBA strain (Grüneberg, 1951), the smaller the potential size of a tooth the more likely it is to disappear altogether in the course of development. One would therefore expect that the fall in the mean size of lower third molars on diet 1 would be accompanied by an increase in the number of missing molars. This does in fact seem to happen; four out of 100 mice on diet 1 were affected, but only one out of 116 on diet 86. The difference is, however, not significant on a sample of this size ($P=0{\cdot}142$, by Fisher's exact method).

Table 9. *Partial correlations of tooth size with birth weight and weight at 4 weeks: strain* A, *diet* 86

Characters compared	Lower m_3	Upper m_3	Lower m_1	Factor eliminated
Birth weight	0·26	0·28	0·57	4-week weight
4-week weight	0·58	0·34	0·21	Birth weight

For the diet 86 material of the main experiment a series of partial correlations were calculated (Table 9) so as to give some idea of how far tooth sizes depend on birth weight and how far on post-natal factors, using weight at 4 weeks as a measure of the latter. As Grüneberg (1951) remarks, there is a very close parallelism between the sources of variation of third molar size and of birth weight, possibly because of some relationship between the two characters. The partial correlations found suggest that lower third molar size is strongly influenced by post-natal growth factors; on the other hand, the size of the first molar, which erupts much earlier than the third, depends largely on pre-natal influences, of which those determining the birth weight are important. The upper third molar is, if anything, intermediate in its relationships.

These findings suggest that the increase in third molar size with a change from diet 1 to diet 86 is due to an effect of the changed diet on the mother's milk which, in turn, influences the post-natal development of these teeth. In order to find out if milk factors alone can change tooth size, reciprocal fostering was carried out between the offspring of A strain females and of F_1 females from A × C57BL crosses, both on diet 86. The hybrid mice are very vigorous, and their F_2 offspring's lower third molars are about 7·5 units larger than those of strain A mice. About half of each litter was transferred to the foster-mother as soon as possible after birth; the other half was kept with its own mother. In the pure line, lower third, upper third and lower first molars were measured; in the hybrids, the first and last kinds. The lower third molars of A strain mice suckled on hybrid mothers were significantly larger than those of their litter mates left with their own mothers ($t=3{\cdot}67$, $n=37$ and $P=0{\cdot}001$). The distribution is shown in Fig. 4. Very similar results were found for all the other teeth measured (Table 10), those of F_2 mice being smaller when fostered on pure line females. Even the lower first molars behave in this way; although their change in size is proportionally not so great it is undoubtedly significant.

Table 10. *Mean sizes in mm./100 of* A *strain and* (A × C57BL)F_2 *molars with and without reciprocal fostering between pure line and* F_1 *mothers*

Type of mice	By whom suckled	No.	Lower m_3	Upper m_3	Lower m_1
A strain	Own mother	16	54·6	60·2	147·2
	(A × C57BL) F_1	23	57·0	63·3	149·5
F_2	Own mother	24	62·2	—	149·9
	A strain	17	60·0	—	146·6

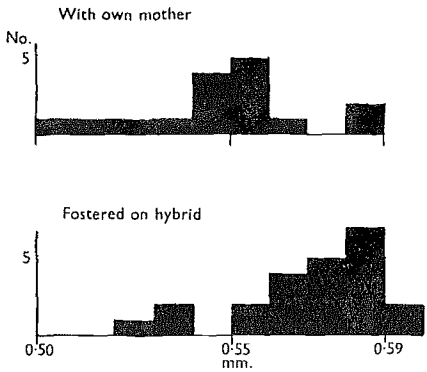

Fig. 4. Size distributions of lower third molars in A strain mice with their own mothers and fostered from birth with hybrid mothers.

Tooth size clearly depends partly on post-natal factors connected with the mother's milk; although no fostering was tried between A-strain mice on the two diets it seems likely that the change in diet affected tooth size through the action of both pre-natal and post-natal factors, judging from its effect on birth weight and from the experiment just described. The milk factors increasing growth of the first molars must act very shortly after birth, for at 3 days the laying down of enamel and dentine to give the definitive crown size is already well advanced, while this process has hardly started in the third molar. The very low partial correlation between lower first molar size and 4-week weight may seem at variance with the results of the fostering experiments, but the milk factors causing an increase in tooth size are not all necessarily general growth promoters. One very important specific milk factor in mice is the 'mammary tumour inciter', with many of the properties of a virus (for references see Grüneberg, 1952*b*). Khanolkar & Chitre (1944) found that C57BL mice suckled by C3H females had a higher serum esterase content than when suckled by their own mothers. In rats, Rutman (1951) has found that females of an inbred strain having a high rate of incorporation of methionine into liver protein induce this higher level in foster-nursed young of a less active strain. So this type of biochemical change may also have occurred in the fostering experiments described above.

Discussion

All three treatments have some effect on skeletal variation, tooth size being particularly sensitive, as was expected from previous work. But, judging from the fostering experiments and other data, the size of the third molar (or, indeed the first) cannot be regarded as an indicator of pre-natal environment alone, as had been thought (Grüneberg, 1951), for differences in lactation undoubtedly help to determine its final size. The delayed

hybrid vigour with respect to lower third molar size found by Grüneberg in CBA × C57 BL F_2 and backcross mice has thus probably been due largely to the superior milk production of the F_1 as compared with the pure-line female. This could easily be tested by suitable fostering experiments. The frequency of most other variants is almost certainly determined by pre-natal factors. The skull anomalies have not been studied from this angle; judging from the advanced state of development of the structures concerned at birth and from other considerations, pre-natal factors determine the frequencies of dyssymphysis of Th I and Th II, cervical fusions, f.t.i. and absence of the tuberculum anterius.

Thiouracil treatment, rather surprisingly, increases the frequency of well-developed spinous processes on Th II; the oats diet acts in the same direction, though a significant level is not quite reached. One might expect that those variants which were found to be particularly sensitive to maternal influences in the original study of the causes of variation (Searle, 1954*a*) would also be particularly sensitive to changes in diet, acting on them through the maternal physiology. But results do not altogether bear this out, although variation in the spinous process and in third molar size are affected by maternal age as well as by dietary factors. For the frequency of cervical fusions is normally quite unaffected by maternal factors, while f.t.i. and absence of the tuberculum anterius are only very slightly affected, yet all three are strongly influenced by the change of diet to oats. Where results from the two studies are not concordant in this respect, it may be suggested that the maternal factors involved are subthreshold on a normal diet, but become important on a poor diet.

The previous study showed that pure-line variants influenced by maternal age invariably become less common in older mothers. But the change to an oats diet does not lead to any general increase or decrease in the frequencies of affected characters. Mice tend to become more normal with respect to some, such as cervical fusions and missing tubercula anteriora, but less normal with respect to others. As shown previously (Searle, 1952), correlations between these characters are generally small or absent, though not every possible test has been made.

Table 11. *Comparisons of variances of lower third molar size on good and poor diets*

Comparison	Control diet	Poor diet	Difference	S.E. of diff.
Diet 86 *v.* oats (C57 BL)	1·748	5·695	3·947	1·065
Diet 86 *v.* diet 1 (A)	8·847	6·700	2·147	1·512

Most of the variation studied in these experiments seems to be 'quasi-continuous' (Grüneberg, 1952*a*), with a physiological threshold splitting into two parts the originally continuous range. A change of diet which affects a variant of this type may do so by altering the position of the threshold, by shifting the mean or by changing the variance of the continuous distribution. Only with the defect of lower third molars in the A strain could the continuous distribution easily be measured as well as the all-or-none frequency. Results suggest that the influence of the dietary change has been to shift the mean of the distribution without altering the position of the threshold. Table 11 shows that there may also be a significant increase in the variance on the poorer diet. This happens with the radical change of diet to oats; with diet 1 versus diet 86 in the A strain the tendency is in the opposite direction, though not significant. It is interesting to note that the variance of strain A lower third molars, like CBA, is much greater than that for C57 BL,

in which there is no tendency for absence of these teeth (apart from one unique individual, discussed by Searle, 1954*a*). This high variance in strains A and CBA is another indication that lower third molars are becoming vestigial in these strains; lower first molars are much less variable.

In considering how far dietary changes will affect outbred populations similarly it must be remembered that several workers (e.g. Grüneberg, 1951; Robertson & Reeve, 1952) have found that the variance of a metrical character may be reduced in the F_1 and even the F_2 generations, as compared with one or both pure lines. Presumably because of this phenomenon and changes in the mean due to heterosis, several of the pure-line variants reappear very rarely or not at all in F_1 and F_2 generations when the pure lines are crossed (Grüneberg, 1950, 1951, 1952*a*). If the change of diet shifts the mean of the continuous distribution towards the threshold this hidden variation may reappear. But decreased variance means greater independence of the environment and therefore, presumably, reduced susceptibility to dietary changes. Experimental data are needed to see what really happens in genetically heterogeneous populations.

Several of the anomalies studied have been found by Weber (1950) in wild populations, with frequency differences between groups which he thought could not be explained as environmental. Results of the present experiments make it much more doubtful whether all the differences Weber found were in fact due to genetic differentiation. The behaviour of dyssymphysis of ThII—becoming common on oats and also on a normal diet in a different C57BL substrain—demonstrates that similar changes in the frequency of a variant may be due either to genetic or to environmental factors. Simple changes in diet may thus give rise to a type of phenocopy. It is clearly of taxonomic importance to differentiate between those characters which are sensitive to such environmental factors and those which are not. For the differences in diet which were used in the present experiment (apart from the thiouracil treatment) are of a type likely to occur between wild populations of an omnivorous species, including man. Extreme changes in phenotype brought about by differences in early nutrition are well known in social insects, leading to the formation of different castes.

The general effects on the offspring of different maternal levels of nutrition have been studied in various domesticated animals; in the sheep, for instance, by Wallace (1948) and Coop (1950). Wallace found that growth of the skull was more severely affected than that of any part of the vertebral column, but he thought that tooth growth was probably little affected by conditions which influence body growth. Coop found that a high level of nutrition during pregnancy increased birth weight slightly but had little influence on the subsequent rate of growth. General effects of poor nutrition, similar to those found by these and other authors, were seen in skeletal material from the oats experiment. In the most retarded mice there was a general reduction in the length of the bones; it was very noticeable in the larger limb-bones that the thickness of the shaft or diaphysis was proportionately much more reduced than that of the epiphysis. Warkany (1945) has reviewed data on the effects of pre-natal nutritional deficiency. He himself has found that restricting riboflavin intake of rats when their foetuses are 13–14 days old leads to many developmental defects, such as cleft palate, shortening of bones, fusions of ribs, sternebrae, fingers and toes. Rather similar effects may be produced in pigs with vitamin A restriction. He considers that since similar malformations are sometimes genetically determined their production in the offspring by maternal deficiencies of well-defined

chemical substances may contribute to an understanding on gene action. But these 'borderline deficiencies' of specific substances, produced under conditions which only just permit maternal reproduction, are hardly comparable with the simpler and more natural dietary changes used here, which nevertheless also change the intra-uterine environment. What remains to be investigated is whether this change is necessarily permanent, or whether a switch back to a good diet in the middle of the mother's reproductive life would produce a quick response. The existence of such variants, apparently very sensitive to maternal influences, should also allow useful studies to be made of the differences in the maternal physiology of hybrid and pure line animals. Other alterations in diet should also be studied to find out how far the sensitivity of individual variants is general, or specifically connected with a particular type of change.

These skeletal anomalies, much influenced by the environment, are certainly very remote effects of gene action and interaction. But so are many characters of economic importance. It seems reasonable to hope that studies on the former may throw some light on the behaviour of the latter.

SUMMARY

1. The effects of changes in parental diet on skeletal variation in the offspring has been studied in mice of the inbred strains A and C57 BL.

2. All three changes (to thiouracil in the drinking water of C57 BL mice, from a good cube diet to oats in C57 BL mice, from a good to a poorer cube diet in A strain mice) reduced the mean size of lower third molars. Results of fostering experiments and other data suggest that this is partly due to post-natal factors connected with the mother's milk, which may even influence the size of first molars.

3. Eight out of eleven C57 BL skeletal anomalies were significantly affected by the change to an oats diet, three becoming rarer and five more common, as shown in Table 6.

4. The frequency of dyssymphysis of the second thoracic vertebra increased from nil to 16% on the oats diet. In quite a different C57 BL substrain this anomaly is common on a normal diet; the oats diet thus produces a type of phenocopy.

5. C57 BL mice on thiouracil were somewhat retarded, but the only clear-cut effect of this substance on skeletal variation was an increase in the proportion of well-developed spinous processes on the second thoracic vertebra.

6. The relation of these results to previous work on maternal influences in pure lines and nutritional deficiency effects is discussed, also their bearing on taxonomic studies.

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REFERENCES

COOP, I. E. (1950). The effect of level of nutrition during pregnancy and during lactation on lamb and wool production of grazing sheep. *J. Agric. Sci.* **40**, 311–40.

DICKIE, M. M. & WOOLLEY, G. (1948). Differences in the reaction of inbred strains of mice to prolonged thiouracil treatment. *Genetics*, **33**, 102 (Abstr.).

GRÜNEBERG, H. (1950). Genetical studies on the skeleton of the mouse. I. Minor variations of the vertebral column. *J. Genet.* **50**, 112–41.

GRÜNEBERG, H. (1951). The genetics of a tooth defect in the mouse. *Proc. Roy. Soc.* B, **138**, 437–51.

GRÜNEBERG, H. (1952*a*). Genetical studies on the skeleton of the mouse. IV. Quasi-continuous variations. *J. Genet.* **51**, 95–114.

GRÜNEBERG, H. (1952*b*). *The Genetics of the Mouse*, 2nd ed., pp. xiv + 650. The Hague: Martinus Nijhoff.

HEATHCOTE, J. G. (1950). The protein quality of oats. *Brit. J. Nutrition*, **4**, 145–54.

KHANOLKAR, V. R. & CHITRE, R. G. (1944). Studies in esterase (butyric) activity. III. *Cancer Res.* **4**, 128–33.

ROBERTSON, F. W. & REEVE, E. C. R. (1952). Heterozygosity, environmental variation and heterosis. *Nature, Lond.*, **170**, 296–8.

RUTMAN, R. J. (1951). The inheritance of rates of methionine incorporation by rats and the relations to growth. *Genetics*, **36**, 54–71.

SEARLE, A. G. (1952). A study of the causes of variation in the skeleton of the mouse, pp. viii + 295. Ph.D. Thesis, London University.

SEARLE, A. G. (1954*a*). Genetical studies on the skeleton of the mouse. IX. Causes of skeletal variation within pure lines. *J. Genet.* **52**, 68–102.

SEARLE, A. G. (1954*b*). Genetical studies on the skeleton of the mouse. X. Rarer variants in the A and C57 BL pure lines. *J. Genet.* **52**, 103–10.

SEARLE, A. G. (1954*c*). The influence of maternal age on development of the skeleton of the mouse. *Ann. N.Y. Acad. Sci.* (in the Press).

SMITH, F. W. & GARDNER, W. U. (1949). Thyroid secretion rate in different stocks of mice. *Anat. Rec.* **103**, 506–7 (Abstr.).

TRUSLOVE, G. M. (1952). Genetical studies on the skeleton of the mouse. V. 'Interfrontal' and 'parted frontals'. *J. Genet.* **51**, 115–22.

WALLACE, L. R. (1948). The growth of limbs before and after birth in relation to the level of nutrition. *J. Agric. Sci.* **38**, 93–153, 243–302 and 367–401.

WARKANY, J. (1945). Manifestations of prenatal nutritional deficiency. *Vitam. & Horm.* **3**, 73–103.

WEBER, W. (1950). Genetical studies on the skeleton of the mouse. III. Skeletal variations in wild populations. *J. Genet.* **50**, 174–8.

YOUNG, G. B. (1953). A study of genotype-environment interaction in mice. *J. Agric. Sci.* **43**, 218–22.