Genet. Res., Camb. (1965), **6**, *pp.* 263–283
With 1 *text-figure*
Printed in Great Britain

Congenital defects as indicators of lifelong abnormal processes

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(*Received* 23 *July* 1964)

1. INTRODUCTION

Congenital defects due to genes are scars in the sense that they are the static end results of abnormal developmental processes. The question arises of whether the abnormal processes which gave rise to them during development are 'burnt out' and thus no longer in operation later on; or whether the abnormal processes may continue into adult life. If so, physiological disturbances of one kind or another may be demonstrable as active processes throughout life and the congenital defect as such may be merely a label for a continuing disability of a dynamic nature.

To answer this question a group of thirteen mouse mutants, which were selected for the mildness of their structural anomalies, has been subjected to a general survey. It could be assumed that the congenital defects as such had little or no adverse effect on the fitness of the mouse, at least under cage conditions. To be successful, a search for unspecified physiological disturbances of one kind or another has to be based on the measurement of variables so general that they are likely to be influenced by many different mechanisms. The parameters chosen were body weight and life span. The data on life span have remained fragmentary. After a period of over 2 years, the outbreak of a virus infection—the first in 30 years in this laboratory—necessitated the premature termination of the experiment. This paper is thus based entirely on body-weight determinations.

On this basis, evidence will be presented in this paper indicating that in eight out of the thirteen mutants screened, there is a *prima facie* case for the existence of active physiological disturbances of one kind or another. But, whereas the unspecific parameter is useful for the discovery of such disturbances, it gives virtually no information as to their nature. Further work will thus be required to narrow down the possibilities with a view to the eventual identification of the physiological disturbances caused by the various genes in adult life.

2. MATERIALS AND METHODS

With the exception of the gene for Tail-short (*Ts*), the thirteen mouse mutants used in this experiment (Table 1) were kept in genetically heterogeneous stocks. Mutants and their normal litter mates thus have the same genetic background, but

stocks differ greatly from each other in this respect. To prevent complications due to breeding, the animals were kept in groups of 5 ♂♂ or 5 ♀♀ to the cage; no rearrangement was made subsequently when animals died. The original plan was to have, for each mutant, 20 paired comparisons between a mutant and a normal sib (10 pairs of ♂♂ and 10 pairs of ♀♀, except for the genes for undulated (*un*) and for White (Mi^{wh}) for each of which 10 trios of +/+, +/*un* and *un*/*un* etc. were planned for each sex). This plan had to be modified on account of the severe fighting which broke out in the ♂♂ cages; for four stocks, this was so ferocious and persistent that the attempt to obtain data for the males had to be abandoned; in these, 10 pairs of ♀♀ were substituted for the ♂♂ which had to be discarded. In the other stocks, 5 pairs of ♀♀

Table 1. *The material of the main experiment. The '+' sign stands for the normal and 'm' for the mutant allele. The genes for Tail-short, Danforth's short-tail, Oligosyndactylism, Patch and Brachyury are lethal in the homozygous condition*

		♂♂			♀♀		
No.	Gene	+/+	+/m	m/m	+/+	+/m	m/m
1	Brachypodism (*bp*)	—	10	10	—	15	15
2	Flexed-tail (*f*)	—	10	10	—	15	15
3	Tail-short (*Ts*)	—	—	—	20	20	—
4	Danforth's short-tail (*Sd*)	10	20	—	15	30	—
5	Undulated (*un*)	10	10	10	15	15	15
6	Short-ear (*se*)	—	—	—	—	20	20
7	Oligosyndactylism (*Os*)	—	—	—	20	20	—
8	Patch (*Ph*)	—	—	—	20	20	—
9	Deafness (*dn*)	—	10	10	—	15	15
10	Pintail (*Pt*)	10	10	—	15	15	—
11	Brachyury (*T*)	10	10	—	15	15	—
12	Vestigial-tail (*vt*)	—	10	10	—	15	15
13	White (Mi^{wh})	10	10	10	15	15	15

were added as a precautionary measure; it so happened that in these stocks the ♂♂ ultimately settled down to a peaceful life. Another modification of the original plan was introduced in the case of the gene for Danforth's short-tail (*Sd*) which, on certain genetic backgrounds, has many kidney abnormalities and a correspondingly great early mortality; as a precautionary measure, twice as many mutant as normal animals were included in the experiment; however, in the event, our stock proved to be virtually free of such abnormalities. Altogether, the main set of observations is based on 685 mice which were weighed, to the nearest 0·1 gm., at 4-weekly intervals starting from the age of 42 days. The data are inevitably somewhat selected in favour of the more normal mutant animals, as individuals which died early were not included in the experiment, and the choice naturally favoured the more vigorous animals. Altogether, only 37 out of the 685 mice, or 5·4%, died during their first year. All the animals of the main experiment were kept in the same room (the size of which determined that of the experiment); differences as regards light, temperature

gradients, etc. were randomized by changing the position of all the cages each week. The five mutant animals and their respective controls invariably occupied the two sides of a teak double cage (Grüneberg, 1952, Fig. 96, but with a modified top), and thus underwent all changes of position together. The diet was No. 86 of the Rowett Research Institute, Aberdeen, as supplied by the North-Eastern Agricultural Cooperative Society Ltd., Aberdeen.

A survival experiment of this kind may be considerably influenced by the degree of infestation of the animals with ectoparasites. To minimize this source of error, all the mice of the main experiment were disinfested by a method described by Bateman (1961); this involved dipping the animals in an aqueous solution of two pesticides on two occasions separated by 1–3 weeks, in the interval between weaning and the beginning of the main experiment at 42 days. Later in life, during the second year, the animals tended to get re-infested, but the dipping procedure was not repeated.

Ideally, it would be desirable to start an experiment of this kind at the same time for all the animals. In practice, this cannot be done with supply stocks of limited size. Particularly in stocks where three genotypes are required, litters with such a trio of the same sex are rare. As a result the building up of the whole experiment took many months, with the genes *un* and Mi^{wh} the last to be completed and thus the least advanced when the experiment had to be terminated.

The present analysis is based, for eleven out of the thirteen mutants tested, on a comparison of the average weight of a group of mutant animals with that of their normal litter mates throughout life. The animals being thus present in litter-mate pairs, when one died the other was disregarded in the calculation of subsequent averages (though, of course, complete records were obtained for each animal to the end of its life). In the case of the mutants *un* and Mi^{wh} where trios are included in the experiment, the death of a mutant homozygote led to the elimination from the averages of its $+/+$ partner for that comparison, although the $+/+$ individual was retained in the average used for the comparison between $+/+$ and $+/un$. However, when a $+/un$ mouse died, the weights of the remaining two animals have been retained in the average values. The final weight of a mouse is often greatly distorted by terminal events which are irrelevant for our present enquiry. For instance, a moribund mouse may fail to eat and drink and thus lose weight rapidly in the last few days; conversely, water retention (pleural exudates, ascites) or the growth of tumours may lead to a spurious increase in weight which is sometimes considerable. To minimize such irregularities the final weight of a mouse has been disregarded if the animal died within a fortnight of its being weighed.

In addition to the main experiment, subsidiary data are available for some of the mutants for the period from birth to 42 days, and sometimes beyond. These, unfortunately, were less complete at the conclusion of the experiment than the main data. Altogether, this paper is based on over 20,000 individual weighings.

For details and full references to the mutants used (except for deafness), the reader is referred to a recent book by one of us (Grüneberg, 1963).

The building up of the experiment from the supply stocks has been in the hands of

G. M. T. The animals in the experiment have been wholly maintained and cared for by J. M. G. who has also done nearly all the weighings and kept all the records. For the plan of the experiment, the evaluation of the data and the drafting of this paper the senior author takes full responsibility.

3. RESULTS

(i) *Brachypodism*

Brachypodism (symbol *bp*, linkage group 5; Landauer, 1952) causes a drastic shortening of hands and feet and, to a lesser extent, of the long limb bones. The rest of the skeleton is quite normal. The condition has been traced to the 12-day stage of embryonic development, i.e., to the membranous skeleton (Grüneberg, unpublished). Of all the mutants included in this experiment, brachypodism is probably the only one which handicaps the mouse to some extent by virtue of its inability to climb and jump though, under cage conditions, this is probably of little account. Also, due to the shortness of their limbs and curved overgrowth of their claws, brachypods have difficulties in grooming themselves.

The growth curves of brachypod mice and their normal controls, from 42 days onwards, are given separately for the two sexes in Fig. 1. For each sex there is also a curve which expresses the weight of the brachypods (A, for abnormal) in terms of that of their normal litter mates (N); these A/N values are thus independent of the actual weights of the animals. In both sexes the brachypods are, and remain, consistently lighter than their normal litter mates from the beginning of the curves. Males, with an average A/N value of 0·841 for the first year, are slightly more affected than the females (A/N = 0·875). During the first year the A/N values for both sexes are almost constant; later, in the ♀♀, the brachypods gradually diverge from their normal sisters even more, as the normal females continue to gain weight slowly whereas the brachypod ♀♀ do not. Towards the end of the curves, irregularities tend to arise on account of the small number of surviving pairs on which the values are based; for instance, the sudden upturn of the A/N curve for ♂♂ at the end is based on a single pair and is clearly of no significance. As the behaviour of most mutants is quite consistent throughout life, subsequent discussion will be based mainly on the first year (or rather, for the sake of convenience, up to the age of 378 days).

The weight differences (Table 2) are all highly significant and, with one exception, the respective P values are smaller than 0·001. Needless to say, successive weighings of the same group of animals are highly correlated with each other. None the less, by testing the significance of a difference at various ages, chance fluctuations are reduced, a precaution clearly unnecessary where, as in the present case, the significance of the difference is beyond any doubt.

The striking reduction in weight of brachypod mice is not yet present at birth (Table 3), but develops mainly during the first week *post partum*. The birth weights in Table 3 are based on only eight animals each. Using the whole of our material and pooling the sexes, the mean birth weight of 46 brachypod mice was 1·317 ± 0·019 gm. and that of their 46 normal sibs 1·354 ± 0·024 gm. The difference (0·037 ± 0·031 gm.)

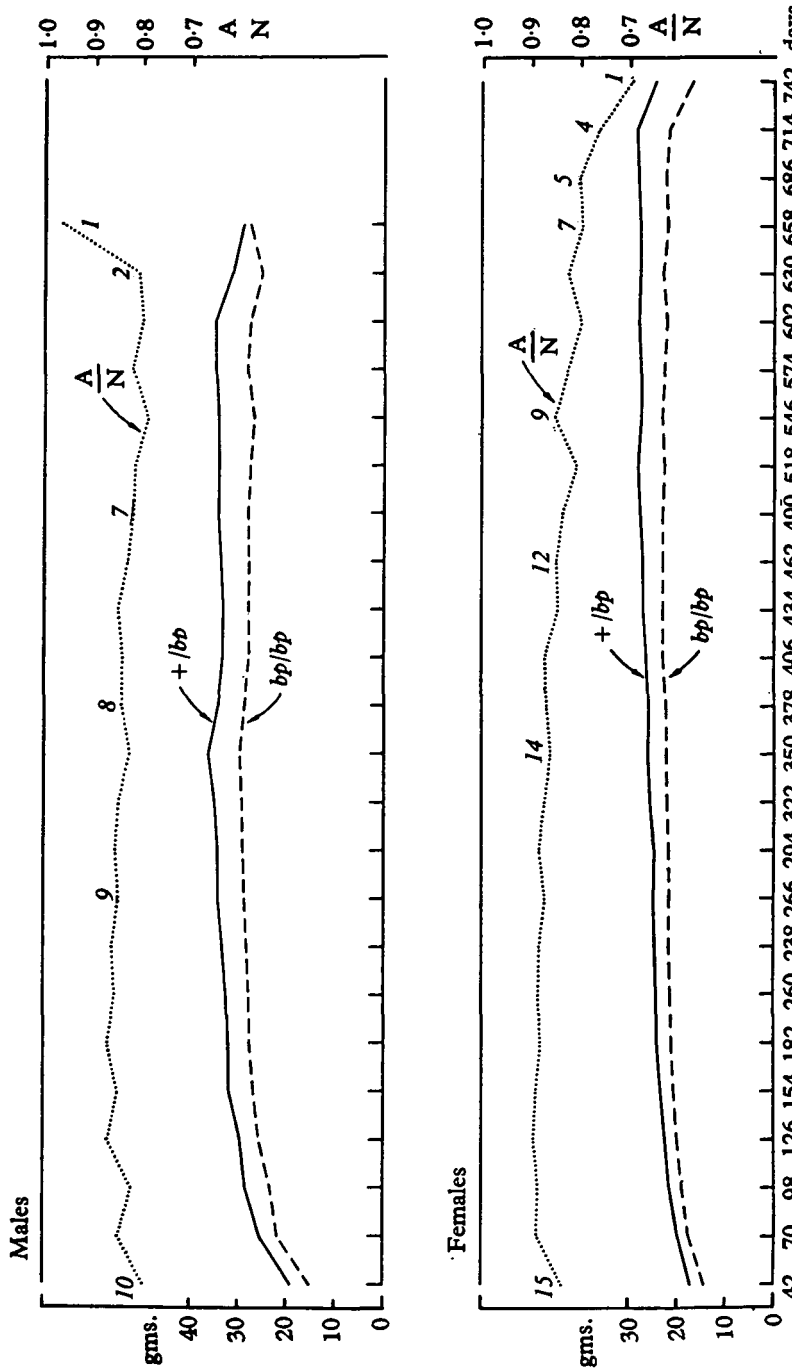

Fig. 1. Growth curves of brachypod and normal mice, and A/N values. The figures next to A/N curves indicate the number of pairs of animals on which the values are based.

https://doi.org/10.1017/S0016672300004146 Published online by Cambridge University Press

Table 2. *Brachypodism. Body weight up to the age of* 378 *days. Main experiment. The number of degrees of freedom on which the various* t*-values are based are given in brackets*

	♂♂				♀♀			
Age (days)	*bp/bp* (A)	+/*bp* (N)	A/N	*t*	*bp/bp* (A)	+/*bp* (N)	A/N	*t*
42	15·1	19·0	0·795	5·372 (9)	14·2	17·0	0·835	6·573 (14)
70	21·5	25·3	0·843		17·5	19·8	0·884	
98	23·0	28·2	0·816	7·187 (9)	18·8	21·3	0·883	7·605 (14)
126	25·3	29·2	0·866		19·7	22·1	0·891	
154	26·6	31·5	0·844		20·4	23·0	0·887	
182	27·4	31·7	0·864	5·787 (9)	21·0	23·9	0·878	5·231 (14)
210	27·4	32·2	0·851		21·2	24·0	0·883	
238	28·2	33·0	0·855		21·4	24·3	0·881	
266	28·5	33·8	0·843		21·4	24·6	0·870	
294	28·8	33·9	0·850		21·5	24·4	0·881	
322	29·0	34·4	0·843		22·0	25·3	0·870	
350	29·4	35·8	0·821		22·1	25·7	0·860	
378	28·5	34·0	0·838	2·895 (7)	22·2	25·6	0·867	6·014 (13)
Mean			0·841				0·875	

is not significant, and the A/N value of 0·973 thus does not differ significantly from unity. None the less, it must probably be regarded as real, in view of the findings of Landauer (brachypod: 1·537 ± 0·0187 gm. and normal: 1·607 ± 0·0233 gm.; the difference is 0·070 ± 0·0299 gm., and A/N = 0·956). Assuming, then, that

Table 3. *Brachypodism. Weights of eight pairs of* ♀♀ *from birth to* 70 *days. Four of these pairs only were weighed at the ages of* 7 *and* 21 *days, and one pair failed to survive to* 70 *days*

Age (days)	*bp/bp* (A)	+/*bp* (N)	A/N	*t*
0	1·29	1·29	1·000	
7	3·73	4·18	0·892	
14	5·89	6·73	0·875	3·200 (7)
21	7·16	8·00	0·895	
28	8·66	12·0	0·722	8·866 (7)
42	13·8	17·0	0·812	6·559 (7)
70	17·5	20·5	0·854	8·499 (6)

the difference in our material is real, and adding to it twice its standard error, the weights become 1·285 and 1·385 gm. respectively, and A/N = 0·928. Hence even if our data underestimate the difference by twice its standard error, it would still remain true that brachypods deteriorate considerably in post-natal life.

(ii) *Flexed-tail*

The recessive gene for flexed-tail (symbol *f*; linkage group 14; Hunt, Mixter & Permar, 1933) causes a triad of symptoms. Two of these, belly and tail-spotting and ankyloses between vertebrae (mostly in the tail region) are very variable in expres-

sion and do not always manifest themselves. As such, neither spotting nor the tail flexures can have any significant effect on the fitness of the mouse under cage conditions. The constant feature of the syndrome is a characteristic embryonic

Table 4. *Flexed-tail. Body weight. Main experiment*

Age	♂♂				♀♀			
(days)	*f/f*	*+/f*	A/N	*t*	*f/f*	*+/f*	A/N	*t*
42	16·4	18·8	0·872	4·539 (9)	14·4	16·5	0·873	4·397 (14)
70	21·5	24·9	0·863		17·2	19·0	0·905	
98	23·4	27·1	0·863	5·148 (9)	18·9	21·0	0·900	3·660 (13)
126	25·0	28·7	0·871		20·1	22·3	0·901	
154	25·8	30·2	0·854		20·4	23·3	0·876	
182	27·0	31·2	0·865	4·453 (9)	20·7	24·1	0·859	3·366 (13)
266	28·3	32·7	0·865		21·7	25·5	0·851	
378	27·7	33·8	0·820	7·660 (7)	22·6	26·1	0·862	3·288 (13)
Mean			0·855*				0·868*	

* Based on the complete range of values available.

Table 5. *Flexed-tail. Early weights of females*

Age (days)	*f/f* (A)	*+/f* (N)	A/N	*t*
0	1·16	1·36	0·853	5·129 (12)
14	5·69	6·57	0·866	5·454 (12)
28	9·5	12·2	0·779	7·526 (12)
42	13·1	16·3	0·804	13·600 (12)
70	17·1	20·5	0·834	10·589 (10)

Table 6. Post partum *depression in the early weights of flexed-tail mice* (*sexes combined*). *Each mouse was weighed only once, and the successive values are thus independent of each other. In the first series, each value is based on* 15 *animals, except the* 3*-week values* (12 *animals each*). *In the second experiment, each value is based on* 10 *animals. From Grüneberg* (*1942*a).

Age (days)	0–1	7	13–14	20–21
Flexed-tail (A)	1·34	3·33	5·08	6·73
Normal (N)	1·72	4·69	6·36	9·26
A/N	0·78	0·71	0·80	0·73
Flexed-tail (A)	1·27	3·53	5·13	7·17
Normal (N)	1·65	5·12	6·43	9·01
A/N	0·77	0·69	0·80	0·80

anaemia (Grüneberg, 1942*a*, 1942*b*) which is normocytic hypochromic and peculiar in the presence of numerous red cells with free iron (siderocytes); these cells, in fact, were first discovered in this mutant. The embryonic anaemia disappears spontaneously during the first month after birth and, later in life, the red blood picture is

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normal or nearly so as judged by the usual criteria; we shall come back to an exception later on.

As in the case of brachypodism, *f/f* mice of both sexes are consistently lighter than their normal litter mates (Table 4); the difference persists virtually unchanged throughout life. P values are 0·01 or less. Whereas in brachypodism the birth weight is still almost normal, in flexed-tail (Table 5) a massive difference is present at birth. Superimposed on this is a *post partum* depression (Table 6) which has disappeared again at the age of a fortnight, and a weaning depression at 28 days (Table 5). None the less, even after the clinical anaemia has disappeared the A/N value remains essentially stable at about 0·86 (Table 4).

(iii) *Tail-short*

The gene for Tail-short (symbol *Ts*; Morgan, 1950; Deol, 1961) is semi-dominant and an early embryonic lethal in homozygous condition. In *Ts*/+ heterozygotes, there is a widespread but disparate involvement of many parts of the skeleton. The effects include variable and irregular malformations of skull and vertebral column

Table 7. *Tail-short females. Subsidiary data on early weights above; main experiment below*

Age (days)	*Ts*/+ (A)	+/+ (N)	A/N	*t*
0	1·17	1·44	0·813	8·891 (14)
14	5·82	6·99	0·833	5·145 (14)
28	10·3	12·7	0·811	9·507 (14)
42	14·8	17·3	0·855	9·121 (13)
70	18·1	20·3	0·887	6·029 (13)
Mean			0·840	
42	14·3	16·8	0·851	8·461 (19)
70	17·4	19·8	0·879	
98	18·8	21·8	0·862	9·232 (19)
126	20·0	23·5	0·851	
154	20·9	24·7	0·846	
182	21·6	26·0	0·831	7·539 (19)
266	23·5	28·0	0·839	
378	24·5	29·6	0·828	5·042 (12)
Mean			0·841*	

* Based on the complete range of values available for the main experiment.

which, in themselves, are generally without obvious bearing on fitness under cage conditions. In addition there are skeletal effects which cannot be considered as malformations, such as a tendency for a reduction in the length of the left humerus and of the right tibia (and sometimes femur); and an occasional restitution of the pollex (which in the normal mouse is rudimentary) to a large triphalangous digit. In embryonic life (Deol, 1961), *Ts*/+ mice suffer from a severe though transitory anaemia and from an early stage, *Ts*/+ embryos are strikingly smaller than their

normal litter mates. The anaemia has been traced back to a deficiency of the blood islands in the yolk sac from which the primitive generation of red blood cells is derived. Like the siderocyte anaemia of flexed-tail mice that of the *Ts*/+ heterozygote disappears spontaneously, though pre-natally. *Ts*/+ mice are strikingly reduced in weight at birth and remain so throughout life; Deol's (1961) data from birth to 6 weeks indicate an essentially stable A/N level of about 0·74. Deol's *Ts*/+ mice were relatively more severely affected than those of Table 7; as the identical stock with *Ts* segregating on the BALB/c background was used, the difference is probably environmental rather than genetic in origin. The A/N values remain essentially stable throughout life; i.e., the abnormal animals show no tendency to catch up with their normal litter mates. On the contrary, there is a tendency for the gap to widen with increasing age (mean A/N for the first year 0·841, for the second year 0·814).

(iv) *Danforth's short-tail*

The semi-dominant gene for Danforth's short-tail (symbol *Sd;* linkage group 5; Dunn, Gluecksohn-Schoenheimer & Bryson, 1940) is lethal in homozygous condition. In the *Sd*/+ heterozygote, the tail is shortened to a varying extent depending on the genetic background. There is an anomalous articulation between atlas and axis with virtual absence of the odontoid process of the axis, and the nuclei pulposi of the intervertebral discs throughout the vertebral column are greatly reduced or absent. On certain genetic backgrounds, but not in the present experiment, there are kidney and ureter abnormalities; the more extreme ones, like renal agenesis, are incompatible with life. The lethal *Sd*/*Sd* homozygote is completely tailless and imperforate; i.e., it has a persisting cloaca and it lacks rectum, anus, bladder, urethra and urogenital papilla along with the metanephros; it dies soon after birth. The common cause of all these abnormalities (Grüneberg, 1958*a*) is to be found in the notochord which is of abnormal structure and reduced size from the start and which disintegrates along its whole length soon after it has been formed; in the tail bud of the *Sd*/*Sd* embryo it is never formed at all. The skeletal abnormalities of the *Sd*/+ heterozygote are so mild that under cage conditions they can have little effect on the fitness of the mouse.

In Table 8 the mean weight of two *Sd*/+ sibs has been compared with that of their normal litter mate, except where one of the two *Sd*/+ mice has died. *Sd*/+ mice are consistently lighter than their normal litter mates; the difference is significant at about 0·05 level in the ♂♂ and below the 0·01 level in the ♀♀.

Subsidiary data for the earlier weights are given in Table 9. Again the *Sd*/+ ♀♀ are smaller than their +/+ sisters, but the difference is less (mean A/N = 0·94 as compared with 0·89 in the main experiment). Although only two of the differences reach the conventional level of significance, main and subsidiary data are clearly in essential agreement with each other. The difference in mean A/N values is presumably, at least in part, due to a change in the genetic background; whereas the original *Sd*/+ stock segregated for *A* versus *a*, it had, without conscious selection, become homozygous for *a* later on, and the reduction of the tail was rather more extreme.

The birth weights in Table 9 are based on only eight animals each. Fuller data are given in Table 10. Pooling the birth weights of the two sexes, the difference between normal and *Sd*/+ mice is 0·053 ± 0·0285 gm. and hence not formally significant.

Table 8. *Danforth's short-tail. Body weight. Main experiment*

Age	♂♂				♀♀			
(days)	*Sd*/+	+/+	A/N	*t*	*Sd*/+	+/+	A/N	*t*
42	18·3	19·7	0·929	2·171 (9)	15·5	17·5	0·886	5·925 (14)
70	23·0	24·3	0·947		18·5	20·2	0·916	
98	25·4	27·7	0·917	2·028 (9)	20·2	22·5	0·898	4·930 (14)
126	27·0	30·6	0·882		21·4	23·4	0·915	
154	28·6	33·2	0·861		21·9	24·4	0·898	
182	29·4	33·3	0·883	2·570 (9)	22·5	25·1	0·896	4·800 (14)
266	29·7	35·2	0·844		23·2	26·2	0·885	
378	30·2	36·0	0·839	2·786 (7)	24·2	27·2	0·890	5·000 (14)
Mean			0·874*				0·891*	

* Based on the complete range of values available.

Table 9. *Danforth's short-tail females. Subsidiary data*

Age (days)	*Sd*/+ (A)	+/+ (N)	A/N	*t*	
0	1·43	1·46	0·979	1·098	(7)
14	6·49	6·79	0·956	1·333	(7)
28	10·3	11·3	0·912	1·666	(7)
42	16·8	17·3	0·971	1·273	(7)
70	19·0	20·3	0·936	1·951	(7)
98	20·4	21·8	0·936	2·123	(7)
126	20·8	22·2	0·937	2·561*	(7)
154	21·7	23·0	0·943	1·865	(7)
182	21·5	24·1	0·892	3·229**	(5)
Mean			0·940		

* P = 0·04. ** P = 0·024.

Table 10. *Danforth's short-tail. Birth weights. The data for ♀♀ include the material of the first line in Table* 9. *Number of individuals in brackets*

	♂♂	♀♀	♂♂+♀♀
Sd/+ (A)	1·419 (23)	1·342 (27)	1·377 ± 0·0176 (50)
+/+ (N)	1·488 (12)	1·416 (16)	1·430 ± 0·0224 (28)
A/N	0·980	0·948	0·963

However, as it is in the expected direction, we are inclined to regard it as real. If so, a difference in weight is already present at birth and, following a weaning depression, it persists unchanged into adult life. There is no evidence that it tends to be outgrown.

(v) *Undulated*

As classified in the living mouse, the gene for undulated (symbol *un;* linkage group 5; Grüneberg, 1950, 1954) is recessive; the tail is shortened and often kinky. The anomalies of the axial skeleton are traceable to faulty differentiation of the sclerotomes and thus precede chondrification. In addition, the acromion of the scapula is replaced by a ligament. Occasionally there is a marked kyphosis and scoliosis in the lower thoracic region; such hunchbacked animals remain runts throughout life. As grossly pathological effects of genes ascribable to their early

Table 11. *Body weights of undulated mice. Main experiment*

Sex	Age	*un/un* A	*+/un* H	+/+ N	A/N	*t*	H/N′*
♂♂	42	15·8	18·0	18·0	0·878	3·523 (9)	1·000
	70	20·7	22·1	22·6	0·916		0·978
	98	22·8	24·2	24·2	0·942	2·717 (9)	1·008
	126	24·2	25·2	26·2	0·924		0·969
	154	24·8	26·4	27·2	0·912		0·978
	182	25·8	27·4	28·5	0·905	2·057 (9)	0·979
	266	27·0	30·1	30·5	0·885		0·987
	378	27·9	31·5	32·0	0·872	3·402 (9)	0·994
	Mean				0·899**		0·988**
♀♀	42	14·4	16·2	16·5	0·873	7·115 (13)	0·982
	70	16·9	18·8	18·7	0·904		1·005
	98	18·5	19·9	20·9	0·885	5·011 (13)	0·957
	126	19·3	21·1	21·6	0·894		0·981
	154	20·1	21·9	22·2	0·905		0·986
	182	20·2	22·7	22·4	0·902	5·131 (13)	1·013
	266	21·2	24·1	24·7	0·858		0·972
	378	21·8	25·9	25·5	0·855	4·385 (12)	1·020
	Mean				0·881**		0·991**

* N′ is the average weight of those +/+ mice which were paired with *+/un* mice for this comparison; they are not always exactly the same individuals as those in the A/N comparison.

** Based on the complete range of values available.

action would interfere with the discovery of the physiological gene effects in adult life, with which we are here concerned, we have eliminated from our averages one such severely affected *un/un* mouse which should not have been included in the experiment in the first instance. Otherwise the skeletal anomalies of *un/un* mice are rather trivial.

In our experiments the presence of the closely linked gene for a^t enabled us to distinguish all three genotypes, except for an occasional crossover; thus a^t *un*/a^t *un* mice were black-and-tan, + +/a^t *un* heterozygotes were light-bellied agouti and + +/+ + homozygous normals grey-bellied agouti in colour.

Undulated mice are consistently lighter than their normal (+/+) litter mates (Table 11); in the ♂♂ the difference is significant at the 0·03 level in three out of the four *t*-values calculated; in the ♀♀, all four P values are smaller than 0·001. The

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A/N value of the ♂♂ remains virtually constant (0·899 in the first, 0·889 in the second year); in ♀♀ there is a downward trend and the A/N value falls from 0·881 in the first to 0·809 in the second year.

The weight of the +/*un* heterozygotes does not differ significantly from that of +/+ mice. Considering the two sexes together, 22 out of 56 H/N values equal unity or exceed it whereas 34 values fall below unity.

Combining the two sexes, undulated mice are slightly lighter at birth than their

Table 12. *Birth weights of undulated mice. Subsidiary data. Number of individuals in brackets*

	♂♂	♀♀	♂♂+♀♀
un/un (A)	1·259 (10)	1·239 (9)	1·249 ± 0·0325 (19)
+/*un*, +/+ (N)	1·327 (12)	1·317 (20)	1·321 ± 0·0238 (32)
A/N	0·949	0·941	0·945

normal litter mates (Table 12). The difference is 0·072 ± 0·0403 gm. and thus not formally significant on the data available. Supposing it is real and has been underestimated by twice its standard error, the birth weights of *un/un* and normal mice would be 1·209 and 1·361 gm. respectively, and A/N would be 0·89, i.e., essentially the mean A/N value of Table 11. Clearly, then, there is no evidence that the difference in birth weight (if real) is outgrown later in life. On the contrary, the data suggest that the divergence between undulated and normal mice tends to increase after birth.

(vi) *Short-ear*

The recessive gene for short-ear (symbol *se;* linkage group 2; Lynch, 1921) has been studied by many authors. Basically, there is a disturbance of the membranous skeleton with widespread but very mild effects; of these, the reduction of the pinna of the ear is the most conspicuous and by far the most extensive one. The morphological anomalies in themselves thus hardly affect the fitness of the mouse. The effect of *se* on growth has been known for a long time (Law, 1938); growth from birth to 12 days has been studied by Green & Green (1942). The data of our main experiment (Table 13) are in essential agreement with the findings of Law; from the age of 42 days onwards, *se/se* mice are consistently smaller than their normal litter mates; the mean A/N value for ♀♀ for the first year is 0·938, and for the period from 406 to 630 days is 0·925. In Law's experiment, the difference between *se/se* and normal mice was greater; his A/N value for ♀♀ at the age of 181 days is 0·871 and that for ♂♂ 0·939. In our own experiment with initially 20 pairs of animals, the smaller difference approaches significance at the age of 182 days and thus, but for the supporting evidence of earlier authors, would not be regarded as decisive. We have no data of our own on the early growth of *se;* those collected by Green & Green (1942) leave no doubt, however, that, on a percentage basis, the difference between normal and *se/se* mice at birth is no greater than it is in adult life; in fact it appears to be smaller if not actually zero.

Table 13. *Short-ear. Body weight of ♀♀. Main experiment*

Age (days)	*se/se*	+/*se*	A/N	*t*	P
42	19·3	20·3	0·951	1·707 (19)	0·11
70	22·9	24·2	0·946		
98	25·0	26·2	0·954	1·718 (18)	0·11
126	26·1	27·3	0·956		
154	26·4	28·1	0·940		
182	27·5	29·1	0·945	2·040 (16)	0·06
266	27·2	29·5	0·922		
378	27·9	30·7	0·909	2·411 (12)	0·035
Mean			0·938*		

* Based on the complete range of values available.

(vii) *Oligosyndactylism*

The semi-dominant gene for Oligosyndactylism (symbol *Os;* linkage group 18, Grüneberg, 1956, 1961) is lethal in homozygous condition. As the name implies reduction of the digits, both in the fore and in the hind limbs, happens either by fusion between adjacent elements or by actual elimination of digit 2; there are also regularly anomalies in carpus and tarsus and in metacarpus and metatarsus. Embryologically, the condition has been traced back to the eleventh day, i.e., to a

Table 14. *Oligosyndactylism. Body weight of ♀♀. Main experiment*

Age (days)	*Os*/+	+/+	A/N	*t*	P
42	16·3	16·9	0·964	1·237 (19)	
70	19·5	19·6	0·995	0·422	
98	21·0	21·2	0·991	1·040	
126	21·9	22·9	0·956	2·633	<0·02
154	22·5	23·8	0·945	2·192	<0·05
182	22·5	24·4	0·922	4·005 (18)	<0·001
210	23·2	24·8	0·935	2·788	<0·02
238	24·0	25·4	0·945	3·236	<0·01
266	24·4	26·1	0·935	3·052	<0·01
294	24·7	26·0	0·950	2·099	~0·05
322	24·9	26·6	0·936	2·925	<0·01
350	24·9	26·9	0·926	3·108	<0·01
378	25·3	27·0	0·934	3·122	<0·01

stage in which no condensations of mesenchyme can yet be detected in the limb buds. As in the other mutants included in this series, the structural anomalies of *Os*/+ mice must be regarded as comparatively trivial.

The data on body weight in the main experiment (Table 14) show no significant difference between *Os*/+ and +/+ mice in the interval between 42 and 98 days. However, from 126 days onwards, the *Os*/+ ♀♀ are significantly smaller than their sisters with little signs of change with age; the mean A/N value for 42–98 days is 0·983 which does not differ significantly from unity; for the rest of the first year, the

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mean A/N value is 0·938 and for the second year it is 0·930. The data of the main experiment thus suggest that *Os*/+ mice do not diverge from their normal sibs until the age of about 100 days. The subsidiary data (Table 15) show, however, that the situation is not so simple. It is true that the birth weight of *Os*/+ mice is about normal; for the 15 pairs of ♀♀ in Table 15 the mean birth weights happen to be identical; pooling all available data for both sexes, the mean birth weight of 41 *Os*/+ animals was 1·421 gm. and that of 42 +/+ litter mates was 1·403 gm. However, whereas *Os*/+ mice have normal birth weights they begin to fall back while still in the nest and a particularly striking drop of the A/N value to 0·864 is found at the age of 28 days; thereafter there is some improvement but the A/N values in the sub-

Table 15. *Oligosyndactylism. Early body weights of* ♀♀. *Subsidiary data*

Age (days)	*Os*/+	+/+	A/N	Pairs weighed	*t*	P
0	1·44	1·44	1·000	15		
7	3·74	3·82	0·979	6		
14	5·93	6·16	0·963	15	2·040 (14)	
21	6·76	7·17	0·943	6		
28	9·85	11·4	0·864	15	4·621 (14)	0·001
42	15·4	16·8	0·917	15	3·427 (14)	0·005
70	20·2	20·8	0·971	12		
98	21·7	22·5	0·964	7		
126	22·1	23·6	0·936	7		
154	22·7	24·0	0·946	7		
182	23·3	24·3	0·959	6		
210	23·9	24·8	0·964	5		

sidiary experiment never quite recover the ground lost; in the main experiment, where similar events presumably happened before the beginning of the weighings, recovery, for the period of 70 to 98 days (Table 14), was substantially complete though indeed not permanent.

(viii) *Patch*

The semi-dominant gene for Patch (symbol *Ph;* linkage group 3; Grüneberg & Truslove, 1960), in the heterozygous condition, is a spotting gene rather like that for piebald spotting; the only other effect discovered is a slight shortening of the skull in the longitudinal and a corresponding widening in the transverse direction with an increase in the size of the interfrontal bone. The majority of *Ph*/*Ph* homozygotes are hydropic and die on the tenth day of embryonic life; others survive to later stages of pregnancy when they have a striking median cleft of the face and numerous subepidermal blebs; few if any of them survive to term.

Considering in this case the subsidiary data (Table 16) first, there is no reason to suspect an adverse effect of *Ph* on growth during the first 6 weeks of life; in particular, there is no sign whatever of a weaning depression such as occurred in at least six out of the seven mutants discussed in the preceding sections.

A very different picture is revealed in the main experiment (Table 17). From the

Table 16. *Early weights of* Ph/+. *Sexes combined; number of mice on which each average is based in brackets*

Age (days)	*Ph*/+	+/+	A/N
0	1·382 (39)	1·417 (48)	0·975
7	3·802 (39)	3·982 (48)	0·955
14	5·953 (38)	6·204 (48)	0·960
21	7·408 (32)	7·480 (42)	0·990
27	10·773 (28)	10·765 (31)	1·001
34	16·634 (23)	16·730 (31)	0·994
41	19·037 (20)	18·452 (25)	1·032
Mean			0·987

Table 17. *Patch. Body weight of* ♀♀. *Main experiment*

Age (days)	*Ph*/+	+/+	A/N	*t*	P
42	16·5	16·9	0·976	0·786 (19)	
70	20·1	21·0	0·957		
98	21·9	23·4	0·936	2·961 (19)	<0·01
126	23·2	24·6	0·943		
154	24·0	25·4	0·945		
182	24·6	26·4	0·932	2·277 (18)	<0·05
266	26·5	28·7	0·923	2·354 (18)	<0·05
378	28·1	30·0	0·937	2·143 (17)	<0·05
490	28·0	30·6	0·915	3·037 (15)	<0·01
602	26·9	29·4	0·915	3·121 (13)	<0·01
714	23·7	28·3	0·837	5·347 (9)	<0·001

age of 98 days onwards, *Ph*/+ mice are significantly lighter than their normal litter mates; the gap widens with increasing age; for the first year the mean A/N value is 0·938, for the second year it is 0·898, and for the age groups of 770–826 days it is 0·814 (the latter based on only a few pairs and correspondingly with a considerable error).

(ix) *The remaining mutants*

Unlike the eight mutants discussed in the preceding sections, the growth curves of these five conditions (Table 1) give no evidence for the existence of active processes persisting beyond the phase in embryonic life during which the structural defects came into being (in *Pt*, *T*, *vt* and Mi^{wh}) or, in the case of *dn*, persisting beyond the process in childhood which leads to the regressive changes in the scala media complex of the cochlea (Deol & Kocher, 1958). In view of the essentially negative nature of the information obtained, the evidence will be given in a very condensed form (Table 18). In each case, tests of significance were carried out for the ages 42, 98, 182 and 378 days. In the three skeletal mutants and in White, none of these values was significant at the 0·05 level. In the case of *dn*/*dn*, the 378-day value for males corresponded to a P value of 0·035, and the 98-day value for females was 0·05 approx.

However, as the sexes deviate from unity in opposite directions, the deviations can probably be ascribed to chance alone.

For *Pt*, *vt* and Mi^{wh}, some birth weights are also available. Pooling the sexes, the mean birth weights of 17 *Pt*/+ and of 17 +/+ young were 1·296 and 1·308 gm. respectively, and A/N = 0·991. Similarly, 25 *vt*/*vt* young weighed 1·362 gm. and

Table 18. *Mean A/N values for the mutant genes* dn, Pt, T, vt *and* Mi^{wh}

Mutant	Sex	1st year	2nd year
Deafness (*dn*/*dn*)	♂♂	0·950	0·928
	♀♀	1·053	1·027
Pintail (*Pt*/+)	♂♂	1·031	0·961
	♀♀	0·967	0·979
Brachyury (*T*/+)	♂♂	1·044	1·044
	♀♀	1·019	1·015
Vestigial-tail (*vt*/*vt*)	♂♂	1·027	1·006
	♀♀	0·986	1·001
White (Mi^{wh}/Mi^{wh})	♂♂	0·947	0·949
	♀♀	0·977	0·974

their 21 +/*vt* litter mates 1·411 gm. with A/N = 0·965. In neither case does the A/N value differ significantly from unity. The same applies to the few birth weights of White which are available.

Whereas the evidence presented is thus entirely negative for these five mutants, this does not prove that the genes in question are inactive in adult life. Indeed, it is not easy to see how such proof could ever be obtained.

In White though the weighings give no such indication, there is *prima facie* evidence of another kind for a continuing gene effect, at least in the Mi^{wh}/+ heterozygote. These animals, in addition to being spotted, also show a general dilution of coat colour; as this dilution persists unchanged through all moults it must be controlled by a continuing gene effect. The mild eye anomalies of the Mi^{wh}/Mi^{wh} mouse as well as the spotting are static end results of early embryonic disturbances.

4. DISCUSSION

In normal development, differences in birth weight tend to be levelled during subsequent growth. Young which were small at birth tend to grow faster than their heavier litter mates. This is illustrated by the data of Table 19. In a sample of 134 ♂♂ of the inbred strain C57BL, the heaviest group exceeds the lightest by 53% at birth, by 39% at weaning and by 19% at 60 days. Between birth and 60 days, the young which were smallest at birth grow fastest and vice versa. Hence the differences in birth weight are progressively reduced (though by no means completely eliminated). The greater part of this levelling process happens after weaning (column C/B).

With one exception the structural anomalies of our eight positive mutants are completely established long before birth. The exception is *se* whose effect on the

development of the pinna extends into the first week *post partum*. Disregarding *se*, it can thus be said (1) a reduction in the birth weight of a mutant may be directly due to the process which gave rise to the structural anomaly; and (2) if that process is 'burnt out' and thus no longer in operation, the difference in birth weight should tend to be levelled (though not necessarily eliminated) during subsequent growth. The facts reported in this paper are quite otherwise. In five out of the seven mutants

Table 19. *The weights of* 134 *C57BL* ♂♂ *at birth, at* 21 *days and at* 60 *days. The material has been divided into six groups by birth weight* (*up to* 1·20 *gm.*, 1·21–1·30 *gm. etc. and* 1·61 *gm. and over*). *Control animals on normal diet; material of Deol & Truslove* (*1957*)

Number of animals	Birth A	21 days B	60 days C	B/A	C/B	C/A
9	1·100	6·60	20·74	6·00	3·14	18·85
24	1·257	7·64	22·23	6·08	2·91	17·68
29	1·355	7·68	22·87	5·67	2·98	16·88
30	1·466	8·15	23·51	5·56	2·88	16·04
28	1·551	9·37	25·16	6·04	2·69	16·22
14	1·684	9·18	24·68	5·45	2·69	14·66
134	1·416	8·23	23·42	5·81	2·85	16·54

of Table 20 birth weight is either normal or nearly so. Most or the whole of the divergence between the mutants and their normal sibs thus develops after birth. In the two instances in which birth weight is seriously reduced (*f*, *Ts*), there is little evidence that the mutants are catching up with their normal sibs later in life. We submit

Table 20. *A/N values at birth and in adult life*

	bp/bp	*f/f*	*Ts/+*	*Sd/+*	*un/un*	*Os/+*	*Ph/+*
Birth	1·000	0·853	0·813	0·979	1·033	1·000	0·975
Adult* ♂♂	0·841	0·855	—	0·874	0·899	—	—
Adult* ♀♀	0·875	0·868	0·841	0·891	0·881	0·938	0·938

* Mean value for the period from 42–378 days, except *Os* and *Ph* (126–378 and 98–378 days respectively).

that these facts constitute a strong *prima facie* case for the existence, in these mutants (and presumably in *se* as well), of abnormal processes which continue actively throughout adult life.

In the life of all mammals there occur three natural periods of stress which leave their mark on the growth curve. Leaving aside the embryo and the foetus, the first of these periods begins at birth. In man, an actual loss of weight is followed by a gradual recovery such that, during the second week, the birth weight is reached again. In the mouse, this *post partum* depression is variable; in some litters, growth is slow in starting for a day or two but rapidly accelerates thereafter; most litters show little

or no sign of a *post partum* depression. During the third week, as the young start leaving the nest and the milk supply becomes increasingly inadequate for the requirements of the litter, growth slows down; the completion of weaning at 20–21 days precipitates a period of slow growth (weaning depression) and sometimes actual weight losses until the young have mastered the situation. The third period of stress, the senile depression, has an insidious onset with a gradual loss of weight; in the nine stocks for which data are available, ♂♂ reached their maximum weight between 294 and 462 days (average 390 days); similarly, ♀♀ reached their maximum weight between 490 and 658 days (average of thirteen stocks 550 days). After a plateau of varying duration, and sometimes without a plateau, body weight gradually declines. Evidently, aging animals live under some stress even in an optimal environment.

An additional stress (in the main experiment) was due to the disinfestation; it

Table 21. *A/N values from birth to* 70 *days for seven mutant genes. Subsidiary data, from Tables* 3, 5, 7, 9, 15 *and* 16, *in some cases slightly augmented. The data for undulated are based on four pairs of animals not tabulated elsewhere. No data are available for* se

Age (days)	*bp/bp*	*f/f*	*Ts/+*	*Sd/+*	*un/un*	*Os/+*	*Ph/+*
0	1·000	0·853	0·813	0·979	1·033	1·000	0·975
7	0·892				1·021	0·979	0·955
14	0·875	0·866	0·833	0·956	1·051	0·963	0·960
21	0·895				1·002	0·943	0·990
28	0·722	0·779	0·811	0·912	0·840	0·864	1·001
42	0·812	0·804	0·855	0·971	0·965	0·917	1·032
70	0·854	0·834	0·887	0·936	0·968	0·971	

caused a definite mortality and thus may have biased the samples in favour of the more vigorous mutant animals; it presumably superimposed its effect on the weaning depression; to what extent this was counterbalanced by the long-term benefit of being free of ectoparasites is another question. The animals for the subsidiary data were not disinfested.

Yet another source of stress was due to the fact that the experiment had to be built up over a period of many months. Thus the first mouse to be put into a given cage was often all alone for weeks and occasionally for months. On the other hand, the following mice, on entering the experiment at the age of 42 days, found bigger animals already in the cage; fighting would thus tend to establish their position at the bottom of the social scale in the cage until they were fully adult.

A weakness inherent in a mutant may be so marked as to be manifest under all conditions; or it may be latent under optimum conditions but show up during periods of physiological stress; or it may be so slight that only a severe experimental challenge will bring it to the surface. We shall now discuss the response of the eight 'positive' mutants to physiological stress by reference to Table 21.

(*a*) *Post partum depression*

This manifests itself by a significant decline in the A/N value between birth and 7 days. The most striking example is *bp* which lags by as much 11% during the first week. Slight effects may be present in *Os* and *Ph*. The data of Table 21 do not suggest any *post partum* depression for *f;* where 7-day data are available (Table 6), a striking *post partum* depression is in fact revealed which has been outgrown again by the age of a fortnight. The data of Deol (1961) suggest a mild *post partum* depression in *Ts*, and the same may be found for *Sd* when more complete data become available. On the other hand, the figures for *un* (scanty though they are) do not suggest any deterioration between birth and weaning. Table 21 thus includes instances in which the *post partum* depression is striking, cases in which it is mild, and probably one in which it is absent.

(*b*) *Weaning depression*

This is revealed by a sharp fall in the A/N value between 21 and 28 days which may be preceded by a milder fall between 14 and 21 days when weaning starts. In five of the seven mutants (*bp, f, Sd, un* and *Os*) the A/N value for 28 days is clearly a minimum; and to a lesser extent this is also true for *Ts*. In three of these cases, data are available to show that the break in the curves occurs after 21 days, i.e. on completion of the weaning process. On the other hand, there is no indication whatever of a weaning depression in *Ph*. As in the case of the *post-partum* depression, the behaviour of the mutants is thus by no means uniform. The greatest weaning depression occurs in *bp;* evidently, mutants already small at birth like *f* and *Ts* are not necessarily more vulnerable to this kind of stress than mutants with a normal birth weight.

The heterogeneity of the mutants as regards birth weight, *post partum* and weaning depression indicates that the physiological weakness is due to different mechanisms which are specific for each gene.

Inherent weaknesses of mutants can thus evidently be made manifest or accentuated by the normal stresses of mammalian life. Minimum stress may be equated to optimum living conditions, and quantitative differences in A/N values may be due to the fact that in one experiment the animals were living under more nearly optimal conditions than in another.

Following a period of stress, the depression produced in the growth curve may be followed by partial or complete recovery. For instance, the marked *post partum* depression in *f* (Table 6) is followed by a recovery in the next week which, in terms of the A/N value, brings the *f* young back to where they were at the time of birth. In other instances, the downward drift slowly continues, as in *Os* (Table 21). The 28-day minimum of the weaning depression is generally followed by a marked improvement by 42 days and often beyond. Evidently, once the stress is removed, the mutant animals tend to catch up with their normal litter mates as best they can. There is thus not simply a passive carrying forward of an accumulated debt. Where, on the surface, such appears to be the case, a dynamic situation is probably concealed. For instance, in *f* (Table 20), A/N at birth is virtually the same as in adult life. But in the

interval (Table 21), *f* mice have been pulled down to much lower levels first by the *post partum* and then by the weaning depression, and on each occasion they have risen again. But they can never reach normal level because they are being held in check by a continuing physiological weakness of one kind or another. In this particular case there is independent evidence that all is not well with the adult *f* mouse; though there is no sign of any clinical anaemia, some 3% of siderocytes continue to circulate in their blood throughout life (Grüneberg, 1942*b*). Clearly, then, *f* mice do not simply perpetuate a state of affairs already present at birth: they are kept back by a process which continues actively throughout life.

Similar evidence is available for another gene included in this survey, *se*. It has been shown by Green (1958) that the formation of the callus and subsequently of cartilage in artificial rib fractures is inferior in *se*/*se* as compared with + /*se* mice; the animals were operated on at the age of 30–40 days, i.e. long after the known morphological effects of the gene have come into being. Green concluded that 'the *se* locus affects some process necessary to all normal cartilage formation'. It is clear that that process has not come to an end when the structural anomalies of *se*/*se* mice have become established; it either continues or at least it can be reactivated by the conditions of the experiment.

Finally, it has just been published that *Os*/+, in adult life, causes diabetes insipidus of renal origin (Falconer, Latyszewski & Isaacson, 1965). On most genetic backgrounds the increased water intake and excretion is comparatively mild but, in the company of certain 'modifiers' (which actually have a similar effect in their own right), the disturbance becomes extreme. Diabetes insipidus was first observed at the age of 5 weeks; it increases in intensity with age. Presumably, the renal anomaly is in some way connected with the effect of the gene on body weight described in this paper.

Could it be that the structural defects of our mutants are less trivial than has been assumed and that they themselves are responsible for the effects discussed in this paper? If so, one would have to explain why *Ts*, *Sd* and *un* affect growth whereas three mutants with comparable morphological effects like *Pt*, *T* and *vt* do not. The structural deviations of *se* are so mild that they cannot plausibly be invoked, and the same applies with even greater force to *Ph*. Of course it is never possible to answer the appeal to undetected structural anomalies—except by actually demonstrating the nature of the continuing process. This will have to be the ultimate aim of future work.

SUMMARY

Congenital defects due to genes are the static end results of abnormal processes in embryonic development. Are these 'burnt out' and no longer in operation later in life, or do they continue as active physiological processes? To answer this question, thirteen genes with comparatively trivial morphological effects have been subjected to a screen test. For eight of them (*bp*, *f*, *Ts*, *Sd*, *un*, *se*, *Os* and *Ph*) a *prima facie* case has been established for the existence of continuing processes of one kind or another. For the remaining five genes (*dn*, *Pt*, *T*, *vt* and Mi^{wh}) there is no such

evidence though the possibility remains that such processes might be detectable by different means.

We are indebted for advice on the statistical treatment of our data to Professor C. A. B. Smith, for Fig. 1 to Mr A. J. Lee, and for the data of Table 16 to Miss Beryl F. Fannon. The investigation has been supported by a grant from the Rockefeller Foundation which is gratefully acknowledged.

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