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The role of latent viruses in subline differentiation in inbred strains of mice

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SUMMARY

The possibility that changes in latent viruses would contribute to the differentiation of sublines in inbred strains of mice was investigated using the Bittner virus and the BALB/c strain of mice. The results show that, at least for this combination, there is no reason for implicating latent viruses in subline differentiation.

1. INTRODUCTION

Subline differentiation as regards the frequency of morphological variants in inbred strains of mice was reported by Green (1953) and by Grüneberg (1954). The high rates at which sublines are formed (Deol *et al.* 1957, 1960; Carpenter, Grüneberg & Russell, 1957; Grewal, 1962; Yong, 1972) indicated that the phenomenon is difficult to explain satisfactorily as the result of conventional mutations alone. Hence Grüneberg (1970) suggested that the subline differentiation might, in part, be caused by changes in 'latent' viruses in the mouse genome. Beardmore (1970) argued that it could be explained on the basis of the number of loci involved and that more mutations with small effects are being detected by this method.

The existence of latent viruses in the genome as proviruses (Todaro & Huebner, 1972) and their transmission in the genome (Bentvelzen *et al.* 1970) is accepted. Thus there are grounds for implicating changes in viruses in the differentiation of sublines.

It had been shown by Deol & Truslove (1957) that, in the C57BL strain, the incidence of many skeletal variants is correlated with body weight at birth or later in life. There is also evidence that some viruses are, in fact, not strictly latent, but that they affect the physiology of their carriers, albeit mildly. Hence, a change in such a virus might affect body weight and thus indirectly skeletal variants (Grüneberg, 1970). The present investigation was undertaken to test the suggestion that latent viruses could affect the frequency of skeletal variants.

2. MATERIALS AND METHODS

The Bagg Albino (BALB/c) inbred strain of mouse was selected for this study as it is susceptible to the Bittner virus (Bentvelzen *et al.* 1970). The present stock was known to be free of the virus as very few tumours had been recorded since 1963 when the virus had been eliminated. Two lines of descent, A and B, were set up from a pair of mice (Fig. 1), brother–sister mating being maintained throughout the experiment. After separation of A and B, generation 39 was bred as the base line for the study. The virus was introduced into generation 40 of B by fostering

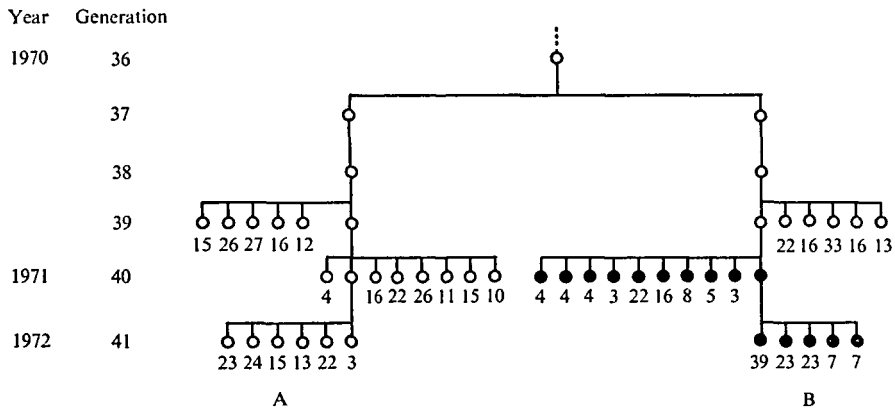

Fig. 1. Pedigree of BALB/c mice used in the experiment. Circles represent mated brother–sister pairs; black circles are mice with, and white circles without, the Bittner virus. This was introduced by fostering on C+ mice (i.e. BALB/c mice with the virus) in generation 40; those in generation 41 receiving it from their parents. The figures indicate the number of mice (♂♂ and ♀♀ combined) whose skeletons were examined. Note that mice born of pairs of generation 39 are themselves generation 40, and so throughout.

litters on C+ mice (i.e. BALB/c mice with the Bittner virus) obtained from the Chester Beatty Institute. Cross-fostering was carried out by transferring newborn litters from B to C+ mice which were suckling young less than 4 days old. To prevent rejection of the foster litters by the C+ mothers, the litter was rubbed into the foster mother's fur before being introduced into her cage and the foster parents supplied with fresh nesting material. This caused them to start rearranging their cages and in the process they accepted the foster litter. At the same time the litter of the foster mother was transferred to the B mother using the same procedure. After 2–4 days the litters were returned to their original parents in the same manner. The animals were killed at approximately 60 days of age and their skeletons prepared by the papain maceration method (Luther, 1949; Searle, 1954*a*) for examination. Each mouse was given a serial number at the time of killing and this number was entered on a card for the classification of the skeletal variants. When all the skeletons had been prepared these cards were randomized by shuffling and the skeletons examined in that order. Each skeleton was classified for 52 minor skeletal variants (Grüneberg, 1963). These variants are not subject to the

Table 1. *List of skeletal variants which were either absent or occurred only sporadically, or, in the case of no. 13, was present in all animals examined, or, in nos. 28–46, showed a similar frequency in all groups of mice*

1. Bent nose	Searle (1954*a*)
2. Maxilla–turbinal fusion	Deol (1955)
3. Interfrontal present	Keeler (1933), Truslove (1952)
4. Interfrontal–frontal fusion	Truslove (1952)
5. Frontal fontanelle	Deol & Truslove (1957)
6. Fused frontals	Deol & Truslove (1957)
7. Squamosal–frontal fusion	Berry & Searle (1963)
8. Squamosal–parietal fusion	Searle (1954*c*)
9. Interparietal–occipital fusion	Deol & Truslove (1957)
10. Occipital–periotic fusion	Searle (1954*c*)
11. Basisphenoid–presphenoid fusion	Deol & Truslove (1957)
12. Basioccipital–basisphenoid fusion	Berry & Searle (1963)
13. Fenestra flocculi present	Searle (1963)
14. Foramen ovale open	Deol (1955)
15. Lower third molar missing	Grüneberg (1951)
16. Fossa olecrani perforata	Weber (1950)
17. Dyssymphysis CI–CII	Grüneberg (1950)
18. F.t.i. C. III, IV or V	Grüneberg (1950)
19. Dystopia cranialis tuberculi anterioris	Grüneberg (1950)
20. Dystopia caudalis tuberculi anterioris	Grüneberg (1950)
21. Tuberculum anterius absent	Grüneberg (1950)
22. Cervical fusions	Searle (1954*a*)
23. Processus spinosus on Th. I	Grüneberg (1950)
24. Dyssymphysis of Th. I	Searle (1954*a*)
25. Dyssymphysis of Th. II	Searle (1954*b*)
26. Sacralization of L. VI	Searle (1954*a*)
27. Foramen acetabuli perforans	Grüneberg (1952)
28. Nasals fused	Berry & Searle (1963)
29. Preorbital foramen double	Berry (1963)
30. Frontal foramen double	Berry & Searle (1963)
31. Accessory maxillary foramen	Berry (1963)
32. Metoptic roots abnormal	Truslove (1954)
33. Foramen palatinus majus double	Berry & Searle (1963)
34. Foramen ovale double	Deol (1955)
35. Size processus pterygoideus	Deol (1955)
36. Foramen hypoglossi double	Deol (1955)
37. Accessory mental foramen	Deol (1955)
38. Arch foramina C. III	Weber (1950)
39. Tuberculum anterius C. VI inflexum	Searle (1954*a*)
40. F.t.i. C. VI	Grüneberg (1950)
41. Foramina transversaria on C. VII	Berry & Searle (1963)
42. Processus spinosus Th. II present	Grüneberg (1950)
43. Arch foramina in Th. V or VI	Searle (1954*a*)
44. Dyssymphysis Th. X	Deol & Truslove (1957)
45. Number of thoracic vertebrae	
46. Sacral arch fusion	Searle (1954*a*)

effects of sexual dimorphism. The cards were then sorted into groups according to line of descent and generation, coded and transferred to computer data cards. The analysis of the results was carried out on the University College London IBM 360/65 computer, using a programme devised by Professor C. A. B. Smith and Dr G. M. Truslove. In the statistical examination of fourfold tables, Yates's correction was used where appropriate for the medial characters and a method devised by Professor C. A. B. Smith (appendix to Grüneberg, 1955) for the bilateral characters, to give a χ^2 with one degree of freedom.

3. RESULTS

As all females in the pairs represented by black circles in Fig. 1 developed mammary tumours and none were found in the others, complete success for the incorporation of the virus can be claimed. All the female parents that had been fostered on the C+ stock tended to develop tumours between 60 and 90 days, whereas their offspring tended to develop tumours a little later.

Table 2. *List of skeletal variants discussed in detail in the text*

(Values in bold type refer to the infected parts of B in Fig. 1. All values are percentages of animals affected or, in the case of bilateral entities, of sides of animals affected.)

		Generation		
	Subline	40	41	42
47. Parted frontals (Keeler, 1930; Truslove, 1952)	A	66·0	62·0	66·0
	B	58·0	**72·5**	**85·9**
48. Foramen sphenoidale medium present (Deol, 1955)	A	72·0	85·0	82·0
	B	55·0	**68·1**	**57·6**
49. Dyssymphysis posterior of C. II (Grüneberg, 1950)	A	26·0	27·0	26·0
	B	18·0	**10·1**	**17·2**
50. Processus spinosus of C. III (Grüneberg, 1950, footnote p. 132)	A	49·0	63·0	70·0
	B	63·0	**46·2**	**59·7**
51. Preoptic sutures present (Truslove, 1954)	A	86·0	82·5	72·0
	B	79·0	**80·4**	**72·7**
52. Accessory scapular foramen (Berry & Searle, 1963)	A	17·0	17·0	42·0
	B	16·0	**18·8**	**22·2**

Table 1 includes 46 skeletal variants which occurred in about the same frequency in all groups of mice and which, for that reason, need not be further considered.

Variant 46 is absent from generation 41 of A but shows a similar distribution in each of the other generations in A and B. This variant, sacral arch fusion, is age-dependent and the group in question includes the youngest animals in the study (average age 69·8 days as compared with 78–87 days in the other groups). This, together with the fact that lack of fusion is not maintained in the next generation, suggests an age effect and the variant is not further considered in this study.

Variants 47–52 show differences in their distributions which require further examination (Table 2).

47: parted frontals

The incidence of this variant is clearly homogeneous in A and in B_{40}. On the other hand B_{42} is significantly different from A_{42} ($\chi_1^2 = 9{\cdot}66$; $P < 0{\cdot}01$). It is not clear from the data available when this difference developed. B_{40} and B_{41} do not differ significantly from each other ($\chi_1^2 = 0{\cdot}20$) and the difference between B_{41} and B_{42} is barely significant ($\chi_1^2 = 3{\cdot}81$; $P \sim 0{\cdot}05$). If it is assumed that the divergence has already started in B_{41}, it might be due to the introduction of the virus. However, it could equally well be coincidental.

48: foramen sphenoidale medium present

Descent A is not significantly heterogeneous ($\chi_2^2 = 4{\cdot}791$; $P = 0{\cdot}09$), and the same applies to descent B ($\chi_2^2 = 3{\cdot}001$; $P = 0{\cdot}22$). On the other hand, A differs significantly from B ($\chi_1^2 = 27{\cdot}93$; $P \sim 10^{-7}$), which indicates the beginning of a subline differentiation between A and B prior to the introduction of the Bittner virus.

49: dyssymphysis posterior of C. II

The situation is almost the same as in the preceding case. A is obviously homogeneous; the same probably applies to B ($\chi_2^2 = 2{\cdot}069$; $P \sim 0{\cdot}36$). A differs significantly from B ($\chi_1^2 = 9{\cdot}598$; $P = 0{\cdot}002$), with the same conclusion: there is obviously no reason to suspect the virus.

50: processus spinosus of C. III

The six values do not agree with each other ($\chi_5^2 = 14{\cdot}490$; P between 0·02 and 0·01). The sum of A does not differ significantly from that of B ($\chi_1^2 = 0{\cdot}476$), and the reason for the probable heterogeneity is not clear; perhaps in so large a group of variants it is a mere accident of sampling. In any case, there is no reason to suspect the virus as its cause.

51: preoptic sutures present

This situation is not clear. Comparing the six values with each other, $\chi_5^2 = 15{\cdot}262$, and P just under 0·01; hence there is a suspicion of heterogeneity. But in each generation A is in tolerable agreement with B. The main reason for the high χ^2 value is the fact that both in A_{42} and B_{42} the incidence of preoptic sutures goes down. The reason is obscure but clearly does not implicate the virus.

52: accessory scapular foramen

The incidence in A_{42} is clearly different from that of the other five values ($\chi_1^2 = 46{\cdot}614$; $P < 10^{-10}$). The other five values agree with each other ($\chi_4^2 = 3{\cdot}167$; $P \sim 0{\cdot}5$). The change in incidence thus occurred in A and has nothing to do with the virus.

Overall divergence

As none of the variants considered separately show any clear evidence of an effect of the virus, the method of Grewal (1962) with the modification by Berry (1968) was applied to obtain estimates of divergence between the groups from a comparison of all the variants (Table 3). Eight of the 15 values obtained are negative and thus show no divergence between the respective groups. The remaining seven values are small and do not provide any real evidence of divergence.

Table 3. *Mean estimates of divergence between the generations in A and B*

(Approximations of the standard error of the estimate of divergence in bold type. Note: a negative value for the mean measure of divergence indicates that there is no difference between the groups compared and therefore these estimates of divergence have no real meaning.)

	40B	41A	41B	42A	42B
40A	−0·095 **0·019**	0·046 **0·019**	−0·067 **0·021**	0·103 **0·019**	0·080 **0·061**
40B	—	−0·057 **0·019**	−0·108 **0·047**	0·084 **0·059**	−0·081 **0·061**
41A	—	—	−0·081 **0·021**	−0·044 **0·019**	0·199 **0·019**
41B	—	—	—	0·048 **0·021**	−0·092 **0·021**
42A	—	—	—	—	0·086 **0·019**

4. CONCLUSION

Considering that in the whole array of variants under test one only (parted frontals) could conceivably be ascribed to the introduction of the virus – and not even this case is quite clear – it is obvious that the presence or absence of the Bittner virus in the BALB/c strain has not led to a detectable change of the skeletal profile.

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