Kirschstein² on the behaviour of the neurovirulent type 1 Mahoney strain.

The attenuated type 1 L₁S parental strain and some of its clonal derivatives displayed the *ds*^s, *MS*⁻ and *t*⁻ markers (Table 1). Both spontaneous mutants and those induced by nitrous acid, which maintained their *ds*^r character after several passages, performed either in the presence or absence of the inhibitor, however, retained the *MS*⁻ and *t*⁻ markers of the parental strain (Table 1, rows 7 and 8). A similar pattern, that is the partial independence of the *ds*^s → *ds*^r mutation in relation to the *crt*/40° character, was recorded by Takemoto and Kirschstein². Moreover, it will also be recalled that Boeyé⁶ has described the occurrence of separate mutations as well as of covariation between the *d* and *E* markers. We think that the lack of covariation between the *ds* and *d* characters on one hand, and the so-called "group of markers covarying with neurovirulence" (compare Voss *et al.*⁹) on the other, emphasizes the necessity for an experimental re-appraisal of the actual relationships between these markers, as well as between each of them and neurovirulence.

Our experiments on the behaviour of all these strains towards guanidine unequivocally demonstrate (Table 1, rows 1-8) that this character is not affected by selecting for the *ds*^r character and vice versa, thus indicating the mutual independence of the *ds*^s → *ds*^r and *g*^s → *g*^r mutations.

Preliminary findings concerning the relationship between the behaviour towards dextran sulphate and HBB indicated the lack of covariation of these two characters. The dextran resistant derivatives of the L₁S *ds*^s strain displayed the same susceptibility to HBB as the parental population. Conversely, no difference was observed between the *hbb*^r character in a *ds*^r population after selection in the presence of HBB (Table 1, row 9) and the *hbb*^r character acquired by the L₁S strain exposed directly to the drug. Moreover, no change was observed in the susceptibility of the latter strain towards dextran sulphate (Table 1, row 10) after mutation from HBB sensitivity to HBB resistance.

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CYTOLOGY

Genetic and Histochemical Studies on Mouse Spleen Black Spots

In the course of immunological experiments involving the C57BL/Ka and C57BL/6 *Jax* strains of mice, black spots were noted on the spleens of some mice. These spots were usually found in the posterolateral aspect of the spleens, although a few cases were noted in which the spot was in the anterior portion of the spleen. Histological sections of these spots were characterized by a diffuse scattering

of pigmented cells, involving primarily the red pulp of the affected portion of the spleen. These spots occur in a number of (C57BL/Ka × Balb/C) F₁ hybrids, but not in Balb/c mice. They are present in affected strains in similar frequency from birth onwards. In order to test whether the appearance of this phenomenon is genetic, and perhaps indicative of some residual heterozygosity in our colony, we carried out laparotomies on a number of hybrid mice, then set up the following mating pairs: black spot × black spot, black spot × normal, normal by normal. The results are shown in Table 1.

Table 1. DISTRIBUTION OF BLACK SPOT OFFSPRING RESULTING FROM TEST MATINGS

Mating pair type	Black spot	Total	Percentage with black spot
Black spot × black spot	6	61	9.8
Black spot × normal	4	22	18.2
Normal × normal	12	75	16.0

The finding that two negative parents could have positive offspring rules out a dominant type of inheritance with complete penetrance. The finding that two positive parents did not have all positive offspring rules out a purely recessive type inheritance. In fact, the random distribution of positives, independent or parental "phenotype" raises some doubts about any genetic transmission of the phenomenon. We could not, however, rule out hypotheses involving the genetic transmission of a trait with variable penetrance.

The F₂ offspring were combined and assorted by skin and hair colour, and the results are shown in Table 2.

Table 2. DISTRIBUTION OF BLACK SPOT OFFSPRING IN F₂ OFFSPRING SORTED BY SKIN COLOUR

	Black spot	Total
Albino	0	40
Coloured	25	118

$$\chi^2 = 8.1, P < 0.005.$$

Thus the results demonstrate a genetic restriction on the phenomenon—the mouse must be capable of forming melanin pigment if it is to have a black spot on its spleen. The albinos were randomly distributed among the three classes of matings, and the removal of albinos from these groups did not change the proportion of black spot mice in any group.

To confirm the hypothesis that the black spot was indeed melanin, we found that the pigmented cells did not stain with Prussian blue (ruling out iron-containing pigments), did stain with DOPA oxidase, and could be bleached with hydrogen peroxide.

Because melanocytes are considered to originate from neural crest cells¹, and the normal resting place of these cells is the skin (although reports of melanocytes in ovaries, thymus, and other organs have been published²), the finding of localized clusters of melanocytes in the spleen may be due to either a peculiar migration potential of these cells, a true metaplastic condition following localized prenatal trauma, or perhaps to the transport of melanin by leucocytes, as reported by Wasserman³.

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