A new gene
affecting the morphogenesis of the vestibular part of the inner ear in the mouse

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INTRODUCTION

A mutation affecting behaviour occurred in a heterogeneous stock of mice at The Jackson Laboratory in 1956. Genetical tests showed that the mutant gene was semi-dominant with incomplete penetrance, and lethal in the homozygous condition. Further studies established that its effects on the inner ear were different from those of other genes affecting behaviour in a similar manner. It was named 'dancer', and assigned the symbol De. This paper gives the results of the genetical, anatomical and developmental studies carried out, as well as an account of the phenotypical effects of the dancer gene.

GENETICS

Inheritance

The segregation data are given in Table 1. They are based on classification according to behaviour. The results of \( Dc/+ \times Dc/+ \) matings suggest that the \( Dc/Dc \) class dies before classification. This is confirmed by the presence at birth of a distinct inviable phenotype among the offspring of matings of this type. The number of the inviable offspring fits the expectation: 9 of the 40 animals classified at birth were of this type.

The number of mutant animals observed in both types of matings in Table 1 falls short of the number expected on the assumption that dancer is a semi-dominant gene, lethal in the homozygous condition. This may in part be due to higher mortality among mutant animals before classification. But the total mortality was not high enough to explain the discrepancy. Therefore it must be assumed that the penetrance of the dancer gene is incomplete.

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Table 1. Inheritance of the Dc gene

<table>
<thead>
<tr>
<th>Mating type</th>
<th>Offspring</th>
<th>Expected ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dc/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Dc/+ x +/+</td>
<td>320</td>
<td>443</td>
</tr>
<tr>
<td>Dc/+ x Dc/+</td>
<td>70</td>
<td>58</td>
</tr>
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</table>

**Linkage**

The dancer gene was tested for linkage with 19 marker genes representing 16 linkage groups (Table 2). It appears to be linked with pale ear (ep) in the linkage group XII, the recombination being 34.74 ± 4.88%.

Table 2. Results of linkage tests with the Dc gene

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Marker gene</th>
<th>Mating type*</th>
<th>Offspring</th>
<th>Recombination (%)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>p</td>
<td>BC</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>II</td>
<td>se</td>
<td>BC</td>
<td>60</td>
<td>31</td>
</tr>
<tr>
<td>III</td>
<td>s</td>
<td>BC</td>
<td>41</td>
<td>9</td>
</tr>
<tr>
<td>IV</td>
<td>Sl</td>
<td>BR</td>
<td>57</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>BC</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>V</td>
<td>Ra</td>
<td>BR</td>
<td>16</td>
<td>9</td>
</tr>
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<td></td>
<td>a</td>
<td>BC</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>VI</td>
<td>Ca</td>
<td>BR</td>
<td>58</td>
<td>28</td>
</tr>
<tr>
<td>VII</td>
<td>Re</td>
<td>BR</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>VIII</td>
<td>Pt</td>
<td>BR</td>
<td>35</td>
<td>15</td>
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<td>BR</td>
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<tr>
<td>X</td>
<td>gr</td>
<td>BC</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>XI</td>
<td>M$^{wh}$</td>
<td>BR</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>XII</td>
<td>ep</td>
<td>BC</td>
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</tr>
<tr>
<td>XIII</td>
<td>ln</td>
<td>BC</td>
<td>122</td>
<td>37</td>
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<td></td>
<td>fz</td>
<td>BC</td>
<td>111</td>
<td>53</td>
</tr>
<tr>
<td>XIV</td>
<td>sa</td>
<td>BC</td>
<td>69</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>bg</td>
<td>BC</td>
<td>72</td>
<td>33</td>
</tr>
<tr>
<td>XVI</td>
<td>ma</td>
<td>BC</td>
<td>92</td>
<td>31</td>
</tr>
<tr>
<td>XVII</td>
<td>W$^v$</td>
<td>BR</td>
<td>86</td>
<td>32</td>
</tr>
</tbody>
</table>

* B = backcross, C = coupling, R = repulsion.

**Allelism**

The dancer gene was tested for allelism with twirler (Lyon, 1958), because the phenotypical effects of the two genes are similar in both heterozygous and homoz-
zygous conditions. The results were negative: the inviable phenotype characteristic of both dancer and twirler homozygotes was not observed among the 22 offspring of $Tw/+/\times Dc/+\,$ matings.

PHENOTYPICAL EFFECTS

Heterozygotes

Dancer heterozygotes ($Dc/+\,$) can be distinguished from their normal littermates at the age of about 3 days by their abnormal postural reflex. When lifted by the tail they do not arch the back and extend the front legs as normal mice do, but raise the back into a hump and retract the front legs, bending the head toward the belly at the same time. By the age of 1 week they are noticeably smaller and more active, and at 2 weeks most features of the waltzing syndrome become apparent. Hyperactivity increases, and the animals develop a weaving gait, with a strong tendency to run in circles without any preference for a particular direction. Jerking movements of the head occur in both horizontal and vertical planes, and are seldom absent while the animals are awake. Some mice may hold their heads tilted to one side. When lifted by the tail the adult dancer heterozygote twists and turns violently and throws its body about as if completely at a loss what to do. Swimming ability is always affected. The animals go under the water, gyrate about in all directions, and would certainly drown if not rescued. Occasionally dancer mice are able to swim well enough to save themselves from drowning, but even then their swimming ability cannot be described as normal: they swim in circles instead of heading for the sides of the tank in an effort to get out. Deafness, a common feature of the waltzing syndrome, does not occur. The expression of the syndrome is variable.

In addition to abnormal behaviour, dancer mice usually have a white spot in the middle of the head. The size of the spot, when present, varies: sometimes it is no more than a few white hairs, at others it forms a prominent blaze. Its existence was not noticed until the collection of segregation data was well under way, so information about its frequency is based on only 539 animals, 228 dancer and 311 normal, as judged by behaviour. Among the dancer mice the spot was present in 191 animals and absent in 37. Among the normal mice only 7 animals showed it. It is believed that these 7 animals were in fact $Dc/+\,$ in genotype, and were misclassified because of the incomplete penetrance of the gene with regard to behaviour (see Genetics). Unfortunately no progeny tests were carried out in verification.

Homozygotes

Homozygous dancer mice ($Dc/Dc\,$) have a unilateral or bilateral cleft lip as well as cleft palate (Text-fig. 1). They die within a few hours of birth, presumably as a result of these malformations.
EFFECTS ON THE INNER EAR

Materials and methods

The details of the sectioned material are given in Table 3. As it was not possible to identify mutant embryos by any external features, entire litters from segregating matings were sectioned. Many of the matings were of the $Dc/^+ \times Dc/^+$
type, but since it was found that the abnormalities of the inner ear in heterozygotes and homozygotes are indistinguishable the two types of embryos have been treated together. All embryos were fixed in Bouin's fluid, embedded in paraffin, sectioned at 8 or 10 $\mu$m, and stained with Ehrlich's haematoxylin and eosin. About half of the 10-day and 11-day embryos were sectioned in the
transverse plane (in relation to the neural tube in the region of the inner ear), and the others in the horizontal plane. The older embryos were cut only in the transverse plane.

Table 3. Details of the sectioned material

<table>
<thead>
<tr>
<th>Age</th>
<th>Mutant</th>
<th>Normal</th>
<th>?</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-day embryos</td>
<td>18</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>11-day</td>
<td>15</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>12-day</td>
<td>5</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>13-day</td>
<td>7</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>14-day</td>
<td>3</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>16-day</td>
<td>4</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Newborn mice</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>8 days old</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>15 days</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>18 days</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>32 days</td>
<td>1</td>
<td>—</td>
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</tr>
<tr>
<td>43 days</td>
<td>1</td>
<td>1</td>
<td>—</td>
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<tr>
<td>107 days</td>
<td>1</td>
<td>—</td>
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<tr>
<td>179 days</td>
<td>1</td>
<td>1</td>
<td>—</td>
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<tr>
<td>230 days</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>254 days</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
</tbody>
</table>

All post-natal material was fixed in Witmaack's fluid by perfusion. It was decalcified in 1 % nitric acid, and embedded first in celloidin and then in paraffin (for details of the technique see Deol, 1954). Serial sections of the inner ear and the adjoining parts of the brain were cut in the transverse plane at 10 µ, and stained in Ehrlich's haematoxylin and Orange G containing a trace of erythrosin.

The otological terms used in this paper have been recommended by Bast & Anson (1949).

Anatomy of the inner ear

The abnormalities of the inner ear in dancer mice are variable, and confined to the vestibular part. The cochlea, as seen in transverse sections, appears to be normal. Since this plane is not ideal for examining the organ of Corti, this observation was verified by sectioning one ear in a plane parallel to the modiolus. This was regarded as sufficient in view of the fact that the hearing ability of dancer mice seems to be normal.

The saccule is always affected. It is smaller than normal, and considerably narrower. It does not extend dorsally as far as the utricle, to which it is attached by a thick strand of cells without any lumen (Plate 1, figs. A, B). Other strands of cells running across the vestibule may connect it to the lateral wall of the otic capsule. The free wall of the saccule is sometimes highly convoluted. The macula is fairly normal in size and position. The neuro-epithelium has a normal appearance,
and so have the otoliths and otolith membrane, except in small patches in rare cases. In normal mice the saccular macula is supplied by a branch of the inferior vestibular nerve, which enters the otic capsule near the ventral margin of the macula, and by a branch of the superior vestibular nerve, which enters near its dorsal border. In dancer mice the superior vestibular nerve makes its entry near the centre of the macula. Otherwise the nerve supply appears to be normal.

The utricle is invariably reduced in size, particularly in the dorso-ventral direction. Sometimes diverticula of various dimensions are attached to it. The utricular macula is entirely missing, and, naturally, so is the utricular branch of the superior vestibular nerve (Plate 1, fig. B). This is the only constant feature of dancer mice. No animal was observed with even a partially formed utricular macula. In some cases a few cells (less than a dozen) vaguely reminiscent of neuro-epithelium are found on the floor of the utricle. Since they always form a small ridge or protuberance, they may be regarded as a rudimentary crista rather than as a macula. Their connexions with nerves, if any, are difficult to trace reliably.

The superior semicircular duct is abnormal in the majority of animals, mostly unilaterally, sometimes bilaterally. The abnormality is confined to the proximal (ampullar) end of the duct, involving about one-third of its length at the most. It consists of a severe narrowing or complete obliteration of the lumen of the duct for a short distance, and an absence of a proper ampulla. The obliteration is not the result of a collapse of the duct, for in this region the duct is represented by a thin cord of cells and not by a ribbon. This cord is always there, so the continuity of the duct is not broken. The bony canal in this region is also constricted, but not entirely occluded. Whenever these abnormalities occur the crista is either missing or poorly formed and rudimentary in appearance. As a rule when a poorly formed crista is present the duct is only narrowed, whereas when it is missing the duct is fully closed. Conversely, when the duct is wide open, the crista is always normal in appearance. But even in these cases the position of the crista is frequently shifted, and its nerve seldom takes the normal course, although it originates in the superior vestibular ganglion in the normal manner. Instead of following the normal route along the base of the utricle, the nerve may make its way laterally through the otic capsule into the tympanic cavity, then rise for some distance, and enter the capsule again near the point where the malleus is anchored to the roof of the tympanic cavity. In such cases it has its own channel through the otic capsule into the tympanic cavity. Sometimes the nerve is divided at or close to its origin, one branch following a more or less normal course and the other one reaching its destination erratically. Another abnormal route is through a bony channel in the medial wall of the capsule. Even when the nerve takes a fairly normal course it may still divide into two just before reaching the crista, the two branches entering separately.
Figs. A and B. Transverse sections of the inner ear of an 18-day-old normal mouse (fig. A) and its dancer littermate (fig. B). Note the absence of the macula of the utricle and the reduction of the vestibular ganglion in the dancer animal. $S$, Saccule; $U$, utricle; $M$, macula; $VG$, vestibular ganglion; $SVN$, superior vestibular nerve. $\times 86$. 

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Figs. C and D. Horizontal sections through the otic vesicle of a 10-day normal embryo (fig. C) and its dancer littermate (fig. D). Note the reduction of the acoustic ganglion in the dancer embryo. NT, Neural tube; AG, acoustic ganglion; OV, otic vesicle. ×182.

Figs. E and F. Transverse sections through the otic vesicle of an 11-day normal embryo (fig. E) and its dancer littermate (fig. F). Note the lack of innervation of the ventro-lateral wall of the otic vesicle in the dancer embryo. NT, Neural tube; OD, otic duct; OV, otic vesicle; VG, vestibular ganglion; SVN, superior vestibular nerve; AN, accessory nerve. ×108.
The lateral semicircular duct is more frequently abnormal than the superior one, and the two ducts seem to be affected independently of each other. The abnormality of the lateral duct may also be unilateral or bilateral, there being no apparent correlation between the two sides. It is in general of the same type as in the superior duct, being confined to the same region, but it is more severe. The affected part of the duct may be closed or missing. When it is missing the bony canal is also absent and a complete break in the arc occurs. When the duct is simply closed—that is, represented by a solid cord of cells—it originates at a place considerably posterior to the normal position. In all cases in which the lateral duct is affected the crista is either missing or small and poorly differentiated. Again, when the crista is missing there is no ampulla, and when the crista is abnormal the ampulla is imperfect. In some instances the crista is situated not at the proximal end of the duct, but at some distance from the utricle, deep within the capsule. In these cases the position of the ampulla is correspondingly shifted. When the crista is normal, both the duct and the ampulla are also normal.

<table>
<thead>
<tr>
<th>Age</th>
<th>Genotype</th>
<th>Dancer</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>1830</td>
<td>3424</td>
<td></td>
</tr>
<tr>
<td>18 days</td>
<td>1844</td>
<td>3689</td>
<td></td>
</tr>
<tr>
<td>179 days</td>
<td>2174</td>
<td>4058</td>
<td></td>
</tr>
</tbody>
</table>

But, as with the superior duct, even then the nerve of the crista seldom follows the normal path. After entering the capsule it may pass obliquely through the vestibule, reach the lateral wall of the capsule above the stapes, and then take a circuitous route to the crista. During its long passage through the vestibule it is completely unsupported, and bathed in the periotic fluid. On the whole, the path of the lateral nerve can be far more erratic than that of the superior nerve. When the crista is small and poorly differentiated the nerve is very thin.

The posterior semicircular duct and canal seem to be unaffected. The ampulla, the crista and its nerve supply are quite normal in appearance.

The otic duct and otic sac appear to be normal. The ductus reuniens is also normal, but the saccular duct, utricular duct and the utricular valve are usually affected. The saccular duct is severely distorted, and its sinus flattened and curved to such an extent that the two ends join to form a complete circle. The result is that in transverse sections an extension of the periotic space appears to be surrounded by a ring of the otic space. In many cases there is no distinct saccular duct, and the saccule and utricle are confluent. The saccule then communicates with the otic duct through the utricle. The utricular duct and the
valve are also usually distorted, and the valve sometimes replaced by a wide aperture. There is no clear evidence that any part of these intercommunicating channels is actually blocked.

The vestibular ganglion in dancer mice is much smaller than normal (Plate 1, figs. A, B). Dorsally it does not extend much beyond the superior border of the saccular macula. In addition, the cells are not so densely packed. The number of cells in the vestibular ganglion was counted in three dancer mice of different ages and their normal littermates, and the results are given in Table 4. The reduction in the number of cells in dancer mice is of the expected order: approximately half of the neuro-epithelium innervated by the vestibular ganglion is lacking, and the number of cells is reduced to about the same extent. The whole ganglion was treated as a unit, because the distinction between superior and inferior vestibular ganglia is not clear enough in the mouse to permit separate counts. But it is believed that the reduction is confined to the superior vestibular ganglion only, for no part of the neuro-epithelium innervated by the inferior vestibular ganglion is missing. This view is supported by the observation that the ganglion does not extend far enough dorsally, whereas its ventral limits are fairly normal. It is also evident from Table 4 that there is no loss of cells by degeneration in dancer mice as they grow older.

No abnormality of the otic capsule was identified which may not be regarded as a reflexion of the defects of the membranous labyrinth and its nerve supply.

*Development of the inner ear*

It was possible to trace the abnormalities of the inner ear of dancer mice back to the 10-day stage. Two types of embryos can be distinguished in segregating litters of this age. In one type the acoustic ganglion appears to be larger. It lies closely applied to the anterior aspect of the otic vesicle, and curves around it to cover a part of its lateral aspect as well (Plate 2, fig. C). The epithelium of the vesicle is considerably thicker on the antero-lateral side, and it thins down suddenly where the ganglion comes to an end. This point is usually very well defined. In the other type of embryo the acoustic ganglion appears to be smaller. It covers the anterior aspect of the vesicle to a fairly normal extent, but it does not curve round the vesicle to reach its lateral aspect (Plate 2, fig. D). A few stray cells may do so, but not the main body of the ganglion. The thickening of the epithelium on the antero-lateral aspect is either missing or very poor, and so is the depression marking the transition. It is believed that the first type of embryos are +/+ in genotype, and the second Dc/+ or Dc/Dc. The frequency of the two types in segregating litters agrees with expectation. Moreover, their differences are such as would be expected from familiarity with the later stages of development. The two types of embryos are easier to distinguish in horizontal than in transverse sections. When both sides are considered together there is usually little doubt concerning the type. However, it is possible that one or two abnormal embryos have been classified as normal, in particular those that would
have developed all the neuro-epithelium with the exception of the utricular macula. But it is unlikely that any normal embryos have been classified as abnormal. The two 10-day embryos given as unclassifiable in Table 3 were extremely retarded.

At the 11-day stage the identification of the dancer embryos can be made without any doubt, for the acoustic ganglion has now clearly divided into its cochlear and vestibular components, and the layout and innervation of the future maculae and cristae have begun to emerge. In the normal embryo a large thick bundle of nerve fibres leaves the vestibular ganglion and fans out to the ventral and lateral regions of the vestibular division of the vesicle (Plate 2, fig. E). The epithelium in these regions has grown thick and will eventually develop into the utricular macula and the superior and lateral cristae. In the mutant embryos this whole nerve is usually entirely lacking (Plate 2, fig. F). But sometimes it occurs as a thin bundle, which may divide into two or three branches, supplying the ventro-lateral wall of the vestibular division, the site of the future superior and lateral cristae. There is frequently another nerve of various dimensions which leaves the vestibular ganglion on the side facing the facial ganglion. This nerve is not known to occur in normal embryos. It takes a lateral course, passes between the otic vesicle and the anterior cardinal vein (Plate 2, fig. F), and divides into thin branches in the connective tissue just below the epidermis. On the way, it often gives off a branch that joins the otic vesicle in the region of the future superior crista. There may also be found one or more extremely small clumps of cells, ganglionic in appearance, scattered in the space bounded by the anterior cardinal vein, the otic vesicle and the facial and vestibular ganglia. These clumps are generally too small to allow their nerve connexion to be traced. The largest one observed clearly contributed fibres to the accessory nerve seen only in mutant embryos, referred to above. The size of the vestibular ganglion is difficult to judge at this stage on account of its complex shape, but it must be reduced because so much of the innervation of the otic epithelium is missing. There is no difference as yet in the form of the otic vesicle between the two types of embryos. For the study of this stage, as well as all later stages, transverse sections are more suitable than horizontal ones.

By the 12-day stage in the normal embryo the three branches of the superior vestibular nerve supplying the utricular macula and the superior and lateral cristae have become distinct. The neuro-epithelium destined for the lateral crista has separated from that for the superior crista, and shifted ventrally and posteriorly. The folds that will divide the large median chamber into the utricle and saccule have just made their appearance. The sides of the disc-shaped evagination of the superior duct have coalesced over a large area to form the duct, and the evagination for the lateral duct is well advanced. In the mutant embryos the utricular nerve is always missing. The nerves of the superior and lateral cristae may or may not be present. The accessory nerve from the superior vestibular ganglion may sometimes give off a branch to one of these two cristae,
but it is always thinner than the normal nerve. There is still no significant
difference in the form of the inner ear between normal and mutant embryos,
although the utriculo-saccular folds have a somewhat abnormal appearance in
the latter. The small supplementary ganglia referred to before are occasionally
seen.

At the 13-day stage in the normal embryo practically all the main features of
the inner ear have made their appearance. The formation of the semicircular
ducts is complete, and the utriculo-saccular fold has divided the central chamber
of the otic vesicle in such a way that a large part of the floor of the utricle is in
contact with the roof of the saccule. In mutant embryos the nerves of the superior
and lateral semicircular ducts are frequently missing. In all such cases the duct
is either greatly narrowed or completely closed near its proximal end, and there
is no crista. When the nerves are normal the ducts are normal, and a crista is
always present. In some instances the nerve is present but much thinner than
normal. The abnormalities of the related ducts and cristae in these cases are
variable. In mutant embryos the utriculo-saccular fold is also clearly abnormal.
It is thicker, appears to form in the wrong place, often has an abnormal slant in
the medial direction, and does not advance far enough posteriorly. The result is
that the utricle and saccule are abnormal in shape and size, and separated by
mesenchymal tissue except where they are confluent. They usually give off small
diverticula in various places. The utricle, in addition, has no nerve supply, and
its macula is missing. The accessory nerve and the small accessory ganglia are
quite prominent.

After the 13-day stage the otic capsule begins to chondrify, and the gross
differentiation of the inner ear comes to an end. Consequently, the differences
between the normal and mutant embryos at 14-day and 16-day stages are
essentially the same as at the 13-day stage, although much accentuated. The only
new feature of the mutant embryos in these later stages is the absence of the
saccular duct and utricular valve. These structures begin to form in the normal
embryo at the 16-day stage, but in the mutant embryo the utricle and saccule
still flow into each other through a wide aperture. The chondrification of the
capsule has also fixed the position of the accessory nerve. It can be seen passing
laterally through the capsule a little above and in front of the facial nerve. It may
have its own channel through the capsule or it may share the channel of the
facial nerve, but the two nerves are always quite distinct. It may or may not
give off small branches to the superior and lateral cristae. Its thickness is highly
variable. It may be mentioned here that this nerve was not described in the
section on anatomy of the inner ear, because the post-natal material was not
suitable for following its path: sections anterior to the vestibular ganglion were
not kept.
CONCLUSIONS AND DISCUSSION

The first visible effect of the dancer gene is on the acoustic ganglion, and in consequence on the nerves of certain parts of the inner ear. The absence of the macula of the utricle and the cristae of the superior and lateral semicircular ducts is obviously the direct result of the missing nerves, for the maculae and cristae arise from modification of the epithelium of the otic vesicle in response to contact with nerve endings. No observation made during this study conflicts with this statement. In the few semicircular ducts with insufficient nerve supply the crista was small and poorly developed. The question is whether the other malformations of the inner ear are also consequent on the abnormality of the ganglion and the nerves or whether they are independent effects of the gene.

There is some evidence that the malformations of the superior and lateral ducts are caused by the missing nerves. In all, 15 cases were available for analysis, 11 animals of various ages and 4 16-day embryos. The embryos were included because by this stage all malformations of the ducts have appeared and become fairly stabilized. As the abnormalities of the superior and lateral ducts seemed to be independent of each other, and as there was no apparent correlation between the two sides, there were 60 ducts to consider. Of these, 18 were normal in appearance; that is, they were wide open with reasonably uniform calibre. All of them had well-developed cristae and nerves of normal thickness. In 36 cases a part of the duct was either missing or represented by a thin cord of cells without any lumen. No crista or nerve was observed in any of them. The remaining 6 ducts were clearly affected but not so severely: the lumen was strikingly narrowed in the expected place, but not totally obliterated. All these ducts had insufficient nerve supply: the nerve was noticeably thinner, and the crista poorly developed. Since the abnormalities of the nerves appear about 2 days before anything is visibly the matter with the ducts, or even before the ducts begin to form, the conclusion seems to be justified that the nerve is an essential factor in the morphogenesis of a semicircular duct. The fact that the abnormalities of the ducts are always confined to the proximal end, where the nerve enters, supports this conclusion.

This study has also thrown some light on how and when the nerve plays its part in the morphogenesis of the duct. In the dancer embryo the ducts appear to develop along fairly normal lines until the 12-day stage. Disc-shaped evaginations grow out of the vestibular division of the otic vesicle into the surrounding tissue. It is during the fusion of the central areas of these evaginations and the following resorption of the fused parts that the effects of the missing nerve are felt. The fusion extends too far at the proximal end of the duct. In the lateral duct this end may even be subject to resorption, but in the superior duct resorption does not take place, and a string of cells remains. It would seem that the presence of the embryonic crista or the nerve ending in some way inhibits the coalescence of the walls of the evagination in its vicinity. This becomes credible when the normal
formation of an ampulla is considered. As the crista grows, the walls of the duct retreat and the lumen widens, so that a cavity is continuously maintained in front of the crista. It is possible that in normal development the maintenance of a cavity not only at the site of the ampulla but also in its vicinity is a function of the crista or the nerve endings, and this is lost in the dancer embryo. The malformations of the semicircular ducts in dancer mice may then be regarded as the result of an overflowing or extension of the normal processes of coalescence and resorption. It is significant that in dancer embryos an ampulla is never present when the crista is missing, and it is imperfectly formed when the crista is poorly developed, the same being the case with the lateral duct in the twirler mutant (Lyon, 1958). The minor differences between the superior and lateral ducts in the severity of the defect may be due to the fact that the evagination of the superior duct is a modification of an existing chamber, the topmost region of the otic vesicle, whereas the evagination of the lateral duct is a new structure which appears about 2 days later, when the abnormality of the nerve is already making its influence felt.

There remain the malformations of the saccule, utricle, saccular duct and utricular valve. All of these can be ascribed to the abnormal development of the utriculo-saccular fold, first observed at the 13-day stage (see Development of the inner ear). There can be little doubt that the malformations of the saccule are the result of the abnormality of the fold, for they occur only in that region which could be affected by the fold. The ventral part of the saccule is almost certainly normal, otherwise the ductus reuniens, a most sensitive indicator, would be affected. The abnormality of the utriculo-saccular fold is thought to be caused by the absence of the utricular nerve. Since the nerve is always absent and the fold always abnormal, evidence of the kind presented for the semicircular ducts is not obtainable. But there are other considerations that favour this interpretation. The fold normally develops close to the path of the utricular nerve, and, even if the nerve exerted no active influence on the growth of the fold, for mechanical reasons alone the fold could hardly develop normally when the nerve was absent. However, the possibility remains that the vestibular ganglion also exercises direct inductive influence in the formation of the utricle and saccule.

Studies on the mutants kreisler, dreher, splotch, and loop-tail (Hertwig, 1944; Deol, 1964a, b, 1966a) have provided evidence that in the mouse the morphogenesis of the inner ear is governed by the neural tube in the same manner as in the chick and in amphibians (Zwilling, 1941; Harrison, 1945; Detwiler & van Dyke, 1950). When the neural tube is abnormal the morphogenesis of the inner ear does not take place along normal lines. But the details of this relationship have always been obscure. The neural tube is well separated from the otic vesicle after the 10-day stage, and many components of the inner ear develop at a considerable distance from it. How, then, are the abnormalities of the neural tube transmitted to the otic vesicle at later stages? The present study indicates that the ganglia and nerves may serve as intermediate steps, for when the neural
tube is abnormal it is highly unlikely that the ganglia and nerves would remain unaffected.

The significance of the accessory nerve which originates in the superior vestibular ganglion but resembles the facial nerve in its distribution is not fully understood. It may imply an erroneous division of the facial–acoustic ganglionic complex, for it seems as if a contingent of cells destined for the facial ganglion had become incorporated into the superior vestibular ganglion. The occurrence of small accessory ganglia supports this view.

Opinions differ on the origin of the acoustic ganglion in mammals. Some authors have maintained that it originates in the neural crest (Bartelmez, 1922; Adelmann, 1925), while others believe that it is derived from the otic vesicle (Halley, 1955; Batten, 1958). If the neural crest does in fact contribute to the formation of the acoustic ganglion in the mouse then the association of the white spot on the head and the abnormality of the superior vestibular ganglion in dancer mice may be a clue to the site of action of the dancer gene, for the melanocytes are also derived from the neural crest. There is a demonstrable deficiency of cells in the acoustic ganglion. Since spotting is believed to be the result of an absence of melanocytes rather than their modification (Billingham & Silvers, 1960), the white spot could be the result of a similar deficiency of melanocytes. There is no obvious discrepancy between the size of the spot and the magnitude of the deficiency of ganglion cells. Accordingly, the site of gene action may be the neural crest in the vicinity of the fourth neuromere of the rhombencephalon. If the cause of the waltzing syndrome is indeed some unidentified abnormality of the central nervous system, as has been suggested by Grüneberg (1952) and Deol (1954, 1966b), then the neural tube in the same region may also be included in the site of gene action. A similar association between spotting and abnormalities of the ganglia occurs in splotch (Auerbach, 1954), piebald lethal and lethal spotting (Lane, 1966) mutants, and there too the site of gene action has been assumed to be the neural crest.

This view of the action of the dancer gene, if correct, has two implications. First, it means that the cells destined to give rise to the superior vestibular ganglion must have a chemical specificity even when they form a morphologically indistinguishable part of the facial–acoustic ganglionic complex, the original ganglion in the region of the fourth neuromere. If this were not so then other ganglia descending from this complex should also sometimes be subject to the action of the dancer gene, which appears to take place before the parent ganglion has undergone any division. But the inferior vestibular, spiral and facial ganglia appear to be always normal. This can be said of at least the spiral ganglion with complete confidence, because the sense of hearing in dancer mice is not affected. The second implication is that in normal, undisturbed development melanoblasts from a given part of the neural crest always go to approximately the same area of the skin, and if they fail to arrive that area remains unpigmented. The failure of the spot to occur in 16% of dancer mice does not rule out this possi-
bility. The deficiency of cells in the superior vestibular ganglion is variable: sometimes only the utricular nerve is missing, and at others the nerves of one or two semicircular ducts as well. It is not improbable that the deficiency of the melanoblasts is variable in the same way, and is on occasions too low to cause a visible spot. Genetic variance may also be responsible for the absence of the spot in some mice.

The origin of the otoliths has been the subject of several studies (Nishio, 1926; Lyon, 1955), but the results have always been inconclusive. Two explanations of their origin stand out: according to one they are secreted by certain regions in the wall of the membranous labyrinth, while according to the other they are secreted by the maculae (Lyon, 1955). This study supports the latter view: the utricular macula is always absent and there is no trace of the otoliths in the utricle, whereas the saccular macula is always present and so are the otoliths.

A separate study of $Dc/Dc$ embryos was not attempted, but it is reasonable to assume that the primary abnormality in them also concerns the nervous system, although it may be much more widespread than in $Dc/+ \ $embryos. The presence of cleft palate and cleft lip in $Dc/Dc$ embryos may then indicate some relationship between these malformations and abnormalities of the nervous system. The same arguments will apply to the mutant twirler (Lyon, 1958).

**SUMMARY**

1. The discovery of a new semi-dominant gene in the mouse is reported. It is called ‘dancer’, and it belongs to the linkage group XII. The homozygotes have cleft palate and cleft lip, and die soon after birth. The heterozygotes have abnormal behaviour and a white spot on the head.

2. An examination of the inner ear in the heterozygotes revealed a reduction of the vestibular ganglion, absence of some of its nerves, and widespread malformations in the vestibular region.

3. Developmental studies showed that the malformations appear about 2 days after the abnormalities of the ganglion and nerves, and since they are virtually confined to the parts with affected nerve supply they are probably consequent on these abnormalities.

4. A complete correlation between the defects of the semicircular ducts and of their nerves strongly suggests that the nerve plays an essential part in the morphogenesis of the proximal end of the duct.

5. The failure of the otoliths to form in the utricle in the absence of the macula indicates that the otoliths are probably secreted by the macula.
‘Dancer’ gene in mouse

RÉSUMÉ

Nouveau gène affectant la morphogenèse de la partie vestibulaire de l’oreille interne chez la souris

1. La découverte d’un nouveau gène semi-dominant chez la souris est présentée. Ce gène a été nommé dancer; il appartient au groupe de liaison XII. Les homozygotes ont une fente au palais et à la lèvre; ils meurent peu après la naissance. Les hétérozygotes manifestent un comportement anormal et ont une tache blanche sur la tête.

2. L’examen de l’oreille interne des hétérozygotes révèle une réduction du ganglion vestibulaire, l’absence de certains de ses nerfs, et des malformations étendues dans la région vestibulaire.

3. L’étude du développement montre que les malformations apparaissent sensiblement deux jours après les anomalies du ganglion et des nerfs, et puisqu’elles sont en fait limitées aux parties affectées par l’innervation, elles sont probablement la conséquence des anomalies nerveuses.

4. La totale corrélation entre les aberrations des canaux semicirculaires et de leurs nerfs suggère fortement que ceux-ci jouent un rôle essentiel dans la morphogenèse de l’extrémité proximale du canal.

5. Le fait que les otolithes ne se forment pas dans l’utricle en l’absence de la macula, indique que celle-ci sécrète probablement les otolithes.

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