Tooth development in the ‘crooked’ mouse

By J. A. SOFAER

From the University of Edinburgh,
School of Dental Surgery and the
Department of Human Genetics,
Western General Hospital, Edinburgh

SUMMARY

The semidominant gene ‘crooked’ (Cd) in the mouse produces anomalies of the axial skeleton (resulting in a crooked tail), microphthalmia and dental abnormalities, including small molars with simplified cusp patterns that are equivalent to patterns passed through during normal morphodifferentiation. A series of embryonic litters from Cd/+ x Cd/+ matings was used to investigate the embryological basis for the dental abnormalities. Microphthalmic embryos were classed as Cd/Cd, and their most normal litter mates were selected as controls (+/+ or Cd/+). An additional set of control embryos came from the inbred strain CBA/Cam (+/+). Serial sagittal sections of the heads of these embryos were examined microscopically, and the maximum anteroposterior diameters of the developing upper and lower first molars were measured. Reduction in the rates of growth and morphodifferentiation of Cd/Cd first molars, relative to those of litter mate controls, was associated with the appearance of an adjacent abnormal proliferation of the dental lamina. Some proliferations in older embryos showed signs of early tooth germ formation, but many were seen to have regressed and no examples of supernumerary teeth have been found in Cd/Cd adults. Small size of Cd/Cd molars may therefore result from competitive inhibition of molar growth by a transient abnormal laminal proliferation, and Cd/Cd cusp patterns from the relatively premature onset of hard tissue formation during normal but retarded sequences of morphodifferentiation.

INTRODUCTION

The semidominant autosomal gene ‘crooked’ in the mouse (also known as ‘crooked-tail’, symbol Cd) produces a variety of malformations when homozygous. Among these are anomalies of the axial skeleton, a relatively elongated head with a pointed snout and abnormal ear inclination, microphthalmia, a tail that has irregular tail rings and is sparsely populated with abnormal hairs, and dental abnormalities. Homozygosity is frequently lethal, either before or soon after birth. In the original mutant stock only an estimated 28 % of homozygotes survived the perinatal period, and the majority of these were infertile. Of the 72 % of homozygotes that did not survive beyond birth, about one third were thought to have suffered lethality before implantation. In heterozygotes, the abnormalities of homozygotes are expressed to a much lesser degree, malformations often being confined to the lumbo-sacral and caudal regions of the

1 Author’s address: School of Dental Surgery, Chambers Street, Edinburgh EH1 1JA, U.K.
vertebral column. In the original mutant stock about 11% of heterozygotes were phenotypically normal, and all appeared to be normally viable and fertile. As a result of the anomalies of the caudal skeleton surviving homozygotes and some heterozygotes have sharp bends in the tail. The effects of the gene were first described by Morgan (1954) and have been further discussed by Grünneberg (1963).

Dental abnormalities of crooked mice have previously been reported by Grewal (1962) and Grünneberg (1965), but before describing these it is appropriate to review the main features and nomenclature of normal mouse teeth. The normal mouse dentition is composed of one continuously growing incisor and three molars of limited growth in each quadrant of the jaws. The upper molars are referred to as m$^1$, m$^2$ and m$^3$, the lower molars as m$_1$, m$_2$ and m$_3$, and their surfaces as anterior, posterior, buccal and lingual. The crown of m$^1$ has eight cusps, all of which are tilted posteriorly. Numbered from anterior to posterior there are three central cusps, 1, 2 and 3; three buccal, B1, B2 and B3; and two lingual, L1 and L2. All the cusps present in m$^1$, except cusp 1, are represented in m$^2$. The crown of m$_1$ has seven cusps, most of which are tilted anteriorly. Numbered from anterior to posterior there are three buccal, B1, B2 and B3; three lingual, L1, L2 and L3; and a single central posterior cusp, 4. All the cusps present in m$_1$, except L1 and sometimes B1, are represented in m$_2$. The cusp patterns of the third molars are simpler and subject to some variation. Both m$^1$ and m$^2$ have three roots, anterior and posterior buccal roots and a single lingual root. Both m$_1$ and m$_2$ have two roots, one anterior and one posterior. Third molars usually have single roots that may show a tendency towards division into three (m$^3$) or two (m$_3$) at a variable distance from the apex.

In Cd/Cd mice the lower incisors are either small or absent. The upper incisors are of about normal size, but, if not worn down by an opposing lower, grow round in a spiral. The crowns of the first and second molars, particularly the uppers, are smaller and more bulbous than normal. In m$^1$, cusp 1 is relatively large and more erect than normal, cusp B1 is absent, and cusps L1 and L2 are not as distinctly separated from each other and from cusp 1 as they are in the normal mouse. The anterior and lingual roots are usually fused. In m$^2$, all members of the normal complement of cusps are usually represented, but all are reduced in size and less distinctly separated from each other than normal. This lack of distinctness is most severe on the lingual side, where there may be an undivided large cusp in the place of cusps L1 and L2. The cusp patterns of the lower first and second molars are less abnormal than those of the uppers. In m$_1$, cusps B1 and L1 are poorly separated and cusp 4 is relatively small. In m$_2$, cusp B1 may be reduced or absent, and cusp 4 is usually absent. Bifurcation of the roots of both m$_1$ and m$_2$ usually occurs a little further towards the root apices than normal. Upper, and particularly lower third molars, are either relatively small or altogether absent. Excellent illustrations of normal and Cd/Cd teeth are given by Grünneberg (1965). The dental morphology of Cd/+ mice is usually
Tooth development in the ‘crooked’ mouse

Table 1. The numbers of embryos of each genotype sectioned at each stage, followed in brackets by the numbers of litters from which they were taken. Left sides only of CBA mice, but both sides of mice from the mutant stock were sectioned.

<table>
<thead>
<tr>
<th>Stage</th>
<th>CBA/Cam</th>
<th>Litter mate controls</th>
<th>Cd/Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 14</td>
<td>10 (3)</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>15</td>
<td>10 (3)</td>
<td>2 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>16</td>
<td>10 (3)</td>
<td>2 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>17</td>
<td>10 (3)</td>
<td>2 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>18</td>
<td>10 (3)</td>
<td>2 (2)</td>
<td>4 (2)</td>
</tr>
</tbody>
</table>

normal, though the teeth may be slightly smaller than those of wild-type mice from the same stock (Grewal, 1962).

The present paper is concerned with the embryology of the dental abnormalities in crooked homozygotes, particularly those of the molar crowns. This is an aspect of Cd/Cd mice that has not previously been investigated.

MATERIAL AND METHOD

Embryos from litters of Cd/+ × Cd/+ matings were obtained from two sources: University College London; and the author’s own mutant stock, founded by University College animals. The gestational stage was determined either by an examination of the external features of the embryos (Grüneberg, 1943), or by a combination of timing from the day on which a vaginal plug was found (day zero) and an examination of external features of the resulting litter. Litters of days 14, 15, 16, 17 and 18 were used. Embryos with microphthalmia were classed as Cd/Cd, and the most normal and well-developed member of each litter was selected as a litter-mate control (presumed +/+ or Cd/+). In addition, a series of embryos from the inbred strain CBA/Cam, wild-type at the crooked locus, was used for comparison. The heads of all embryos were fixed in Bouin’s fluid, serially sectioned at 10 μm in the sagittal plane and stained with haematoxylin and eosin. The numbers of embryos sectioned at each stage are shown in Table 1.

The serial sections were examined, and, for Cd/Cd mice and their litter mate controls, the maximum anteroposterior diameter of each tooth germ, from the earliest stage at which it could be measured (day 14 for m1 and day 15 for m2), was taken from images produced by a projection microscope at a standard magnification.
Tooth development in the ‘crooked’ mouse

RESULTS

No clear difference was found between litter mate control embryos and CBA/Cam embryos at any of the stages examined. Dental development in Cd/Cd embryos can therefore be compared with both groups of controls as a whole.

No difference was observed between the upper incisor germs of Cd/Cd mice and those of their controls. By contrast, the lower incisor germs of Cd/Cd mice showed definite differences from the controls, particularly from day 16 onwards, and a considerable degree of variation. Some Cd/Cd lower incisor germs were fairly normal in size and histodifferentiation for their developmental stage, whereas others were smaller, with abnormal odontoblasts and no ameloblasts (Fig. 1 A, B).

A difference between the upper molar regions of Cd/Cd mice and controls was observed at day 14. In one out of four Cd/Cd upper quadrants (two embryos) examined, there was a small knot of cells arising from the anterior half of the band of dental lamina that later would have given rise to the upper first molar germ. This knot, which appeared to have been produced by a localized proliferation of laminal cells, was not present in control embryos (Fig. 1 C). Larger and more definite laminal proliferations were found in five out of six Cd/Cd upper quadrants at day 15 (Fig. 1 D), and larger proliferations still in all of the six Cd/Cd upper quadrants at day 16 (Fig. 1 E and F). At day 17, the proliferation was either absent or appeared to be regressing (four out of six Cd/Cd upper quadrants, Fig. 1 G), or had progressed further to an early stage of tooth germ formation with an associated mesenchymal condensation (two out of six Cd/Cd upper quadrants, Fig. 1 H). In the two cases of early tooth germ formation found at day 17 the first molar germs were smaller than those in the four cases where the proliferation had either regressed or was absent. At day 18, the proliferation was either reduced or absent in all of the eight Cd/Cd upper quadrants examined. The more bulbous shape of Cd/Cd upper molar germs, compared with controls, was clearly visible on days 17 and 18 (Fig. 1 I and J).

---

Fig. 1. 10 μm sagittal sections through Cd/Cd and control (CBA/Cam) embryos at different gestational ages. Anterior to the left. AL, ameloblast layer; OL, odontoblast layer; ER, remnants of internal enamel epithelium; AO, abnormal odontoblasts; DL, dental lamina; R, rudiment of m1. Arrows indicate abnormal proliferations of the dental lamina. See text under ‘Results’ for full explanation. (A) Control upper incisor, day 18. (B) Cd/Cd upper incisor, day 18. (C) Control upper molar region, day 14. (D) Cd/Cd upper molar region, day 15. (E) Control upper molar region, day 16. (F) Cd/Cd upper molar region, day 16. (G) Cd/Cd upper molar region, day 17. (H) Cd/Cd upper molar region, day 17. (I) Control upper molars, day 18. (J) Cd/Cd upper molars, day 18. (K) Control lower molar region, day 14. (L) Cd/Cd lower molar region, day 15. (M) Cd/Cd lower molar region, day 16. (N) Control lower molars, day 18.
No difference between the lower molars of \(Cd/Cd\) mice and those of controls was observed at day 14 (Fig. 1 K), but at day 15 there was evidence of an abnormal proliferation of the dental lamina anterior to the point of origin of the first molar germ in five out of six \(Cd/Cd\) lower quadrants (Fig. 1 L). At day 16, all six \(Cd/Cd\) lower quadrants showed signs of abnormal laminal proliferation (Fig. 1 M), but at days 17 and 18 the abnormal outgrowth was either much reduced or absent in all \(Cd/Cd\) lower quadrants, and \(Cd/Cd\) lower molar germs were morphologically indistinguishable from those of controls (Fig. 1 N).

Measurement of the anteroposterior diameters of upper and lower first molar germs showed that the mean diameter of \(Cd/Cd\) germs was always less than the corresponding mean diameter for litter mate controls. The difference in size between \(Cd/Cd\) germs and their controls appeared to be related to the proportion of \(Cd/Cd\) quadrants in which ‘active’ laminal proliferations were observed; that is, definite proliferations that were larger than those of the previous stage and where there were no signs of regression. The difference in size was greatest at day 17, one day after the maximum incidence of active proliferations in both upper and lower jaws (Fig. 2).
**Tooth development in the 'crooked' mouse**

**DISCUSSION**

The most general manifestation of the crooked gene in the dentition is small size of the fully formed teeth. The measurements of developing first molar germs, summarized in Fig. 2, show that after an initial period when tooth germ size was similar in Cd/Cd embryos and their controls, there was a divergence between the rates of growth of mutant and control first molars. This divergence was associated with the appearance of an abnormal proliferation of the dental lamina in both the upper and lower jaws. Figure 2 suggests that the relative growth rates of mutant and control first molars are not maintained, but that, after initial divergence, the growth of Cd/Cd first molar germs tends to run parallel to that of their controls. Equivalent tooth size in the two genotype groups is separated by a time lag of approximately one day, though the lag is greater in the upper jaw than in the lower.

All parts of m1 and m1 affected by the gene are those appearing late in the ontogeny of normal first molar crowns, and m1 is more severely affected than m1 (Grüneberg, 1965). The different time lags suggested by Fig. 2 may provide an explanation for this. If hard tissue formation occurs at the same chronological age in both Cd/Cd and normal embryos, irrespective of molar size and morphodifferentiation (and there was no indication to the contrary in the present material), the resulting morphology of fully formed mutant first molars would be as is found, namely, corresponding to an incomplete level of normal morphodifferentiation with the upper first molar more incomplete than the lower. The small size and simplified morphology of Cd/Cd first molars might therefore be explained, at least in part, by competitive inhibition of first molar growth due to an adjacent proliferation of the dental lamina. It has already been mentioned that within the present mutant material first molar size was smallest in quadrants where the laminal proliferations were most advanced. There is good evidence that interaction between an abnormal laminal proliferation and first molar growth also occurs in heterozygotes for the X-linked gene 'tabby' (Ta) in the mouse (Sofaer, 1975).

However, competitive inhibition of first molar growth is unlikely to be the whole explanation, because Cd/Cd second molars are also smaller than normal, and third molars are frequently absent. Nevertheless, it is possible that the effect of abnormal laminal proliferation may persist for second and third molars, even though a proliferation itself is no longer present. It appears, both from observation and experiment, that all three molars of one quadrant arise from cells that, at early stages of tooth germ formation, are represented by the first molar germ and its immediately associated epithelium; that is, the three molars of one quadrant do not have clearly independent origins from different points along the dental lamina (Lumsden & Osborn, 1976). More convincing evidence in favour of an additional, perhaps more fundamental, cause of the dental abnormalities comes from the lower incisor findings. These imply that
there is an intrinsic deficiency in the internal enamel epithelium, or its interaction with the adjacent preodontoblasts, that prevents it from differentiating into ameloblasts (Fig. 1 A, B). This observation also provides another interesting parallel with tabby, where homozygotes and hemizygotes show an abnormality of incisor histodifferentiation indistinguishable from that illustrated in Fig. 1 B (Sofaer, 1969a).

There are further points of similarity between the dental abnormalities of Cd/Cd mice and homozygotes (or hemizygotes) for tabby, its recessive autosomal mimics 'crinkled' (cr) and 'downless' (dl) (Grüneberg, 1965; Sofaer, 1969b), and its more recently discovered dominant autosomal mimic 'sleek' (Slk) (Cattanach, 1975; Sofaer, 1977). The first molar crowns are all smaller and more bulbous than normal and have simplified cusp patterns. The missing cusps in both the upper and the lower molars are those that develop late in the normal mouse, with one exception. Cusp B3 of m¹ and m², which appears early in the ontogeny of normal upper molar crowns, and which is present in Cd/Cd mice, is always missing in tabby, crinkled and downless. However, in sleek, which in every other respect is indistinguishable from tabby, crinkled and downless, cusp B3 has been found in about one quarter of the dentitions examined.

A point of difference between Cd/Cd mice and tabby, crinkled, downless and sleek occurs in the upper second molars. These have always been found to be smaller than normal in Cd/Cd mice, whereas they are sometimes larger than normal in tabby and its mimics. A possible explanation for this is that the effect of tabby and its mimics seems to be a timed one, causing suppression of tooth germ growth during particular phases of development. Early growth of the first molar occurs during such a suppression phase, whereas early growth of the second molar occurs at a time when suppression has been relaxed, and when there is therefore an opportunity for compensatory increase in size (Sofaer, 1969a). The absence of cusp B3 could also be due to this suppression phase, in that the cusp normally appears first during the time when suppression would be operative. In the sleek mice examined, cusp B3 may have developed because of a slight shift or shortening of the suppression phase.

One of the most interesting features of tabby, crinkled, downless and sleek dentitions is the occasional occurrence of a supernumerary tooth anterior to the first molar of the normal series in both upper and lower jaws. These teeth develop from a proportion of the abnormal laminal proliferations, the remainder regressing before a tooth germ becomes established. In a large sample of tabby heterozygotes, probably the majority of abnormal proliferations in the upper jaw, and about half of those in the lower jaw, regressed without forming a supernumerary tooth (Sofaer, 1975). It has been shown here that early stages of tooth germ formation do occur in Cd/Cd mice. It therefore seems likely that a proportion of the abnormal proliferations of Cd/Cd mice also go on to form supernumerary teeth, and that if a larger number of Cd/Cd adults were examined such teeth might be found.
Tooth development in the ‘crooked’ mouse

The Cd/Cd dentition therefore has some features in common with the dentitions of tabby, crinkled, downless and sleek mice. The skeletal abnormalities of crooked mice, the majority of which have been shown to be a consequence of irregular somite formation (Grüneberg, 1963), suggest that there is a defect in the mesenchymal element of developing Cd/Cd dentitions. By contrast, experiments with downless (Sofaer, 1973) and crinkled (Mayer, Miller and Green, 1977) indicate that, in these mutants, it is the epidermal component that is at fault. Similar experiments with tabby have produced equivocal results (Sofaer, 1974), and sleek has not been investigated in this way. Nevertheless, the evidence available suggests that a reduction in tooth-germ growth and the formation of bulbous tooth germs, associated with abnormal proliferations of the dental lamina and even failure of histodifferentiation of the internal enamel epithelium, can be produced by primary defects in either the epithelial or the mesenchymal component of a developing dentition.

The author is grateful to Professor H. Grüneberg for his encouragement to undertake the study, to Professor Grüneberg and Dr G. M. Truslove for the gift of Cd/+ mice and fixed embryonic litters, and to Miss Edith Redpath for technical assistance.

REFERENCES


(Received 25 March 1977, revised 10 May 1977)