Maternal treatment with cortisone accelerates eyelid closure and other developmental fusion processes in fetal mice

D. M. JURILOFF

Department of Medical Genetics, 6174 University Boulevard, University of British Columbia, Vancouver, BC, Canada V6T 1W5

Summary

Cortisone acetate administered to pregnant CBA/J and SWV/Bc mice at 30 to 100 mg kg\(^{-1}\) on day 14 of gestation causes accelerated development of eyelid closure, digit fusion and fusion of pinnae to the scalp on day 16 of gestation. Eyelid closure seems to be accelerated more than hindlimb digit fusion.

The results support the hypothesis that the prevention of the open-eye birth defect in \(l_g^M/l_g^M\) mutant mice by cortisone is through a shift of eye closure to an earlier chronological or morphological stage, and that the \(l_g^M\) mutation causes delay in eyelid development and closure.

Most previous studies of the effects of glucocorticoids on morphological development have focused on high doses that induce defects, such as cleft palate, and on treatment earlier in gestation. In the studies reported here, lower doses were used and an acceleration of apparently normal development was observed. The results support the possibility that the gene regulatory effects of physiological levels of glucocorticoids are involved in normal morphological development of mammalian fetuses. The regulation of genes is far less well understood for morphological development than for biochemical differentiation. The responses of the four morphological traits described in this paper seem to offer a route to some greater insight into the genetic regulation of morphology.

Key words: cortisone, eyelid, mouse, fusion, maternal treatment, mutation \(l_g^M\).

Introduction

There are only a few examples of preventing a birth defect by treating the mother during pregnancy. Correcting the open-eyes defect in \(l_g^M/l_g^M\) (lidgap-Miller) mutant mice by administering glucocorticoid hormone to the pregnant mother is one of these (Watney & Miller, 1964; Harris, Juriloff & Biddle, 1984; Nakatsu, Ichara & Miller, 1984; Harris & Juriloff, 1986). The mechanism by which this 'cure' is effected is not known, although it has been suggested that induction of maturation of the periderm may be involved (Harris & Juriloff, 1986). The defect in \(l_g^M/l_g^M\) mutants is not a deficiency of corticosterone (Tuan, Rekdal & Burton, 1971).

During normal mammalian development, the eyelids grow and flatten across the eyes and fuse together. Later the fusion, which involves only the epidermis and periderm layers, breaks down and the eyes 'open'. In the mouse, closure takes place on days 15 and 16 of gestation (Harris & McLeod, 1982), and eye opening on days 12–14 after birth (Theiler, 1972).

In the untreated \(l_g^M/l_g^M\) mutant, the eyelids fail to grow across the eyes and pups are born with their eyes wide open. Later, the eyes develop corneal opacities, possibly due to physical damage to the unprotected corneas in the newborn period. In \(l_g^M/l_g^M\) fetuses from glucocorticoid-treated mothers, the eyelids grow across the eyes and fuse together and the pups are born with their eyes closed (Harris & Juriloff, 1986).

One of the hypotheses that can explain the curative effect of glucocorticoids on \(l_g^M/l_g^M\) fetuses is that the effect of the genetic defect is to delay eyelid closure beyond some critical point in time, a threshold, beyond which the eyelids are incapable of fusing. The hypothesized effect of glucocorticoids would be to accelerate eyelid development and bring it back to the normal side of the threshold. To test this hypothesis, the effects of maternal treatment with...
Two inbred strains of mice were used. CBA/J mice were obtained from the Jackson Laboratory and acclimatized to our conditions for at least two weeks before breeding. SWV/Bc mice were obtained from our own colony. All females were nulliparous and 2–5 months old when bred. All were given Purina Lab Chow and tap water acidified with HCl to pH3-1 ad libitum. All animals were maintained in standard polycarbonate cages in windowless rooms with controlled temperature (22±2°C). The mice were housed in the Zoology Research Unit 1, Animal Care Centre, UBC (Experiment 1) and in the Medical Genetics Mouse Unit, UBC (Experiments 2, 3). The light cycles and mating protocols used in these facilities varied between the three periods, months apart, when the three experiments were done, and each experiment was consequently conducted under a different light/mating protocol. Mice ovulate in relation to the midpoint of the dark period (Bronson, Dagg & Snell, 1966). The day of the plug was called day 0 of gestation. The effect of the differences in protocol would be to cause differences between experiments of a few hours in gestational age at treatment or scoring. The interpretation of the data is therefore limited to differences between treated and control groups within each experiment.

Materials and methods

Mice

Two inbred strains of mice were used. CBA/J mice were obtained from the Jackson Laboratory and acclimatized to our conditions for at least two weeks before breeding. SWV/Bc mice were obtained from our own colony. All females were nulliparous and 2–5 months old when bred. All were given Purina Lab Chow and tap water acidified with HCl to pH3-1 ad libitum. All animals were maintained in standard polycarbonate cages in windowless rooms with controlled temperature (22±2°C). The mice were housed in the Zoology Research Unit 1, Animal Care Centre, UBC (Experiment 1) and in the Medical Genetics Mouse Unit, UBC (Experiments 2, 3). The light cycles and mating protocols used in these facilities varied between the three periods, months apart, when the three experiments were done, and each experiment was consequently conducted under a different light/mating protocol. Mice ovulate in relation to the midpoint of the dark period (Bronson, Dagg & Snell, 1966). The day of the plug was called day 0 of gestation. The effect of the differences in protocol would be to cause differences between experiments of a few hours in gestational age at treatment or scoring. The interpretation of the data is therefore limited to differences between treated and control groups within each experiment.

Cortisone

Cortisone acetate (Cortone, 50 mg ml$^{-1}$, Merck-Frosst Laboratories) was injected subcutaneously into the interscapular region on day 14 of pregnancy. Day 14 was chosen because it is the day when cortisone is most effective in preventing open-eyes in the IgM/IgM mutant (Harris, Juriloff & Biddle, 1984). The vehicle for the Cortone preparation (9 mg sodium chloride, 4 mg polysorbate 80, 5 mg sodium carboxymethylcellulose, 1 ml water) has shown no effect on eye closure in IgM/IgM mice (unpublished data). Similarly, administered in volumes equivalent to those containing 50 mg kg$^{-1}$ of cortisone and following the protocol described in Experiment 3, the vehicle had no effect, compared with untreated controls, on eye, ear or digit fusion in CBA/J mice (unpublished data). Therefore no further studies using vehicle alone were done.

Experiments

Expt 1

SWV/Bc strain mice were used. Females were placed with singly-caged males for 3–4 h between 8.00 and 13.00 h. At the end of this period, the females were checked for the presence of vaginal plugs. The animal rooms were on a 12 h light (7.00 to 19.00 h) and dark cycle.

At 12.00 h on day 14 of gestation, the pregnant mice were weighed and treated with 30, 60 or 100 mg kg$^{-1}$ of cortisone acetate or left untreated. Control and treated mice were collected concurrently. On day 16 of gestation at 12.00 h, the pregnant mice were killed by cervical dislocation and the uterine contents were dissected out, dead embryos and fetuses recorded, and live fetuses were fixed in Bouin’s fluid. The specimens were scored, as described below, several months later. In addition, the palates were examined.

Expt 2

SWV/Bc strain mice were used. Females were placed with singly-caged males for 3–4 h between 12.00 and 16.00 h. At the end of this period, the females were checked for the presence of vaginal plugs. The animal rooms were on a 12 h light (15.00 to 3.00 h) and dark cycle.

At 11.00 h on day 14 of gestation, the pregnant mice were alternately weighed and treated with 60 mg kg$^{-1}$ of cortisone acetate or left untreated. On day 16 of gestation at 12.00 h, the pregnant mice were killed by cervical dislocation and the uterine contents were immediately scored as described below.

Expt 3

CBA/J strain mice were used. Females were placed with singly-caged males in the late afternoon (about 16.00 h) and examined the following morning (about 9.00 h) for vaginal plugs. The animal rooms were on a 12 h light (7.00 to 19.00 h) and dark cycle.

At 15.00 h on day 14 of gestation, the pregnant mice were alternately weighed and treated with 50 mg kg$^{-1}$ of cortisone acetate or left untreated. On day 16 of gestation, the untreated mice were killed by cervical dislocation at 12.00 h or at 18.00 h, and the treated mice were similarly killed at 8.00, 12.00 or 18.00 h. The uterine contents were immediately scored as described below.

After scoring, the fetuses were fixed in Bouin’s fluid. Several months later they were removed from fixative, blotted on a paper towel for a few seconds and weighed. The mean fetal weight was calculated for each litter. These litter means were used as the basic data and were used to calculate the mean weight for each group in the experiment. Differences between groups were compared by a one-way analysis of variance.

Comparisons of fetal development

Dead implanted embryos ('moles') were recorded, as were dead fetuses. Live fetuses were examined and the state of external developmental processes that take place on day 16 were recorded for each embryo.

The state of eye closure was scored as wide open (w), medium open (m), small opening (s), pinhole (ph) and closed (+), according to a standard in extensive use in our laboratory (Fig. 1; and see Harris et al. 1984). Eyelid closure is mildly asymmetrical in some (less than 10%) of fetuses. For example, some have one eye in the medium open stage and the other in the small opening stage. There was no significant difference within any treatment or
Cortisone accelerates eyelid closure in fetal mice

Statistical analysis

The frequency of closed eyes within each litter was transformed to its Freeman-Tukey arcsine value (Mosteller & Youtz, 1961). The mean litter arcsine value was compared among treated and control groups within each experiment by a one-way analysis of variance, using the statistical package of a Hewlett-Packard HP-41C calculator. Similar analyses based on litter arcsine values were done for the frequency of completely fused forelimb digits, completely fused hindlimb digits and completely fused pinnae. The probability level chosen for statistical significance was 5%, unless otherwise noted.

Where significant F values were obtained and there were more than two groups, the Student-Newman-Keuls test for unequal subclass numbers (Sokal & Rohlf, 1969) was used to identify the groups that differed.

Results

In each of the three experiments, fetuses from dams treated with cortisone ('treated fetuses') were more advanced in eyelid, forelimb digit, hindlimb digit and pinna development than were their untreated controls examined at the same chronological age (Table 1). For example, in Expt 1, 52% of untreated SWV fetuses had completed eyelid closure, whereas the eyelids of 98% of those whose mothers were treated with 60 mg kg⁻¹ cortisone were closed. In Expt 2, 10% of untreated SWV had closed eyes whereas 99% of treated fetuses did. In Expt 3, another strain, CBA, showed the same effect. 4% of untreated fetuses scored at 18.00 h had closed eyes, compared with 84% of treated fetuses.

Forelimb fusion takes place earlier in development than eye closure and was completed in more than half of the untreated control fetuses in each experiment. After cortisone treatment, 87-100% of fetuses had completed fusion of forelimb digits (Table 1). Pinna fusion also takes place earlier in development than eyelid closure and 33-72% of untreated controls had completed this step. After cortisone treatment, 92-100% of fetuses had completed fusion of pinnae to the scalp (Table 1).

Completed hindlimb fusion takes place later in development than eye closure. No controls achieved this stage and the frequency in treated fetuses was low, ranging from 0 to 13% (Table 1).

Statistical analysis based on litter arcsine transformed proportions of fetuses with completed fusion in each of the four structures indicated that the changes observed were real (Table 2). In each of the three experiments, the proportion of fetuses with closed eyes was significantly greater in treated than in untreated fetuses. Completed forelimb digit fusion was significantly more frequent in treated than in untreated fetuses in Expts 2 and 3. Completed hindlimb digit fusion was significantly more frequent

---

Control group between right and left eyes (P > 0.05; contingency χ² tests) in the proportions falling into the various closure categories. To simplify the data and have one eye value for each fetus, the data for left eyes only were used for all further analysis.

The digits of the forelimbs, which are separate on early day 15, fuse together, beginning at the base of the digits and proceeding toward the tips (Maconnachie, 1979; Trasler, 1965). The state of fusion between the third and fourth digit was scored into the following categories: no fusion, fused about one third of the distance to be joined, two thirds fused or fusion complete.

The digits of the hindlimbs follow a similar pattern of development a few hours later than the forelimbs (Maconnachie, 1979). The fusion between the third and fourth digit was scored as: no fusion, one quarter fused, one half fused (see Fig. 1), three quarters fused, fusion complete.

The pinnae also undergo a developmental change which involves fusion to the scalp. The pinnae fold forward over the external auditory meatus. Fusion begins at the ends of the pinnae and proceeds to the centre from both ends (Maconnachie, 1979). The process was scored as: no fusion, one quarter fused, one half fused, three quarters fused, completely fused.

---

Fig. 1. Stages of eyelid closure used in scoring eyelid development. +, closed; ph, pinhole; s, small opening; m, medium opening; w, wide open. Also shown is a hindlimb with digits in the half-fused (1/2f) stage.
Table 1. Effects of cortisone acetate given on day 14 of gestation on development of day-16 fetuses

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of litters</th>
<th>% moles and dead</th>
<th>Number of live fetuses</th>
<th>% closed eyes*</th>
<th>% forelimb digits fusion complete</th>
<th>% hindlimb digits fusion complete</th>
<th>% pinnae fusion complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1 - SWV scored at 12.00 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>5</td>
<td>134</td>
<td>52</td>
<td>86</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>Cortisone, 30 mg kg⁻¹</td>
<td>4</td>
<td>7</td>
<td>51</td>
<td>65</td>
<td>100</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>Cortisone, 60 mg kg⁻¹</td>
<td>4</td>
<td>6</td>
<td>46</td>
<td>98</td>
<td>100</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Cortisone, 100 mg kg⁻¹</td>
<td>4</td>
<td>10</td>
<td>47</td>
<td>100</td>
<td>100</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>Expt 2 - SWV scored at 12.00 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>3</td>
<td>61</td>
<td>10</td>
<td>54</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td>Cortisone, 60 mg kg⁻¹</td>
<td>6</td>
<td>1</td>
<td>68</td>
<td>99</td>
<td>100</td>
<td>3</td>
<td>nd</td>
</tr>
<tr>
<td>Expt 3 - CBA given cortisone, 50 mg kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, scored 12.00 h</td>
<td>7</td>
<td>11</td>
<td>51</td>
<td>0</td>
<td>55</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Control, scored 18.00 h</td>
<td>4</td>
<td>10</td>
<td>27</td>
<td>4</td>
<td>63</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Treated, scored 8.00 h</td>
<td>5</td>
<td>11</td>
<td>39</td>
<td>22</td>
<td>87</td>
<td>0</td>
<td>97</td>
</tr>
<tr>
<td>Treated, scored 12.00 h</td>
<td>5</td>
<td>12</td>
<td>37</td>
<td>38</td>
<td>97</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Treated, scored 18.00 h</td>
<td>5</td>
<td>11</td>
<td>32</td>
<td>84</td>
<td>97</td>
<td>9</td>
<td>100</td>
</tr>
</tbody>
</table>

* Left eyes.
nd, not done.

Table 2. Effects of cortisone acetate given on day 14 of gestation on development of day-16 fetuses: statistical analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Closed eyes */ Mean litter arcsine ± S.E.</th>
<th>Forelimb digit fusion complete</th>
<th>Hindlimb digit fusion complete</th>
<th>Pinnae fusion complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1 - SWV scored at 12.00 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>46.4±7.2</td>
<td>71.2±6.7</td>
<td>7.7±0.1</td>
<td>60.4±6.0</td>
</tr>
<tr>
<td>Cortisone, 30 mg kg⁻¹</td>
<td>55.5±11.7</td>
<td>82.2±0.1</td>
<td>7.9±0.1</td>
<td>75.2±14.1</td>
</tr>
<tr>
<td>Cortisone, 60 mg kg⁻¹</td>
<td>78.8±3.1</td>
<td>81.8±0.2</td>
<td>12.5±4.1</td>
<td>81.8±0.2</td>
</tr>
<tr>
<td>Cortisone, 100 mg kg⁻¹</td>
<td>81.8±0.3</td>
<td>81.8±0.3</td>
<td>20.6±7.4</td>
<td>81.8±0.3</td>
</tr>
<tr>
<td>Expt 2 - SWV scored at 12.00 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.1±5.6</td>
<td>45.6±10.2</td>
<td>6.4±3.7</td>
<td>nd</td>
</tr>
<tr>
<td>Cortisone, 60 mg kg⁻¹</td>
<td>79.7±2.0</td>
<td>81.7±0.1</td>
<td>11.6±3.4</td>
<td>nd</td>
</tr>
<tr>
<td>Expt 3 - CBA given cortisone 50 mg kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, scored 12.00 h</td>
<td>10.4±0.6</td>
<td>47.2±6.7</td>
<td>10.4±0.6</td>
<td>33.1±5.6</td>
</tr>
<tr>
<td>Control, scored 18.00 h</td>
<td>14.4±3.7</td>
<td>58.9±6.3</td>
<td>10.6±0.5</td>
<td>50.9±5.5</td>
</tr>
<tr>
<td>Treated, scored 8.00 h</td>
<td>29.1±6.5</td>
<td>69.8±6.5</td>
<td>9.9±0.4</td>
<td>77.4±2.5</td>
</tr>
<tr>
<td>Treated, scored 12.00 h</td>
<td>38.9±2.6</td>
<td>76.6±3.4</td>
<td>10.2±0.4</td>
<td>79.8±0.4</td>
</tr>
<tr>
<td>Treated, scored 18.00 h</td>
<td>66.2±6.0</td>
<td>76.1±2.9</td>
<td>20.6±4.2</td>
<td>76.1±2.9</td>
</tr>
</tbody>
</table>

1 F₁,₃₈ = 5.08; P < 0.05
2 F₁,₃₈ = 0.92; P > 0.50
3 F₁,₃₈ = 3.67; P < 0.05
4 F₁,₃₈ = 3.35; P < 0.05
5 F₁,₂₀ = 126.56; P < 0.001
6 F₁,₁₅ = 15.18; P < 0.005
7 F₁,₁₀ = 1.71; P > 0.10
8 F₁,₇₁ = 29.64; P < 0.001
9 F₄,₃₁ = 5.46; P < 0.05
10 F₄,₃₁ = 6.00; P < 0.05
11 F₄,₃₁ = 26.67; P < 0.001

*a,b,c Means sharing the same superscript do not differ by Student-Newman-Keuls test at P < 0.05.

L, left eyes.
nd, not done.
Cortisone accelerates eyelid closure in fetal mice

Table 3. Effects of cortisone acetate given on day 14 of gestation on development of day-16 fetuses. Comparison of distribution of fetuses among stages of development

<table>
<thead>
<tr>
<th>Group</th>
<th>Eyelids*</th>
<th>Forelimbs†</th>
<th>Hindlimbs‡</th>
<th>Pinnae‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>w m s ph</td>
<td>nf 1/3</td>
<td>2/3 c</td>
<td>nf 1/4</td>
</tr>
<tr>
<td>Expt 1 – SWV scored at 12.00 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1 3 23 21 52</td>
<td>1 2 10 87</td>
<td>8 16 52 24 0</td>
<td>0 0 1 28 72</td>
</tr>
<tr>
<td>Cortisone, 30 mg kg⁻¹</td>
<td>0 4 16 16 65</td>
<td>0 0 0 100</td>
<td>4 24 28 45 0</td>
<td>0 0 0 8 92</td>
</tr>
<tr>
<td>Cortisone, 60 mg kg⁻¹</td>
<td>0 0 0 2 98</td>
<td>0 0 0 100</td>
<td>0 0 15 81 4</td>
<td>0 0 0 0 100</td>
</tr>
<tr>
<td>Cortisone, 100 mg kg⁻¹</td>
<td>0 0 0 0 100</td>
<td>0 0 0 100</td>
<td>0 0 4 83 13</td>
<td>0 0 0 0 100</td>
</tr>
<tr>
<td>Expt 2 – SWV scored at 12.00 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21 38 15 16 10</td>
<td>2 3 41 54</td>
<td>2 67 30 2 0</td>
<td>— — — —</td>
</tr>
<tr>
<td>Cortisone, 60 mg kg⁻¹</td>
<td>0 0 0 1 99</td>
<td>0 0 0 100</td>
<td>0 0 13 84 3</td>
<td>— — — —</td>
</tr>
<tr>
<td>Expt 3 – CBA given cortisone 50 mg kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, scored 12.00 h</td>
<td>69 20 12 0 0</td>
<td>2 12 31 55</td>
<td>27 39 29 4 0</td>
<td>16 10 16 25 33</td>
</tr>
<tr>
<td>Control, scored 18.00 h</td>
<td>19 44 30 4 4</td>
<td>0 7 15 63</td>
<td>19 26 37 19 0</td>
<td>11 4 0 22 55</td>
</tr>
<tr>
<td>Treated, scored 8.00 h</td>
<td>13 21 31 13 23</td>
<td>0 5 8 87</td>
<td>8 23 31 38 0</td>
<td>0 0 0 3 97</td>
</tr>
<tr>
<td>Treated, scored 12.00 h</td>
<td>8 14 24 16 38</td>
<td>0 0 3 97</td>
<td>8 6 16 70 0</td>
<td>0 0 0 0 100</td>
</tr>
<tr>
<td>Treated, scored 18.00 h</td>
<td>0 3 9 3 54</td>
<td>0 0 3 97</td>
<td>0 3 3 84 9</td>
<td>0 0 0 0 100</td>
</tr>
</tbody>
</table>

* w, wide open; m, medium open; s, small opening; ph, pinhole; +, closed.
† nf, no fusion; 1/3, one third fused; 2/3, two thirds fused; c, fusion complete.
‡ nf, no fusion; 1/4, one quarter fused; 1/2, one half fused; 3/4, three quarters fused; c, fusion complete.

in treated fetuses than controls in Expts 1 and 3. Completed fusion of pinnae to scalp was significantly more frequent in treated than control fetuses in Expts 1 and 3.

When stages of development less advanced than complete closure or fusion were considered, the pattern also indicated that cortisone-treated fetuses were more advanced than untreated fetuses (Table 3). Untreated fetuses tended to be distributed among the less advanced of the incomplete closure stages in all experiments and for all four phenomena examined.

There were few treated fetuses that did not have completely fused forelimb digits and pinnae (Table 3). As these completely fused stages are 'off-scale' in the sense that no further morphological change can be detected while other structures continue to change, the relationship between timing of eye closure and forelimb digit fusion or pinna fusion could not be examined. Hindlimb digit fusion was 'on-scale' in both treated and untreated fetuses (Table 3) and the relationship between eye closure and hindlimb digit fusion was examined (Table 4).

Within every stage of hindlimb development, treated embryos had more advanced eyelid development than untreated. This was true in all three experiments. For example, in CBA fetuses (Expt 3) with hindlimbs in the three-quarter-fused stage, the majority of untreated fetuses had small gaps between the developing eyelids, but the majority of treated fetuses had completely closed eyes (Table 4).

These results indicate that, although cortisone treatment accelerated both eyelid and hindlimb development, it had a greater effect on eyelids. This is expressed as a change in the relationship between stage of hindlimb fusion and eyelid closure in treated compared with control fetuses.

To find out whether cortisone stimulated differentiation alone or stimulated growth with differentiation the weights of the fixed fetuses from the various groups in Expt 3 were compared. Control and treated fetuses collected at 12.00 h on day 16 of gestation had mean weights of 0.381 g (s.e. = 0.010) and 0.369 g (s.e. = 0.004), respectively, and did not differ significantly ($F_{1.10} = 0.85; P > 0.25$). Similarly, no significant difference was found between treated (0.430 ± 0.016 g) and control (0.426 ± 0.008 g) fetuses collected at 18.00 h on day 16 ($F_{1.7} = 0.05; P > 0.75$). Growth within the treated and control groups collected at different times was detected. There were significant differences between the mean weights of the control fetuses collected at 12.00 and 18.00 h on gestational day 16 (0.381 and 0.426 g, respectively; $F_{1.9} = 9.60; P < 0.05$). Similarly, there were significant differences among the mean weights of treated fetuses collected at 8.00, 12.00 or 18.00 h on gestational day 16 (0.351, 0.369 and 0.430 g, respectively; $F_{2.12} = 8.37; P < 0.01$).
The data indicate that 30-100 mg kg⁻¹ of cortisone No fusion
fusion of the digits and earlier fusion of pinnae to the
earlier than the peak of the therapeutic response of
delay of eyelid development stages among fetuses in each stage of hindlimb fusion.

The usual peak of the critical period for cleft palate
induction is day 12 of gestation (Biddle, 1979), 2 days
earlier than the peak of the therapeutic response of
open-eyes (Harris, Juriloff & Biddle, 1984). The

Discussion

The data indicate that 30–100 mg kg⁻¹ of cortisone administered to the mother on day 14 of gestation in mice causes earlier closure of the eyelids, earlier fusion of the digits and earlier fusion of pinnae to the scalp. Based on the CBA strain study, where more treated fetuses have completed these steps (except hindlimb fusion) at 8.00 h on day 16 of gestation than untreated fetuses have at 18.00 h (Table 2), the advance is by at least 10 h. These conclusions are based on the relatively objective developmental endpoints, completed eyelid closure, completed digit fusion and completed pinna fusion.

The data also suggest that while all of the development-mental phenomena scored were speeded up, the advance in closure of the eyelids was greater than that of the hindlimbs, resulting in an alteration of the relationship between developmental events. For example, in untreated CBA fetuses at the stage of having their hindlimb digits half fused, the majority have their eyes in the medium gap or wide gap stages, whereas, in treated CBA fetuses in the same hindlimb stage, the majority have their eyes in the small gap to closed stages (Table 4).

The data support the hypothesis that the genetic defect in /gMI//gMI mutant mice causes a delay of eyelid closure beyond a critical time and that the cure of /gMI//gMI mutant open-eyes by cortisone is due to accelerated eyelid closure which moves the closure event back to the normal side of the threshold in time. The significant differences in weight between control fetuses collected 6 h apart and among treated fetuses collected 6–10 h apart indicates that weight differences between treated and control fetuses collected at the same time could have been detected if they were present. As discussed above, the development of morphological traits in treated fetuses was accelerated by at least 10 h. No evidence of weight differences between treated and control embryos collected at the same gestational age was found. It appears, then, that the effect of cortisone is to accelerate differentiation, not growth, in this system.

As cortisone treatment in this study accelerated several developmental events, but apparently did so unequally, it is not clear whether the therapeutic effect on eyelid closure in /gMI//gMI mutants would be due to accelerated eyelid closure relative to gestational age or to accelerated eyelid closure relative to other developmental events.

The response to cortisone is likely to be common in mice, as the two strains used, which respond similarly, are unrelated. CBA/J mice are known to complete their eye closure several hours later than SWV/Bc under the same conditions in our laboratory (unpublished data).

Cortisone is widely used in teratology studies in mice, particularly in the study of induced cleft palate. The usual peak of the critical period for cleft palate induction is day 12 of gestation (Biddle, 1979), 2 days earlier than the peak of the therapeutic response of open-eyes (Harris, Juriloff & Biddle, 1984). The

Table 4. Effects of cortisone acetate given on day 14 of gestation on development of day-16 fetuses. Distribution of eyelid development stages among fetuses in each stage of hindlimb fusion.
teratogenic dosages used are usually much higher than those in this study, with ED_{50}'s ranging from 115 to 687 mg kg^{-1}, although the ED_{50} for the most sensitive known strain, SW/Fr, is 50 mg kg^{-1} (Biddle, 1981). Teratogenic doses of cortisone appear to cause cleft palate by delaying palate closure (Vekemans & Fraser, 1979), in contrast to the acceleration in other developmental events after lower cortisone doses in the present study. The dose–response relationship of open-eyes to cortisone appears to be parabolic in shape and doses above 60 mg kg^{-1} appear to inhibit growth (Harris et al. 1984). This fact may explain the opposite effects of cortisone in our studies compared with studies of cleft palate. The palates were checked in the specimens of Expt 1. All were closed in the controls and in fetuses treated at 60 mg kg^{-1}. 4% were unclosed at 30 mg kg^{-1} and 8% at 100 mg kg^{-1}. Possibly cortisone delays palate closure even at low doses. This is an area we need to examine further.

It is notable that all of the developmental events scored in the present study (except palate closure) involve epidermis- and periderm-based temporary fusions. It may be that at this developmental age the accelerating effects of cortisone are specific to periderm or epidermis differentiation (Sugimoto, Kojima & Endo, 1976; Epstein & Munderloh, 1981; Fisher & Sawyer, 1986). The relatively greater effect on eyelid closure compared with hindlimb digit fusion may be a function of treatment time. At other times, other areas may be differentially responsive.

The data may present a new route to further understanding of the role of glucocorticoid hormone in regulating differentiation. Glucocorticoids are known to regulate activity at many gene loci and the regulation appears to be tissue-specific (Yamamoto, 1985; Ringold, 1985; Greengard, 1975). A human glucocorticoid receptor has been sequenced (Hollenberg et al. 1985) and the DNA regulatory sequence (enhancer) to which the receptor binds has been sequenced (Yamamoto, 1985). Most of the studies of glucocorticoid-induced differentiation have been biochemical, usually involving the appearance of enzymes. The present study has examined morphological developmental steps and the failure of one of these steps, at least (eyelid closure), leads to a birth defect, open-eyes. The regulation of genes is far less well understood for morphological development than for biochemical differentiation. The responses of the four morphological traits described in this paper seem to offer a route to some greater insight into the genetics of morphology.

I thank Dr Muriel Harris and Dr James R. Miller for helpful discussions and comments on the manuscript, and Valerie Magel, Jennifer Boyd, Richard Oliver, David Ciriani and Brenda Wishart for technical assistance. I wish to acknowledge the influence of some unpublished data, three-dimensional figures, which were shown to me by Dr F. C. Fraser in 1976. This work was supported by Medical Research Council of Canada grant MA-6766.

References


(Accepted 7 April 1987)