Development 113, 671–678 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Curvature of the caudal region is responsible for failure of neural tube closure in the curly tail (*ct*) mouse embryo

FRANCES A. BROOK[1,*], ALISA S. W. SHUM[1,*], HENNY W. M. VAN STRAATEN[2] and ANDREW J. COPP[1]

[1]*Imperial Cancer Research Fund, Developmental Biology Unit, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[2]*Department of Anatomy and Embryology, National University of Limburg, Maastricht, The Netherlands*

*F.A.B. and A.S.W S. contributed equally to this study

Summary

Delayed closure of the posterior neuropore (PNP) occurs to a variable extent in homozygous mutant curly tail (*ct*) mouse embryos, and results in the development of spinal neural tube defects (NTD) in 60 % of embryos. Previous studies have suggested that curvature of the body axis may delay neural tube closure in the cranial region of the mouse embryo. In order to investigate the relationship between curvature and delayed PNP closure, we measured the extent of ventral curvature of the neuropore region in *ct/ct* embryos with normal or delayed PNP closure. The results show significantly greater curvature in *ct/ct* embryos with delayed PNP closure *in vivo* than in their normal littermates. Re-opening of the posterior neuropore in non-mutant mouse embryos, to delay neuropore closure experimentally, did not increase ventral curvature, suggesting that increased curvature in *ct/ct* embryos is not likely to be a secondary effect of delayed PNP closure. Experimental prevention of ventral curvature in *ct/ct* embryos, brought about by implantation of an eyelash tip longitudinally into the hindgut lumen, ameliorated the delay in PNP closure. We propose, therefore, that increased ventral curvature of the neuropore region of *ct/ct* embryos imposes a mechanical stress, which opposes neurulation and thus delays closure of the PNP. Increased ventral curvature may arise as a result of a cell proliferation imbalance, which we demonstrated previously in affected *ct/ct* embryos.

Key words: neural tube, morphogenesis, mouse mutant, embryo culture, curvature, curly tail (*ct*).

Introduction

The process of neurulation, by which the brain and spinal cord develop, involves the formation, elevation and fusion of neural folds in the dorsal midline of the embryo (review: Schoenwolf and Smith, 1990). Although the events of neurulation have been well described, the underlying developmental mechanisms remain poorly understood. One approach to investigating the mechanisms of neurulation is to make use of the many mouse mutants (review: Copp *et al.* 1990) in which neurulation is disturbed, leading to neural tube defects (NTD). Curly tail (*ct*) is one such mutant; around 60% of homozygous embryos develop spinal NTD which include lumbosacral spina bifida and tail flexion defects. Malformations in *ct/ct* embryos have been shown to closely resemble NTD in humans (Grünberg, 1954; Embury *et al.* 1979). Previous studies have shown that spinal NTD in *ct/ct* embryos result from delayed closure of the neural folds at the posterior neuropore (PNP) (Copp, 1985). Here we propose a mechanism to account for the delay in neural tube closure.

The rate of cell proliferation of the notochord and gut endoderm is reduced in *ct/ct* embryos that show the most severely delayed PNP closure (Copp *et al.* 1988*a*). In the chick embryo, a reduction of cell proliferation rate in the notochord and ventral endoderm leads to development of the cranial flexure (Takamatsu and Fujita, 1987). By analogy, we would expect that abnormally slow proliferation of the ventromedial elements combined with normal elongation of the dorsal structures should produce ventral curvature in the caudal region of *ct/ct* embryos. Moreover, there is evidence that ventral curvature can influence the rate of neural tube closure: cranial flexure, which results in dorsal bulging of the neuroepithelium, is believed to delay closure of the neural tube during normal development of the mouse (Jacobson and Tam, 1982). Hence, we consider here the hypothesis that reduced cell proliferation in the notochord and gut endoderm may affect neurulation by generating ventral curvature

of the neuropore region, thereby placing a mechanical stress upon the neuroepithelium which would oppose normal closure.

In this paper, we present data that show that *ct/ct* embryos with delayed PNP closure do indeed exhibit increased ventral curvature of the caudal region. We then go on to examine the relationship between ventral curvature and delayed PNP closure. First, to test if delayed closure itself increases ventral curvature, we experimentally induced delayed closure of the PNP in non-mutant embryos and measured the resulting ventral curvature. Second, to test directly the hypothesis that an increase in ventral curvature is a cause of the delayed PNP closure in *ct/ct* embryos, we experimentally reduced curvature of the neuropore region by inserting the tip of a human eyelash into the hindgut lumen to act as a brace. The results of these experiments support the idea that ventral curvature is the cause of delayed PNP closure in *ct/ct* embryos.

Materials and methods

Mouse strains

Curly tail arose in the early 1950s as a spontaneous mutation in the GFF inbred strain. Grünberg (1954) crossed the original mutant mice to the CBA/Gr inbred strain for three generations. Since then, the curly tail strain has been maintained as a random-bred, closed colony which has been re-derived from a small number of individuals on several occasions. There is evidence that the mice, although not inbred, are homogeneous for genes affecting NTD. First, the incidence of fetal NTD is not influenced by the phenotype of the parents; unaffected *ct/ct* mice produce a similar number of offspring with NTD as mice that are themselves affected (Copp *et al.* 1982). Moreover, selective breeding for either the unaffected or severely affected phenotype does not alter the incidence of offspring with NTD (Embury *et al.* 1979; F.A.B. and A.J.C., unpublished). This suggests that curly tail mice all have the same genetic predisposition to NTD and that non-genetic factors determine whether this predisposition manifests itself as developmental abnormalities. Thus, the comparisons in the present study between affected and unaffected *ct/ct* embryos can be expected to shed light on the developmental effects of the *ct* mutation itself.

Curly tail mice were maintained on a light/dark cycle with the dark period from 1.00 am to 9.00 am. Animals were paired for mating between 8.00 and 9.00 am and females were checked for copulation plugs 4–8 h later.The day of finding a plug was designated day 1 of gestation. For the measurement of ventral curvature *in vivo*, embryos were harvested between 11.00 am and 2.00 pm on day 11 of gestation. For measurement following culture, and in the experiment to prevent curvature, conceptuses were explanted on the afternoon of day 10 of gestation and cultured for 18 to 25 h (method of New *et al.* 1973; Cockroft, 1990).

Mice of the non-mutant, random-bred, albino strain PO (Pathology, Oxford) with no predisposition for neural tube defects, were used in the experiment to delay neural tube closure. Animals were maintained on a light/dark cycle with the dark period from 7.00 pm to 7.00 am. They were paired for mating overnight and females were checked for copulation plugs the following morning. Conceptuses were explanted in the afternoon of day 10 of gestation, and cultured for 12 h before experimental manipulation.

Measurement of curvature

Embryos were dissected from their extraembryonic membranes in PB1 medium (Cockroft, 1990) containing 10 % fetal calf serum, and the number of somites was counted (Copp *et al.* 1982). Only embryos with 27 to 29 somites were further classified according to their degree of delayed PNP closure as indicated by size of the PNP. The following neuropore categories have been defined for *ct/ct* embryos with 27 to 29 somites (Copp, 1985). Category 1/2 embryos (small neuropores) develop normally in about 60 % of cases, the remainder developing tail flexion defects. Category 3 embryos (moderately enlarged neuropores) develop normally in 30 % of cases with the remainder showing tail flexion defects and a few developing open lumbosacral spina bifida. Less than 10 % of category 4/5 embryos (severely enlarged neuropores) develop normally. The rest develop spinal NTD comprising tail flexion defects with open spina bifida in 15–30 % of cases and tail flexion defects alone in the remainder. Only embryos in categories 1/2 ('unaffected embryos') and 4/5 ('affected embryos') were taken for measurement of curvature *in vivo* and after culture.

The caudal region of each (unfixed) embryo was severed midway between the forelimb and hindlimb bud. Camera-lucida drawings were made at a fixed magnification with the caudal regions in lateral profile. The positions of the hindlimb bud and the most caudal 3 or 4 somites were recorded on each drawing, and the angle of curvature was measured as follows. A line was drawn along the midline of the tail bud, parallel to and equidistant from the ventral and dorsal surfaces. A second line was drawn tangential to the ventral edge of the penultimate somite, to bisect the first line. The angle between the two lines was measured to give the ventral curvature of the caudal region (Fig. 1). All measurements were carried out without knowledge of the PNP category, on drawings that had been coded. A minimum of 5 embryos were measured for each somite/PNP grouping.

Experimental delay of PNP closure

Embryos of PO strain mice were allowed to develop in culture until they had approximately 25 to 26 somites. At this stage, a

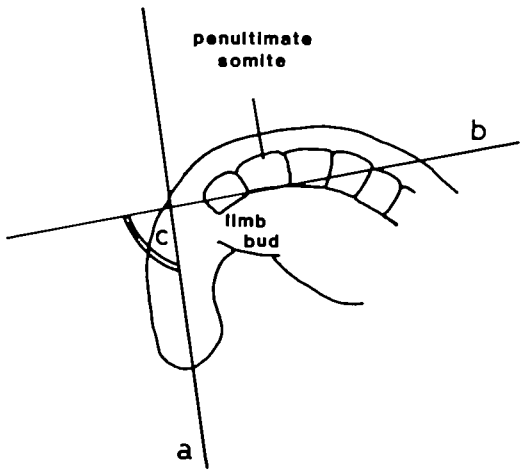

Fig. 1. Measurement of ventral curvature of the neuropore region from camera lucida drawings. Line 'a' is drawn along the midline of the tail bud, parallel to and equidistant from the ventral and dorsal surfaces. Line 'b' is drawn tangential to the ventral edge of the penultimate somite. Angle 'c' is measured to give the ventral curvature.

small hole was made in the yolk sac and amnion over the region of the tail bud. The PNP was enlarged mechanically using a sharp glass needle. The needle was held perpendicularly to the long axis of the tail bud, inserted into the neuropore and moved in a cranial direction, incising the roofplate and surface ectoderm of the most recently formed portion of the neural tube (see Fig. 1 in Copp, 1985). In control embryos, the yolk sac and amnion were opened but the neuropore was not enlarged.

The embryos were returned to culture and allowed to develop for a further 4 h. Embryos were then dissected from their extraembryonic membranes and any that fell outside the 27–29 somite range were discarded. Curvature of the tail bud region was measured as described above. A total of 18 experimental and 18 control embryos were measured.

Experimental prevention of curvature

Embryos were explanted from *ct/ct* mice at the 19–20 somite stage and a small hole was made in the yolk sac and amnion over the caudal region. The embryos were divided into three groups, one experimental and two controls. In the experimental group, the tip of a human eyelash, approximately 0.5 mm in length, was inserted into the hindgut lumen *via* the caudal end of the embryo. (Human eyelashes were first thoroughly washed with mild detergent, sterilized by soaking in methanol for 1 h, dried on filter paper and stored at 4 °C; a modification of the method of Eto *et al.* 1981). In the mock-operated control group, an eyelash was inserted but then immediately withdrawn. No eyelash was inserted in the third group, the untreated controls, although a hole was made in the membranes as before.

Embryos were cultured as described above until they were expected to have 27 to 29 somites. They were then dissected from their extraembryonic membranes, and any which fell outside the 27–29 somite range were discarded. Category and length of the posterior neuropore, crown–rump length and head length were measured using an eyepiece graticule. The caudal region of each embryo was severed midway between the forelimb and hindlimb bud at the level of somite 13, ventral curvature was measured as described above and the protein content of the majority of caudal regions was determined by the method of Lowry *et al.* (1951). In preliminary experiments to locate the position of the eyelash, a few caudal regions of experimental embryos were fixed in Bouin's solution, dehydrated and embedded in wax. Sections were cut at 6 μm and stained with haematoxylin and eosin.

Statistical analysis

The data were analysed by 2-way analysis of variance with replication, corrected for non-proportionality (Armitage and Berry, 1987), or by chi-squared test.

Results

Measurement of curvature

(a) *Embryos* in vivo

Ventral curvature of the caudal region varied significantly with neuropore size, such that the angle of curvature was greatest in embryos with large neuropores (category 4/5) and was 12–21° less in embryos that had small neuropores (category 1/2), irrespective of somite number (Figs 2 and 3; $P<0.005$).

The angle of curvature also decreased progressively as the number of somites increased from 27 to 29, an effect that was significant ($P<0.005$). This occurred regardless of the size of the PNP; in embryos with large or small neuropores, the caudal region straightened by about 30° between the stages of 27 and 29 somites.

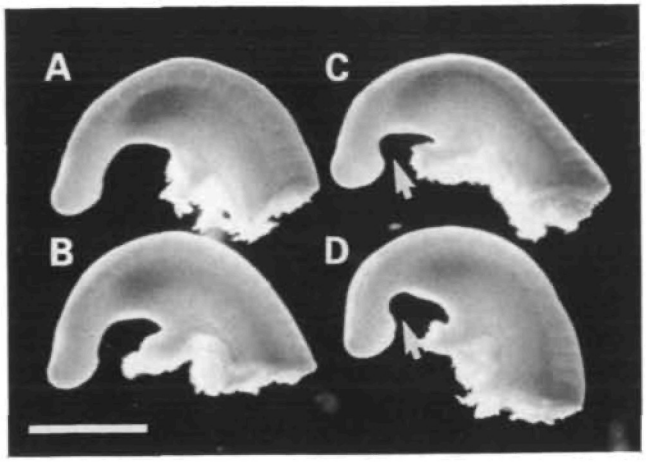

Fig. 2. Caudal regions of *ct/ct* embryos that developed *in vivo* until the 27–29 somite stage. Embryos A and B have a small neuropore (category 1/2), whereas embryos C and D have a large neuropore (category 4/5). Ventral curvature is more pronounced in the embryos with a large neuropore (arrowed). Scale bar represents 250 μm.

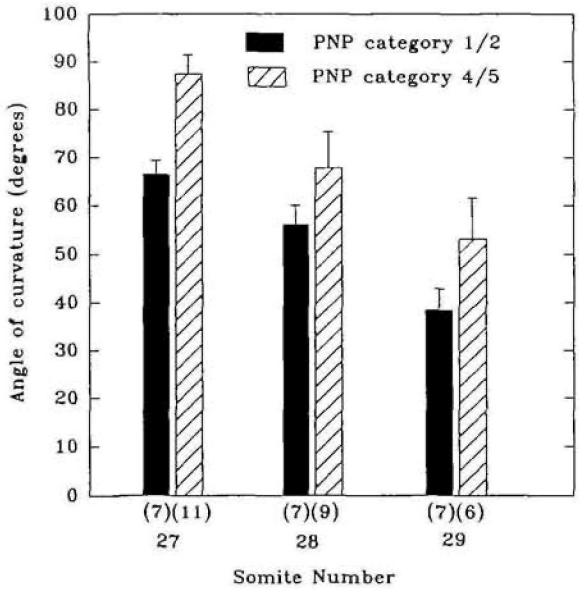

Fig. 3. Ventral curvature of the neuropore region of *ct/ct* embryos with small (category 1/2) or large (category 4/5) posterior neuropores. Embryos were allowed to develop *in vivo* to the 27–29 somite stage. Mean±S.E. Sample size in parentheses. The angle of curvature was significantly smaller in embryos with a small PNP than in those with a large neuropore ($P<0.005$). The angle of curvature also decreased significantly as somite number increased ($P<0.005$).

(b) *Embryos* in vitro

The angle of ventral curvature of the neuropore region

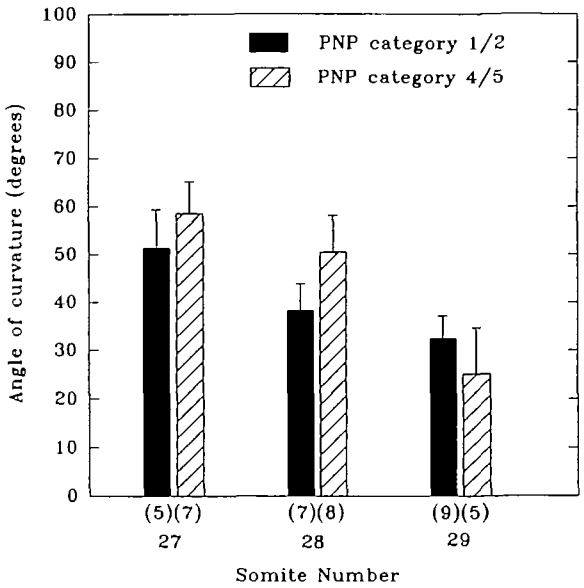

Fig. 4. Ventral curvature of the neuropore region of *ct/ct* embryos with small (category 1/2) or large (category 4/5) posterior neuropores. Embryos were explanted on day 10 of gestation and cultured until they had 27 to 29 somites. Mean±S.E. Sample size in parentheses. The variation in angle of curvature with PNP category was not significant, although ventral curvature was greater in embryos with large neuropores than in those with small neuropores at 27 and 28 somites. There was a significant decrease in the angle of curvature as somite number increased ($P<0.01$).

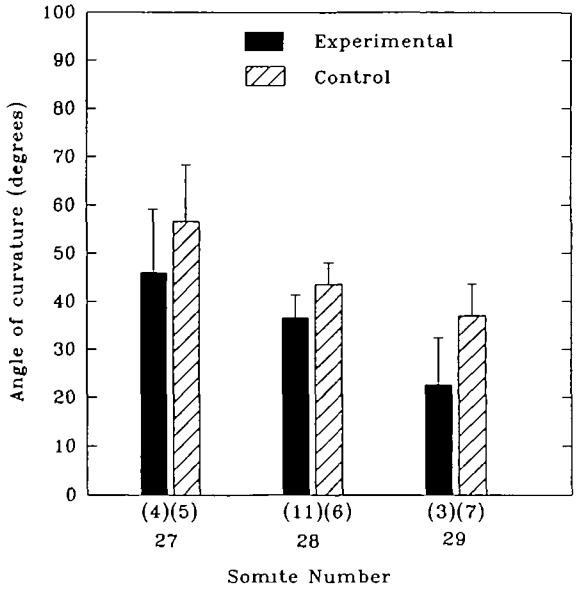

Fig. 5. Ventral curvature of the neuropore region of PO embryos in which closure of the PNP was delayed experimentally. Mean±S.E. Sample size in parentheses. There was no significant effect of delayed PNP closure upon the angle of curvature. The decrease in curvature with somite number was also non-significant.

in cultured embryos was reduced by 15–30° in most somite/PNP groupings, when compared with embryos *in vivo* (Fig. 4). The variation in angle of curvature with PNP size was not significant overall, although ventral curvature was greater in embryos with large neuropores than in those with small neuropores at 27 and 28 somites. As with embryos *in vivo*, ventral curvature decreased as the number of somites increased ($P<0.01$).

Despite the differences in degree of ventral curvature between embryos *in vivo* and *in vitro*, the pattern of changes in curvature with PNP categories and with somites were considered sufficiently similar to justify the use of cultured embryos in subsequent experiments.

Experimental delay of PNP closure

The increased curvature observed in *ct/ct* embryos with large PNPs could arise as a result of delayed PNP closure. To test this possibility, delayed closure was produced experimentally in non-mutant mouse embryos by mechanical enlargement of the PNP. All such manipulated embryos had a large, open PNP after culture, comparable in size to category 4/5 PNPs of *ct/ct* embryos, whereas the PNP of the controls was very small or closed altogether. Experimentally induced delay in closure of the PNP in PO mice had no significant effect upon ventral curvature of the caudal region; the trend, in fact, was towards a decrease in curvature in embryos with delayed PNP closure (Fig. 5). There was no significant effect of somite number upon curvature, although the trend showed a decrease in curvature from 27 to 29 somites.

Experimental prevention of curvature

(a) *Effect of eyelash implantation upon curvature and PNP closure*

To test directly the effect of curvature upon closure of the PNP, ventral curvature of the caudal region was reduced experimentally in *ct/ct* embryos. This was achieved by insertion of an eyelash tip to act as a brace, thereby mechanically preventing the caudal region from bending during the subsequent culture period (Fig. 6 A,B).

After approximately 20 h in culture, the eyelash was clearly visible completely enclosed within the caudal region (Fig. 6 C,D). Transverse sections of this region showed the eyelash tip located within the hindgut lumen (Fig. 6 E). There was no evidence of tissue disruption or of increased cell death resulting from the presence of the eyelash. Ventral curvature of the neuropore region was significantly reduced in the experimental group compared to the control groups (Fig. 6 D,F; Fig. 7; $P<0.05$). The magnitude of the reduction fell as somite number increased, from an average difference between experimental and pooled control embryos of 26° at 27 somites to an average difference of 11° at 29 somites. There was no significant difference in curvature between the mock-operated and untreated control groups, indicating that the trauma of inserting the eyelash tip did not itself cause a reduction in curvature. There was no significant effect of somite number upon curvature in control embryos although, as

before, the trend was towards a reduction in curvature as number of somites increased.

Corresponding to the observed reduction in ventral curvature, the length of the PNP was significantly decreased in the experimental embryos compared with the controls (Fig. 6 D,F); in most cases, the mean lengths in experimental and control groups differed by at least 300 μm (Fig. 8; $P<0.001$). There was no

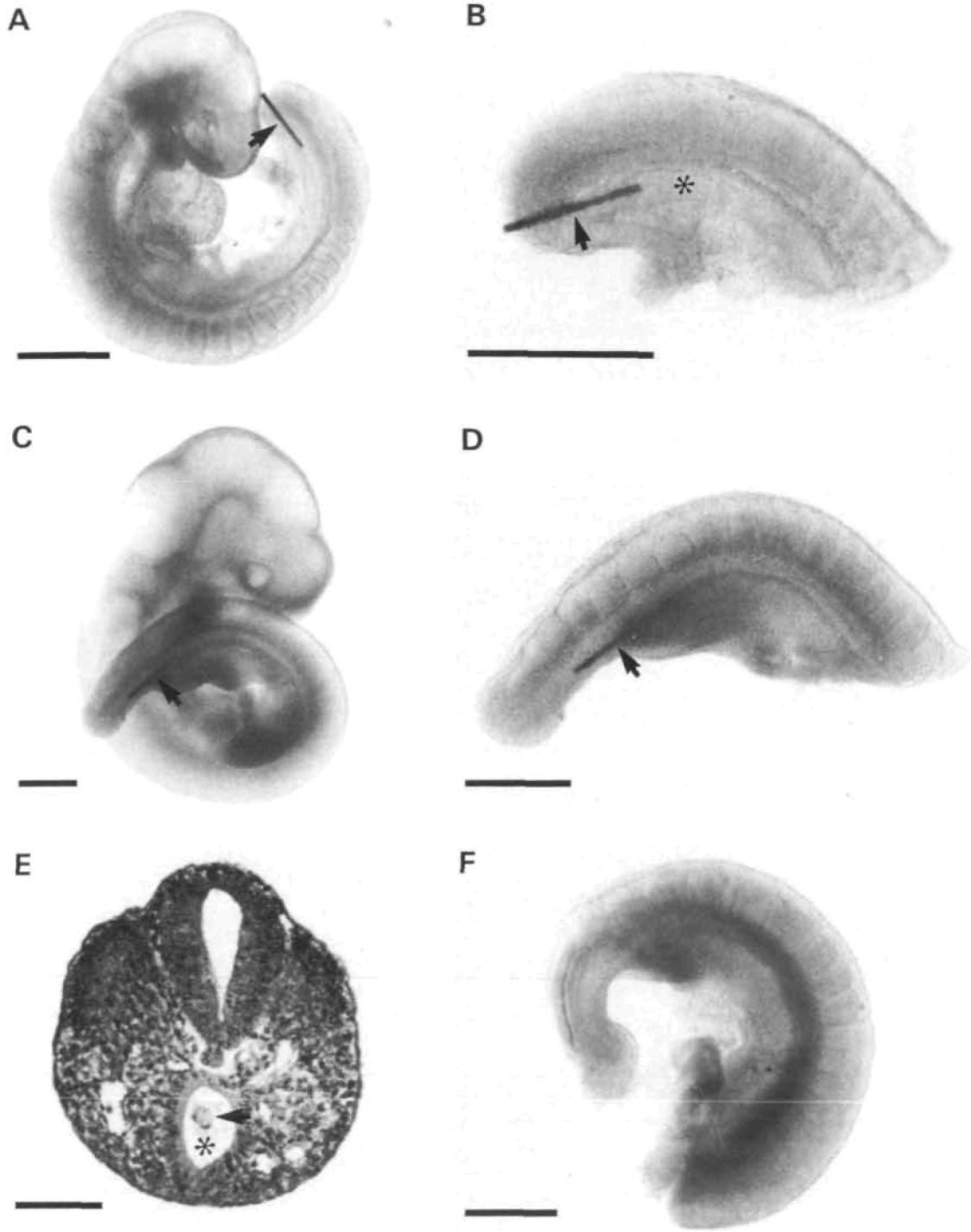

Fig. 6. Effect of eyelash implantation upon ventral curvature and neurulation in *ct/ct* embryos. (A and B) An eyelash tip (arrowed) was inserted longitudinally into the lumen of the hindgut (*) *via* the caudal end of a 19 somite embryo. Scale bar represents 500 μm. (C and D) After 24 h of culture, the caudal region had grown beyond the eyelash tip (arrowed). Ventral curvature was reduced and the PNP was closed. Scale bar represents 500 μm. (E) Transverse section through the caudal region showing the eyelash (arrowed) located within the hindgut lumen (*). Scale bar represents 100 μm. (F) Mock-operated control embryo after 24 h of culture. Ventral curvature was much greater than in D and there was a large PNP. Scale bar represents 500 μm.

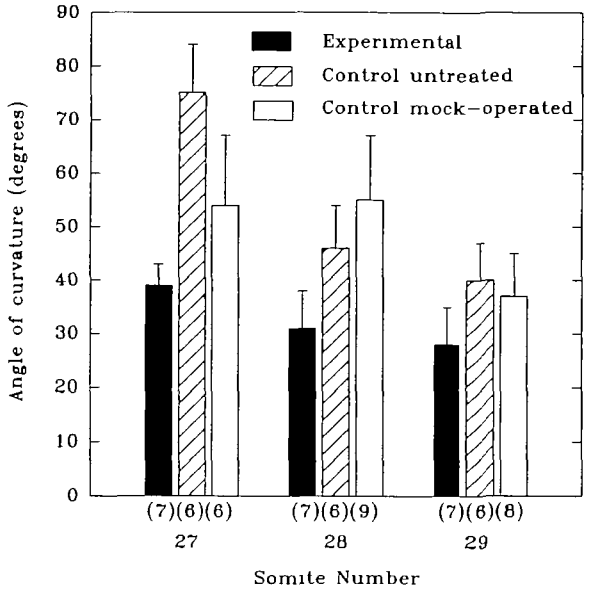

Fig. 7. Ventral curvature of the neuropore region of *ct/ct* embryos with 27 to 29 somites after insertion of an eyelash into the hindgut lumen or after control manipulations. Mean±S.E. Sample size in parentheses. The angle of curvature was significantly reduced in the experimental group ($P<0.05$). There was no significant difference between the two control groups. There was no significant effect of somite number upon curvature, even when the control groups were considered alone, although the trend was towards a reduction in curvature as number of somites increased.

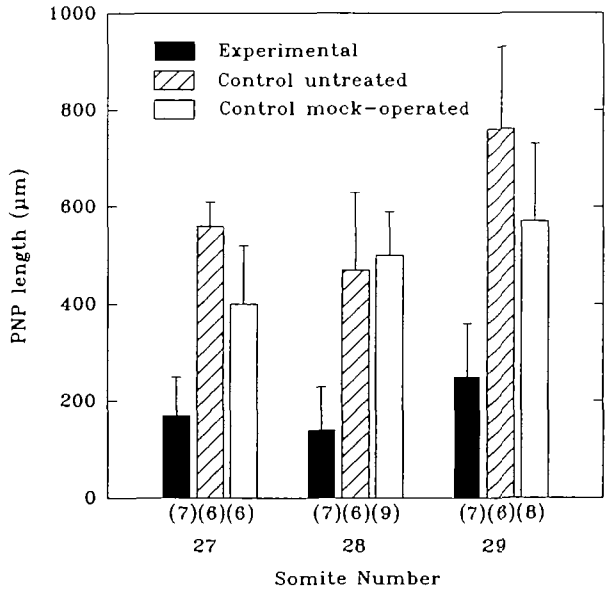

Fig. 8. Length of the PNP in *ct/ct* embryos with 27 to 29 somites after insertion of an eyelash into the hindgut lumen or after control manipulations. Mean±S.E. Sample size in parentheses. Length was significantly reduced in experimental embryos compared to the control groups ($P<0.001$). There was no significant difference in length between the control groups, nor was there a significant effect of somite number upon PNP length.

Table 1. *Length of the PNP and frequency distribution of* ct/ct *embryos between the PNP categories after insertion of an eyelash into the hindgut lumen or after control manipulations*

PNP category	PNP length (μm)	Number of embryos* (%) Experimental	Control (untreated)	Control (mock-operated)
0	0	10 (48)	2 (11)	4 (17)
1	1–100	3 (14)	0 –	0 –
2	101–300	2 (10)	1 (6)	2 (9)
3	301–550	4 (19)	5 (28)	9 (39)
4	551–750	2 (10)	6 (33)	2 (9)
5	>750	0 –	4 (22)	6 (26)

* All embryos had 27 to 29 somites. There was a significant difference in the frequency distribution of PNP categories between experimental and control embryos, such that there was a higher incidence of small or closed neuropores in the experimental group and a greater incidence of embryos with large neuropores in the control groups ($P<0.01$).

significant difference in length of PNP between the two control groups. There was no significant effect of somite number upon PNP length, although the trend was towards an increase in length with increasing number of somites.

Table 1 relates PNP length measurements to the categories defined in Materials and methods. There is a significant difference in the frequency distribution of PNP categories between experimental and control embryos ($P<0.01$). 71% (15/21) of experimental embryos had PNPs that were small (category 1/2) or closed altogether (category 0), whereas this distribution was reversed in the control groups with 83% (15/18) and 74% (17/23) respectively of untreated and mock-operated control embryos having moderately or severely enlarged neuropores (categories 3 and 4/5).

(b) *Effect of eyelash implantation upon growth*

The results show that the delay in PNP closure observed in the control *ct/ct* embryos was ameliorated in the experimental group, in which ventral curvature was reduced. It is possible that implantation of the eyelash effected a general reduction in growth of the caudal region and that growth retardation is the mechanism by which delayed neuropore closure was corrected (c.f. Copp *et al.* 1988*b*). To test this possibility, the relative growth of experimental and control embryos was assessed by comparing protein contents of the caudal region, and by crown–rump and head length measurements. There was no significant difference between experimental and control embryos in protein content (Fig. 9), nor in crown–rump or head length measurements (data not shown). Implantation of an eyelash, therefore, does not retard growth of the embryo

Discussion

In the present study, we have demonstrated an increase

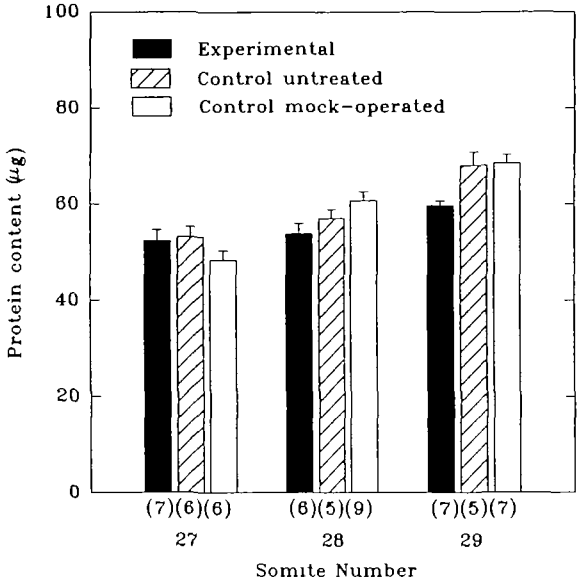

Fig. 9. Protein content of the caudal region of *ct/ct* embryos with 27 to 29 somites after insertion of an eyelash into the hindgut lumen or after control manipulations. Mean±S.E. Sample size in parentheses. There was no significant difference between experimental and control groups.

in ventral curvature in the neuropore region of *ct/ct* embryos in which closure of the PNP is delayed. There are two ways in which curvature and neuropore size could be causally related. First, increased curvature might be a consequence of delayed PNP closure, the enlarged neuropore perhaps allowing the release of tensions generated by the elongating tail bud and permitting the neuropore region to bend. However, our results do not support this interpretation. Mechanical enlargement of the PNP of non-mutant mouse embryos has been shown to delay subsequent closure of the neuropore (Copp, 1985), but yet it does not produce increased ventral curvature.

An alternative hypothesis is that increased curvature of the neuropore region brings about delayed closure of the PNP. This could occur simply as a result of increased mechanical stress placed upon the neuroepithelium by ventral bending, which would tend to oppose the elevation and fusion of the neural folds in a manner analogous to that suggested to occur in mouse cephalic neurulation (Jacobson and Tam, 1982). The results from the eyelash experiment are consistent with this hypothesis. An eyelash has a sharp tip, small diameter and slender shape which make it very suitable for insertion into the hindgut lumen *via* the caudal end of the embryo. Eyelash tips were originally used to deliver small quantities of drugs to a specific location (Eto *et al.* 1981). In the present study, however, the eyelash was acting solely as a mechanical support to prevent the caudal region of the embryo from bending; there was no effect upon growth of the caudal region. Eyelash implantation reduced ventral curvature and, concomitantly, the delay in closure of the PNP was reduced. We conclude that decreasing the ventral curvature of the caudal region leads to normalisation of PNP closure in *ct/ct* embryos.

Closure of the PNP in *ct/ct* embryos begins after the 30 somite stage (Copp *et al.* 1982). The degree of ventral curvature in *ct/ct* embryos declines sharply between the 27 and 29 somite stages (Fig. 3). Therefore, it would appear necessary for the caudal region of *ct/ct* embryos to 'unbend' before final closure of the neuropore can commence. Experimental prevention of curvature, by implantation of an eyelash, resulted in a high proportion of *ct/ct* embryos with premature closure of the PNP, suggesting that by enhancing the normal 'unbending' of the caudal region, closure of the PNP may be brought about at an earlier stage of development.

What factors might be responsible for curvature of the caudal region? Previous studies have revealed a reduced rate of proliferation of gut endoderm and notochord cells in the neuropore region of *ct/ct* embryos developing spinal NTD, compared with normally developing embryos (Copp *et al.* 1988*a*). The cells of the neuroepithelium showed no reduction in proliferation rate. Hence if the embryo is regarded as a flexible structure, a reduced rate of axial elongation of the ventromedial elements combined with normal elongation of the dorsal structures would tend to cause the neuropore region of the embryo to bend ventrally. If the rate of cell proliferation in the gut and notochord progressively increased to 'catch up' with the rate of elongation of the neuroepithelium, one would then expect to see a gradual 'unbending' of the caudal region. There is some evidence that this is so: the mitotic index of the gut endoderm increases progressively between 27 and 29 somites in *ct/ct* embryos, a trend that is more pronounced in category 4/5 than in category 1/2 embryos. However, even at 29 somites, the mitotic index of the gut of category 4/5 embryos lags behind that of the category 1/2 embryos (A.J.C. and F.A.B., unpublished data).

The results from this and previous studies lead us to propose the following mechanism for the development of spinal NTD in *ct/ct* mouse embryos. Failure of normal cell proliferation in the gut endoderm and notochord of affected embryos leads to an imbalance of cell proliferation rates and results in ventral curvature of the neuropore region. Such flexion imposes a mechanical stress that opposes and thus delays normal neural tube closure during the time that the embryo is developing from the 27 to the 29 somite stage. Proliferation of the gut endoderm/notochord gradually increases during the latter part of this period, ameliorating the imbalance in growth rates and relaxing caudal curvature. This is sufficient to allow closure of the PNP after the 30 somite stage in the least affected embryos. However, in the most severely affected embryos, increased proliferation of the gut endoderm/notochord still leaves the growth rate below normal levels and curvature remains too severe to permit neuropore closure. Such embryos develop spina bifida. The relationship between the cell proliferation defect and

ventral curvature of the caudal region is now under investigation.

What implications have our results for the process of normal neurulation? Primary neurulation of the spinal cord involves elevation of the neural plate, apposition of the neural folds and their subsequent fusion. The mechanisms responsible for neural tube closure are still subject to controversy (Karfunkel, 1974; Copp, 1983; Gordon, 1985; Martins-Green, 1988), but neural fold elevation has been linked to cell shape changes (Burnside, 1971; Baker and Schroeder, 1967; Karfunkel, 1971; Smith and Schoenwolf, 1989), cell rearrangements (Jacobson *et al.* 1986) and cell division within the neuroepithelium (Jelinek and Friebova, 1966). Current opinion holds that neurulation involves the interaction of forces both intrinsic and extrinsic to the neuroepithelium (Schoenwolf and Smith, 1990). Our results demonstrate that, for neural tube closure to proceed normally, it is essential that the functioning of the extrinsic, supportive structures is unimpaired; failure of these may lead to NTD as surely as failure of mechanisms intrinsic to the neuroepithelium.

References

Armitage, P. and Berry, G. (1987). *Statistical Methods In Medical Research*, 2nd edition. Oxford: Blackwell Scientific Publications.

Baker, P. C. and Schroeder, T. E. (1967). Cytoplasmic filaments and morphogenetic movement in the amphibian neural tube. *Devl Biol.* **15**, 432–450.

Burnside, B. (1971). Microtubules and microfilaments in newt neurulation. *Devl Biol.* **26**, 416–441.

Cockroft, D. L. (1990). Dissection and culture of postimplantation embryos. In *Postimplantation Mammalian Embryos, A Practical Approach.* (ed. A. J. Copp and D. L. Cockroft), pp. 15–40. Oxford: I.R.L. Press.

Copp, A. J. (1983). Teratology and experimental embryology: the pathogenesis of neural tube defects. In *The Biological Basis of Reproductive and Developmental Medicine* (ed. J. B. Warshaw), pp. 155–178. New York: Elsevier Biomedical.

Copp, A. J. (1985). Relationship between timing of posterior neuropore closure and development of spinal neural tube defects in mutant (curly tail) and normal mouse embryos in culture. *J. Embryol. exp. Morph.* **88**, 39–54.

Copp, A. J., Brook, F. A., Estibeiro, J. P., Shum, A. S. W. and Cockroft, D. L. (1990). The embryonic development of mammalian neural tube defects. *Progress in Neurobiology* **35**, 363–403.

Copp, A. J., Brook, F. A. and Roberts, H. J. (1988*a*). A cell-type-specific abnormality of cell proliferation in mutant (curly tail) mouse embryos developing spinal neural tube defects. *Development* **104**, 285–295.

Copp, A. J., Crolla, J. A. and Brook, F. A. (1988*b*). Prevention of neural tube defects in the mouse embryo by growth retardation during neurulation. *Development* **104**, 297–303.

Copp, A. J., Seller, M. J. and Polani, P. E. (1982). Neural tube development in mutant (curly tail) and normal mouse embryos: the timing of posterior neuropore closure *in vivo* and *in vitro*. *J. Embryol. exp. Morph.* **69**, 151–167.

Embury, S., Seller, M. J., Adinolfi, M. and Polani, P. E. (1979). Neural tube defects in curly-tail mice. I. Incidence and expression. *Proc. R. Soc. Lond.* B **206**, 85–94.

Eto, K., Figueroa, A., Tamura, G. and Pratt, R. M. (1981). Introduction of cleft lip in cultured rat embryos by localized administration of tunicamycin. *J. Embryol. exp. Morph* **64**, 1–9.

Gordon, R. (1985). A review of the theories of vertebrate neurulation and their relationship to the mechanics of neural tube birth defects. *J. Embryol. exp. Morph. 89*, **Supplement**, 229–255.

Grüneberg, H. (1954). Genetical studies on the skeleton of the mouse. *VIII. Curly tail. J. Genet.* **52**, 52–67.

Jacobson, A. G., Oster, G. F., Odell, G. M. and Cheng, L. Y. (1986). Neurulation and the cortical tractor model for epithelial folding. *J. Embryol. exp. Morph.* **96**, 19–49.

Jacobson, A. G. and Tam, P. P. L. (1982). Cephalic neurulation in the mouse embryo analyzed by SEM and morphometry. *Anat. Rec.* **203**, 375–396.

Jelinek, R. and Friebova, Z. (1966). Influence of mitotic activity on neurulation movements. *Nature* **209**, 822–823.

Karfunkel, P. (1971). The role of microtubules and microfilaments in neurulation in *Xenopus*. *Devl Biol.* **25**, 30–56.

Karfunkel, P. (1974). The mechanisms of neural tube formation. *Int. Rev. Cytol.* **38**, 245–271.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

Martins-Green, M. (1988). Origin of the dorsal surface of the neural tube by progressive delamination of epidermal ectoderm and neuroepithelium: implications for neurulation and neural tube defects. *Development* **103**, 687–706.

New, D. A. T., Coppola, P. T. and Terry, S. (1973). Culture of explanted rat embryos in rotating tubes. *J. Reprod. Fert.* **35**, 135–138.

Schoenwolf, G. C. and Smith, J. L. (1990). Mechanisms of neurulation: traditional viewpoint and recent advances. *Development* **109**, 243–270.

Smith, J. L. and Schoenwolf, G. C. (1989). Notochordal induction of cell wedging in the chick neural plate and it's role in neural tube formation. *J. exp. Zool.* **250**, 49–62.

Takamatsu, T. and Fujita, S. (1987). Growth of notochord and formation of cranial and mesencephalic flexures in chicken embryo. *Develop. Growth and Differ.* **29**, 497–502.

(Accepted 26 June 1991)