J. Embryol. exp. Morph. 92, 269–285 (1986)
Printed in Great Britain © The Company of Biologists Limited 1986

A study on the pattern of prospective somites in the presomitic mesoderm of mouse embryos

P. P. L. TAM

Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong

SUMMARY

Consistently six to seven somites were formed in explants of presomitic mesoderm of 8·5-day to 11·5-day mouse embryos and this number correlated well with the number of somitomeres previously identified in the same tissue by stereo SEM (Tam, Meier & Jacobson, 1982). During this period of development, the size of the presomitic mesoderm varied up to two-fold but the number of prospective somites remained unchanged. This pattern in the presomitic mesoderm was stable with respect to the number and the position of somites that were formed, the craniocaudal sequence and the rate of segmentation. The prospective somite also went through an orderly sequence of expansion and cellular rearrangement prior to segmentation. During the differentiation of the presomitic mesoderm, somites were formed at the expense of somitomeres, suggesting that the somitomere was a morphological manifestation of the prospective somite.

INTRODUCTION

The earliest expression of a metameric pattern in the embryonic axis is seen in the formation of neuromeres in the neural tube (Neal, 1918; Adelman, 1925) and the appearance of somites in the paraxial mesoderm (Meier, 1984). Somites are formed in a craniocaudal sequence and the last somite in the paraxial mesoderm is contiguous with a longitudinal strip of unsegmented tissue called segmental plate or presomitic mesoderm. Somites are continuously added to the body axis by segmentation of the cranial end of this tissue (Bellairs, 1979). The presomitic mesoderm has been regarded as a site of progressive specification of the somitic pattern and one in which a certain degree of regulation of somite size and number is occurring (Flint, Ede, Wilby & Proctor, 1978). However, experimental studies in amphibian embryos and chick embryos show that the presomitic mesoderm is distinctively patterned in terms of the number of somites that can be formed and the craniocaudal sequence of segmentation (Deuchar & Burgess, 1967; Cooke, 1977; Menkes & Sandor, 1977; Stern & Bellairs, 1984*a*). This pattern of somitogenic capacity is unperturbed by the removal of epithelial and axial structures that are normally related to the presomitic mesoderm (Packard & Jacobson, 1976, 1979; Menkes & Sandor, 1977; Stern & Bellairs, 1984*a*). Although the segmental plate of avian and snapping turtle embryos varies from two- to three-fold in size

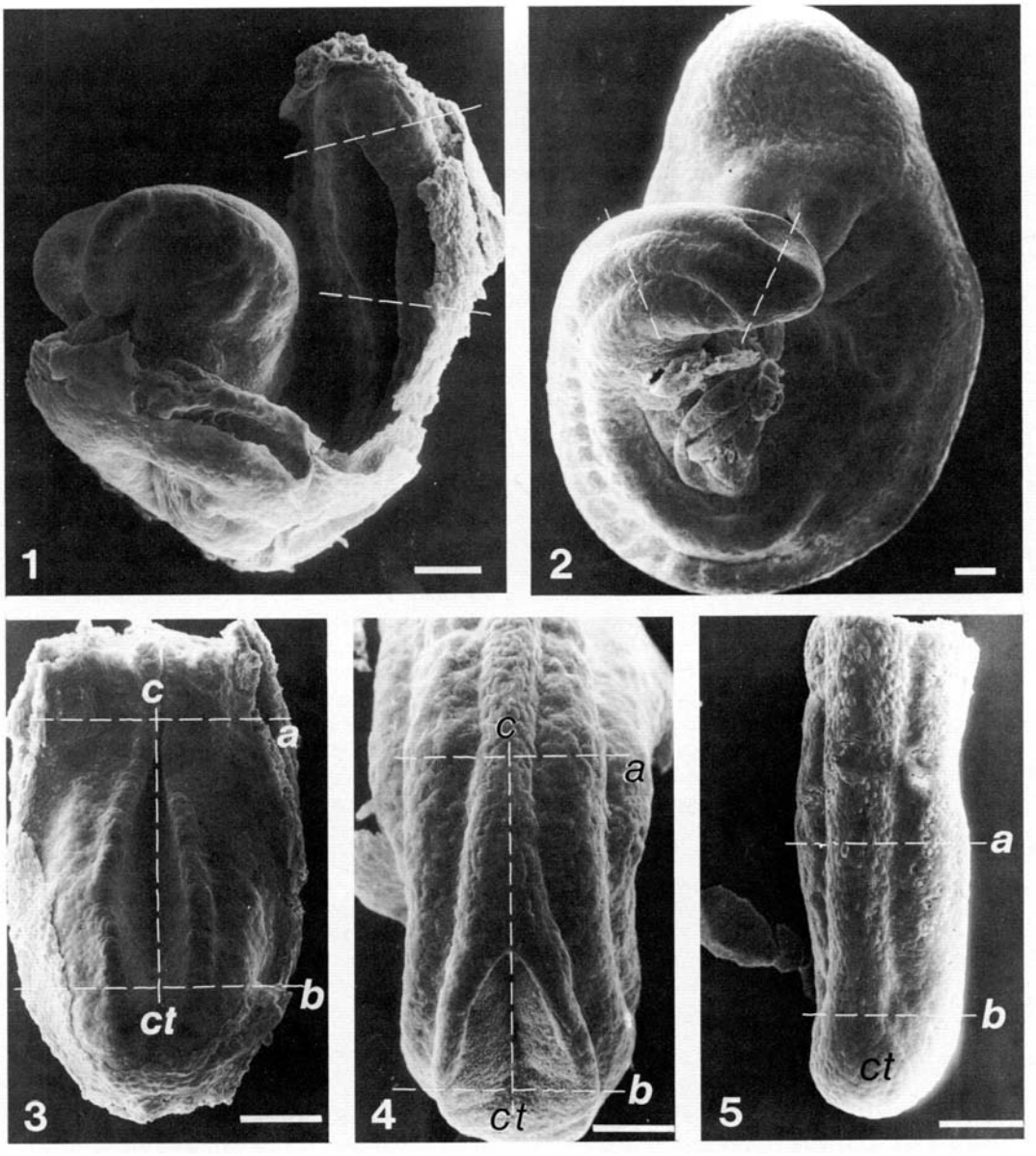
Key words: mouse embryo, presomitic mesoderm, somitomere, somite, metameric pattern.

during development, explants of this tissue always form a relatively constant number of somites (Packard, 1978, 1980*a*,*b*). A pattern of prospective somites is postulated to be present in the segmental plate of snapping turtle (Packard, 1980*a*), chick (Packard, 1978) and quail (Packard, 1980*b*). Morphological studies using scanning electron microscopy and stereoimaging have recently revealed the presence of a recognizable metameric pattern of cellular arrangement in the segmental plate of the chick (Meier, 1979), quail (Packard & Meier, 1983) and snapping turtle (Packard & Meier, 1984) and in the presomitic mesoderm of the mouse (Tam *et al.* 1982). The pattern presents itself as a tandem series of somitomeres which could be identified by the arrangement of mesenchymal cells in circular swirls about a centre and the boundaries between somitomeres are marked by changes in cellular alignment. In the snapping turtle and avian embryos, there is a good correlation between the number of somitomeres and the number of prospective somites contained in the segmental plate (Packard & Meier, 1983, 1984). The presomitic mesoderm is therefore fully organized into a metameric pattern consisting of somitomeres which are undergoing further morphogenesis to become somites. Although the presence of somitomeres in the presomitic mesoderm has been documented in the mouse (Tam *et al.* 1982), the existence of prospective somites as suggested by Tam (1981) has not been demonstrated. The present study was carried out to investigate the *in vitro* differentiation of the presomitic mesoderm and to elucidate the developmental relationship between somitomeres and somites in the mouse embryo.

MATERIALS AND METHODS

Mouse embryos at 8·5 to 11·5 days *p.c.* were obtained from ICR strain of mice. Pregnant mice were killed by cervical dislocation and the conceptuses were removed from the uterus to PB1 medium. The embryos were dissected out from the decidua and the Reichert's membrane and ectoplacental cone were removed.

For the study on the somitogenic capacity of the presomitic mesoderm, 8·5-day and 9·5-day embryos were subjected to the following operation. Two small incisions were made through the visceral yolk sac and the amnion. Into these openings, a pair of electrolytically polished alloy needles were passed and two transverse cuts were made to isolate a portion of the body containing presomitic mesoderm. The first cut was made at the caudal border of the last-formed somites in the paraxial mesoderm. The second cut was made at a plane at about 200–300 μm from the caudal end of the body axis (Figs 1, 2) so that the primitive streak and tail bud tissues (= caudal tissue) were excised. The operated embryos were then placed in bottles containing a culture medium of equal parts of immediately centrifuged rat serum and Hams F10 medium (GIBCO). Embryos with incised membranes or intact membranes were also cultured as controls. Four to five embryos were cultured in 5 ml medium in a 50 ml serum bottle (Wheaton) rotated at 30 r.p.m. For 8·5-day embryos, the culture was gassed with 5 % CO_2, 5 % O_2 and 90 % N_2 for the first 8 h and then with 5 % CO_2 in air for the next 16 h. For 9·5-day embryos, a gas phase of 5 % CO_2, 40 % O_2 and 45 % N_2 was used throughout. At the end of the culture, the embryos were dissected free of membranes and the number of somites present in the body cranial to the first cut, in the segment containing the presomitic mesoderm and in the caudal tissues were counted. The somite numbers of the control embryos and also those of embryos at equivalent ages *in vivo* were also scored. Selected embryos from the operated and control groups were analysed for protein content (Lowry, Rosebrough, Farr & Randall, 1951). Others were fixed in Sanfelice fluid and embedded in paraffin wax (Merck, melting point 42–45 °C). Serial sections of 12 μm thickness were stained with haematoxylin and eosin.



Figs 1, 2. Scanning electron micrographs of 8·5-day (Fig. 1) and 9·5-day (Fig. 2) embryos showing the position of cuts made through the extraembryonic membranes in order to isolate a segment of the body containing the presomitic mesoderm. Bar, 100 μm.

Figs 3–5. Scanning electron micrographs of the caudal region of 8·5-day (Fig. 3), 9·5-day (Fig. 4) and 10·5-day (Fig. 5) embryos. Cut *a* was made usually at the fissure between the last somite and the presomitic mesoderm and sometimes at the intersomitic fissures so that one to two somites were left in the explant. Cut *b* was made at about 300–400 μm from the tip of embryonic axis. The small fragment thus removed was the caudal tissue (*ct*). For 8·5-day and 9·5-day explants, a third cut (*c*) was made to bisect the explant to yield two symmetrical halves. Bar, 100 μm.

In another experiment, 8·5-day to 10·5-day embryos were dissected free from the foetal membranes and a portion of the body containing the presomitic mesoderm was isolated by cutting caudal to the last somite and cranial to the caudal tissue (cuts *a* and *b* of Figs 3–5). The resultant explants were shown in Figs 6–8. In another study, which also included 11·5-day embryos, cut *b* was made at various distances away from cut *a* so that different amounts of presomitic mesoderm were included in the explants. Camera-lucida drawings of original explants containing the entire presomitic mesoderm and caudal tissue, and that of cut pieces were made with a Nikon drawing tube mounted on a Nikon SMZ-10 dissecting microscope. The various types of explants were cultured under conditions as for the whole embryo. Cultures were examined at 6–7 h, 10–11 h and 20–21 h and the number of somites formed was scored. Photographs of the explants before and after culture were taken with a Nikon inverted microscope using phase optics. Some cultured explants were fixed with Sanfelice fluid and processed for wax histology as described previously. Others were fixed with half-strength Karnovsky fixative and 1 % osmium tetroxide, and dehydrated in an ascending alcohol series. Some of the specimens were embedded in epoxy resin, sectioned at 1–2 μm and stained with toluidine blue for light-microscopic examination. Some were critical-point dried for scanning electron microscopy using Freon 13 as the exchange fluid. The specimen was coated with gold and examined with a JEOL JSM-35CF microscope operating at 15 kV. The longitudinal length of explants was measured on the camera-lucida drawings using a GRAF/BAR sonic digitizer (Science Accessory Corporation) linked to an IBM PC-XT computer that was driven by a Stereometry program (Yucomp Co. Ltd) written in BASIC.

The numerical relationship between somitomeres contained in the presomitic mesoderm and somites formed after culture was also studied. The explants of presomitic mesoderm of 8·5-day and 9·5-day embryo were prepared by cutting first at positions *a* and *b* (Figs 3, 4). Cut *a* was usually made so that one to three pre-existing somites were included in the explant and this aided later orientation of the craniocaudal axis. A longitudinal cut in the median plane was then made (cut *c* of Figs 3, 4) to yield two symmetrical portions of the explants. Only pairs of similar length and containing same number of pre-existing somites were used in this study. One portion of the explant was immediately fixed for scanning electron microscopy as described above. The other portion was individually cultured in 0·5 ml of medium (equal parts of rat serum and Hams F10 medium) in a 24-well Falcon Multiwell culture plate under 5 % CO_2 in air for 3–17 h. The number of somites formed in the explant was scored and the explant was fixed and processed for scanning electron microscopy. Stereopair pictures of the presomitic mesoderm were taken at a tilt angle of 10° for stereoscopic examination.

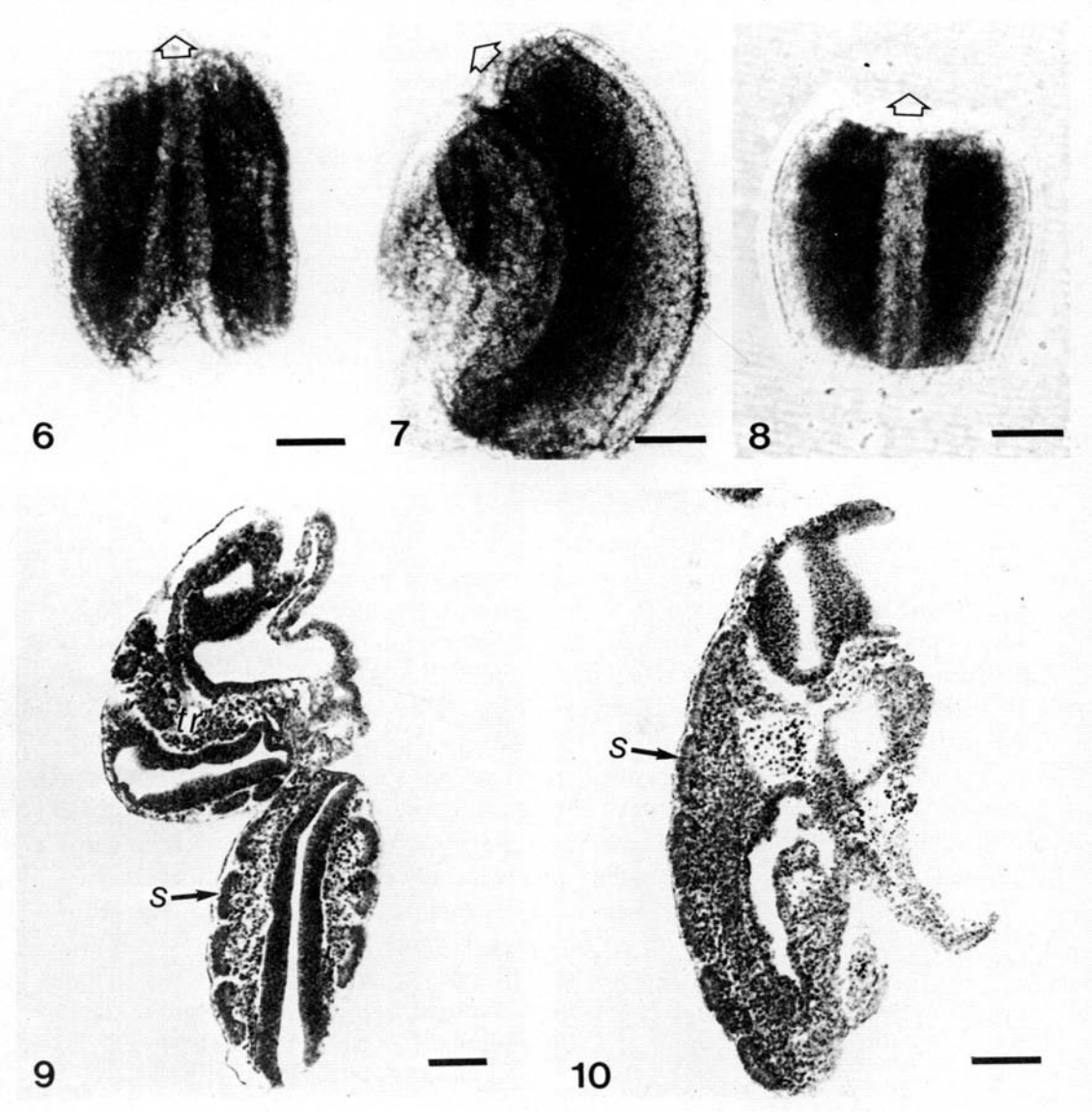
Statistical analyses of the data were done using a NWA Statpak program (Multifunction Statistics Library version 3.1, Northwest Analytical) run on an IBM PC-XT computer.

RESULTS

Transecting the body axis did not affect somite formation in the presomitic mesoderm of the operated embryos. About 11·6 somites were initially present in the 8·5-day embryo used for the operation and during the 24 h of culture 5·8 somites were formed in the presomitic mesoderm portion (Table 1, Fig. 9). The caudal piece containing primarily the primitive streak grew poorly and in only seven cases an additional 2·4 somites were formed. The total number of somites formed in the operated embryo was less than that of the control embryo. The number of somites and the amount of protein contained in the control embryo were comparable to those of 9·5-day embryos *in vivo* (Table 1). The 9·5-day embryos used in this experiment have formed 21 somites at the time of operation, and the presomitic mesoderm formed another 6·1 somites after 24 h of culture (Table 1, Fig. 10). The caudal piece formed a rudimentary tail but no somites were

formed in the scanty paraxial mesoderm. Both the control and operated embryos formed fewer somites and had less protein than 10·5-day embryos *in vivo*.

The presomitic mesoderm obtained from embryos at 8·5 to 10·5 days differed in its length. The presomitic mesoderm of 8·5-day embryo was about 570 μm and the



Figs 6–8. The explants from 8·5-day (Fig. 6), 9·5-day (Fig. 7) and 10·5-day (Fig. 8) embryos produced by making cuts *a* and *b*. Arrow points cranially. Bar, 200 μm.

Figs 9, 10. The isolated segments of 8·5-day (Fig. 9) and 9·5-day (Fig. 10) embryos produced by operations shown in Figs 1, 2 after 24 h *in vitro*. The presomitic mesoderm was segmented into somites (*s*). In Fig. 9, the presomitic mesoderm segment was partly attached to the main trunk (*tr*) of the embryo. Bar, 100 μm.

Table 1. *Somite formation in intact and operated embryos* in vitro

	Number of somites			Protein content (μg)
	Body	Presomitic mesoderm	Whole body	
8·5-day embryo (24 h *in vitro*)				
Control	—	—	22·9 ± 0·4 (22)	113·6 ± 7·5 (19)
Operated	11·6 ± 0·3 (32)	5·8 ± 0·2 (32)	18·0 ± 1·3 (32)†§	94·5 ± 4·3 (28)*
9·5-day embryo *in vivo*	—	—	21·1 ± 0·4 (11)	103·4 ± 5·9 (11)
9·5-day embryo (24 h *in vitro*)				
Control	—	—	33·1 ± 0·7 (11)*	361·8 ± 15·5 (14)†
Operated	21·0 ± 0·6 (25)	6·1 ± 0·3 (19)	27·6 ± 0·6 (25)‡	379·6 ± 17·2 (24)†
10·5-day embryo *in vivo*	—	—	35·5 ± 0·3 (15)	447·8 ± 17·3 (25)

Significantly different from *in vivo* values at * $P < 0{\cdot}05$, † $P < 0{\cdot}02$ and ‡ $P < 0{\cdot}01$ by Student's t-test.

§ An additional 2·4 ± 0·4 somites were formed in the tail segment of seven embryos.

size had nearly doubled at 9·5 days to 970 μm. The size was then reduced to about 680 μm at 10·5 days (Table 2). Despite this variation in the amount of tissues, the presomitic mesoderm formed about 6·3–6·9 somites after 24 h *in vitro* (Table 2). Only about 32–42 % of the 8·5-day and 9·5-day explants developed successfully to form recognizable somites (Figs 11, 12). The remaining explants either degenerated or became so stunted that somites could not be definitively identified. Nearly all the 10·5-day explants formed distinctive somites (Fig. 13). Histological examination of the successfully developed explants showed that proper epithelial somites were formed and some of the somites even began to show sclerotome differentiation (Figs 14–16). The somites were more closely packed than those formed *in vivo*, and were much taller dorsoventrally but shorter craniocaudally.

A comparison of the development of explants without the caudal tissue (PM−PS/TB) and those with intact caudal tissue (PM+PS/TB) was made (Table 3). The position of cut *b* was shifted more cranially than that in previous experiments by about 100 μm to ensure a complete removal of the caudal tissue and the culture was examined at 7–8 h and again at 20–21 h. The PM−PS/TB

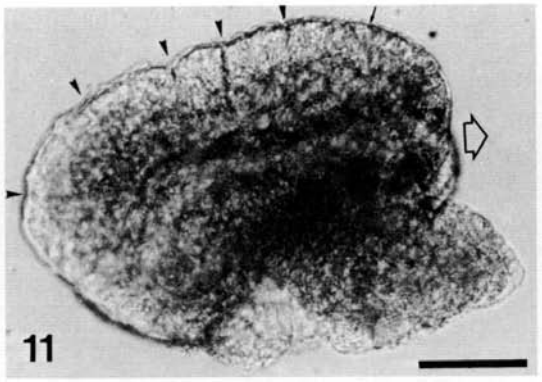
Table 2. *The number of somites formed from explants of presomitic mesoderm of 8·5-day to 10·5-day embryos*

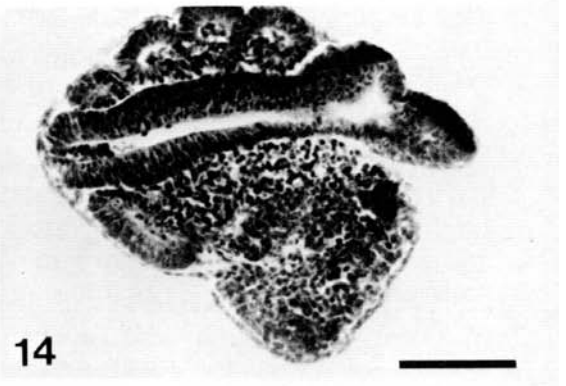
Age (d)	Mean somite no.	Size (μm)	No. of somites
8·5	6·08 ± 0·08 (128)	576 ± 8 (128)	6·93 ± 0·20 (42)
9·5	22·89 ± 0·25 (120)	975 ± 11 (130)	6·39 ± 0·16 (51)
10·5	32·00 ± 0·27 (11)	681 ± 21 (11)	6·30 ± 0·21 (10)

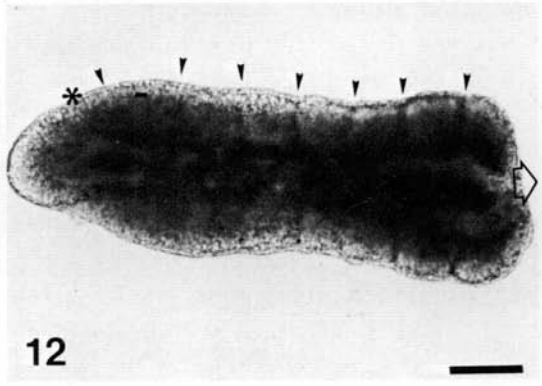
explants formed about 4·2 to 5·7 somites after 7–8 h, and further culturing to 20–21 h only resulted in a slight increase to about 5·1 to 5·8 somites (Table 3). During the same period of time, the PM+PS/TB explants formed significantly more somites (Fig. 17) than those explants without primitive streak or tail bud (Table 3), suggesting that the caudal tissue was an important source of somitic cells. The presomitic mesoderm could only segment into a limited number of somites and further somitogenesis ceased when all existing tissues were exhausted. The 8·5-day and 9·5-day PM+PS/TB explants formed about one somite in every hour but the 10·5-day explants took about 1·8 h to generate one somite. This rate of somite segmentation was in agreement with a previous estimate based upon *in vivo* observation (Tam, 1981).

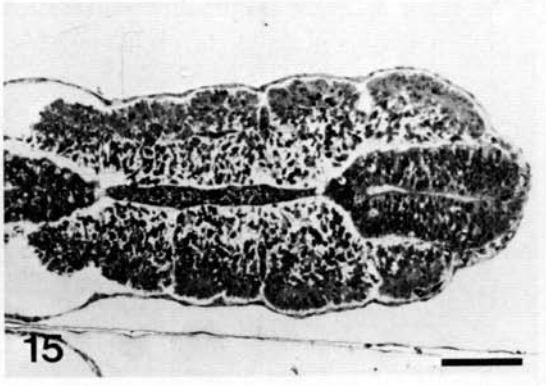
Table 4 shows the results of somite formation in explants of presomitic mesoderm that contained different amount of paraxial tissues. Up to seven somites were formed in the largest explant of 8·5-day and 9·5-day embryos, and six somites were formed from the entire presomitic mesoderm of 10·5-day and 11·5-day embryos. Fewer somites were formed in explants containing a smaller cranial portion of the presomitic mesoderm. A statistical analysis of the length of the explant and the number of somites formed after culture revealed a direct correlation between the amount of available presomitic mesoderm and the number of prospective somites (Table 4). Based upon the regressional equation, the size of the explant that would generate a specific whole number of somites was calculated. Using these data, the domain occupied by each prospective somite in the presomitic mesoderm could then be mapped (Fig. 18). Six to seven prospective somites were accommodated in the presomitic mesoderm of 8·5-day to 11·5-day embryos. The size of prospective somites varied in accordance with their position in the presomitic mesoderm and to the overall size of this tissue. The caudal prospective somite occupied a smaller domain than the more cranial ones. The variation in the size of the most cranial prospective somite at different embryonic ages followed closely the change in size of newly formed somites observed in mouse embryo *in vivo* (Tam, 1981). The size of the caudal tissue also changed during development. The primitive streak regressed in both absolute and relative size between 8·5 and 9·5 days. The tail bud showed a similar trend of reduction in size between 10·5 and 11·5 days.

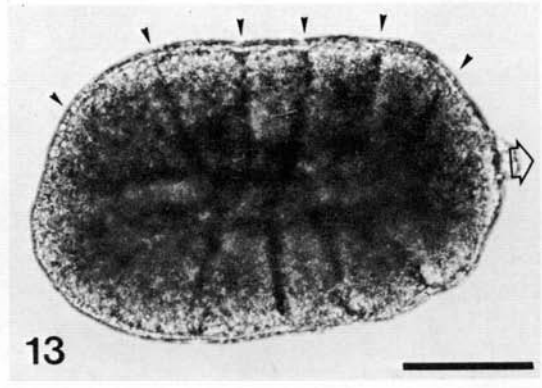
When the presomitic mesoderm was examined with SEM and stereoimaging, characteristic somitomeric units were identified (Fig. 19) and about five to six somitomeres were found in explants of 8·5-day and 9·5-day embryos (Table 5). When the corresponding half of the explant was cultured and examined at various times between 3 and 17 h, somites were seen to form in a proper craniocaudal sequence but at a slower rate than bilaterally intact explants. The newly formed somites were seen to replace the more cranially located somitomeres (Fig. 20). The 8·5-day explants formed one somite every 1·9 h and the 9·5-day explants formed one every 2·4 h. When the explants were harvested and the number of newly added somites and remaining somitomeres was scored, it was found that the total of these two numbers matched the number of somitomeres initially present

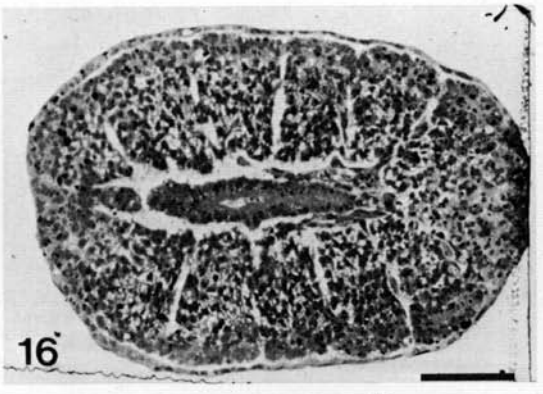

11

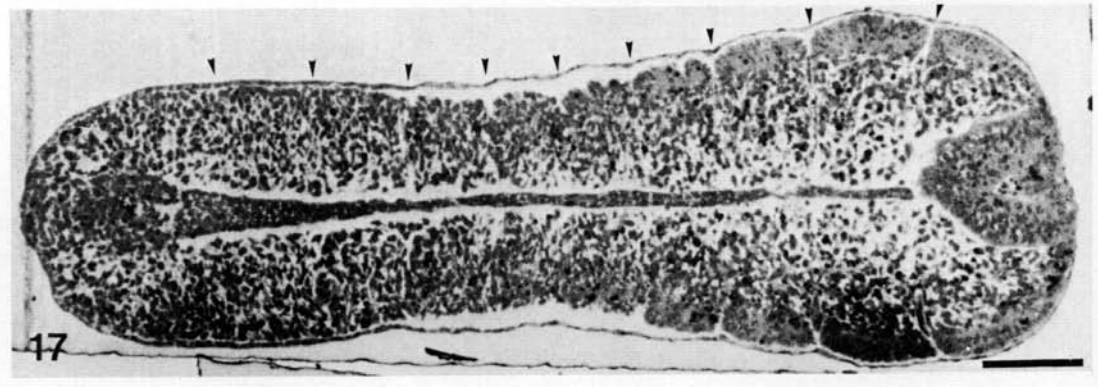

14


12


15


13


16


17

(Table 5). A comparison of the morphology of the somitomere and the somite derived from it showed that somite formation involved a compaction of the mesenchymal cells and a dorsoventral re-alignment of the cells in the explant (Figs 21, 22).

DISCUSSION

The present study has provided evidence in support of the existence of a pattern of prospective somites in the presomitic mesoderm of mouse embryos. The foremost of these is the demonstration that six to seven somites were consistently formed from the presomitic mesoderm which varied at least two-fold in size during embryonic development at 8·5–11·5 days. In the snapping turtle, chick and quail embryos it has been shown that despite a variation in the size of the segmental plate, a relatively constant number of somites would usually be formed in explants of the entire segmental plate. In terms of numbers of somites generated from the presomitic mesoderm, the mouse resembles the snapping turtle where an explant of the entire segmental plate gave five to seven somites (Packard, 1980*a*), but differs from the avian embryos whose segmental plate formed ten to eleven somites (Packard & Jacobson, 1976; Packard, 1980*b*). It has also been shown in the present study that reducing the amount of tissue contained in the explant consequently led to fewer somites being formed. There was no evidence of any regulation to restore the full complement of prospective somites, suggesting that the somitogenic pattern in the presomitic mesoderm is a stable one. Both the craniocaudal sequence and the intrinsic rate of segmentation were retained by the cells in the explants and are unaffected by various surgical manipulations. In both the amphibian and avian embryos, experimental manipulation of the presomitic mesoderm such as reversal of the craniocaudal relation, surgical deletion of tissues and disruption of the normal cellular arrangement has little effect on the predetermined direction of somite segmentation and the number of somites formed (Deuchar & Burgess, 1967; Cooke, 1977; Menkes & Sandor, 1977). Somite segmentation was also unperturbed by the transection of the body axis or the segmental plate (Smith, 1964; Packard, 1978) or by the removal of the axial

Figs 11–13. The PM−PS/TB explants of 8·5-day (Fig. 11), 9·5-day (Fig. 12) and 10·5-day (Fig. 13) embryos showing the formation of somites in the presomitic mesoderm. Intersomitic boundary is marked by arrow heads. In Fig. 11, the intersomitic fissure marked by → was out of focus. In Fig. 12, the paraxial mesoderm (*) lateral to the bulbous end of the neural tube was a homogeneous tissue mass and was not organized into somites. Arrows point cranially. Bar, 200 μm.

Figs 14–16. Histological sections of cultured PM−PS/TB explants of 8·5-day (Fig. 14), 9·5-day (Fig. 15) and 10·5-day (Fig. 16) embryos. The somites formed in the explants were morphologically comparable to those formed *in vivo*. Sclerotome dispersion has occurred and individual somites could be discerned by the dermamyotome and the fissures between sclerotomes. Bar, 100 μm.

Fig. 17. A longitudinal section of the PM+PS/TB explant of 9·5-day embryo showing the presence of eight complete somites, a ninth one which was segmenting and a short unsegmented presomitic mesoderm abutting the tail bud. Bar, 100 μm.

Table 3. *The formation of somites in explants of presomitic mesoderm containing the caudal tissue (PM+PS/TB) and without the caudal tissue (PM−PS/TB)*

No. of somites formed by	8·5-day explants		9·5-day explants		10·5-day explants	
	7–8 h	20–21 h	7–8 h	20–21 h	7–8 h	20–21 h
PM+PS/TB	8·0 ± 0·3 (21)	11·0 ± 0·3 (10)	8·5 ± 0·3 (12)	12·3 ± 0·6 (12)	4·7 ± 0·2 (10)	12·1 ± 0·5 (10)
PM−PS/TB	4·8 ± 0·2 (25)	5·2 ± 0·1 (25)	5·7 ± 0·2 (11)	5·8 ± 0·1 (11)	4·2 ± 0·3 (15)	5·1 ± 0·2 (15)

Tests for difference in number of somites by Student's t-test:

	8·5-day	9·5-day	10·5-day
(a) Between types of explants			
At 7–8 h	$P < 0{\cdot}001$	$P < 0{\cdot}001$	no
At 20–21 h	$P < 0{\cdot}001$	$P < 0{\cdot}001$	$P < 0{\cdot}001$
(b) At the two time points			
PM+PS/TB	$P < 0{\cdot}001$	$P < 0{\cdot}001$	$P < 0{\cdot}001$
PM−PS/TB	no	no	$P < 0{\cdot}02$

Table 4. *The somite-forming capacity of different portions of presomitic mesoderm of 8·5-day to 11·5-day mouse embryos*

	8·5-day	9·5-day	10·5-day	11·5-day
No. of somites in the embryo	5·7 ± 0·2 (40)	22·6 ± 0·5 (37)	34·0 ± 0·9 (26)	46·5 ± 0·5 (17)
Length (μm) of presomitic mesoderm + caudal tissues	798 ± 18 (65)	1116 ± 15 (72)	773 ± 13 (59)	575 ± 17 (39)
Length (μm) of portions of presomitic mesoderm that formed:				
2 somites	280 (1)	310 (1)	315 (2)	216 ± 14 (10)
3 somites	350 ± 26 (10)	508 ± 24 (13)	336 ± 19 (5)	333 ± 25 (4)
4 somites	451 ± 20 (11)	592 ± 29 (9)	441 ± 19 (16)	375 ± 17 (10)
5 somites	533 ± 29 (16)	726 ± 28 (12)	497 ± 16 (20)	427 ± 30 (7)
6 somites	564 ± 17 (8)	811 ± 28 (17)	559 ± 19 (9)	471 ± 26 (8)
7 somites	620 ± 54 (5)	960 ± 10 (3)	—	—
Statistical analysis by single variable logarithmic regression: [Size of explant] = a+b·log[number of somites]				
Regression coefficient a	0·038 ± 0·009	0·074 ± 0·009	0·079 ± 0·008	0·057 ± 0·005
Regression coefficient b	0·301 ± 0·006	0·416 ± 0·006	0·261 ± 0·005	0·231 ± 0·004
Coefficient of determination	0·53	0·64	0·49	0·73
Covariance	0·11	0·05	0·02	0·04
Correlation coefficient	0·73	0·80	0·70	0·85
Significance at P value	$<0·001$	$<0·001$	$<0·01$	$<0·001$

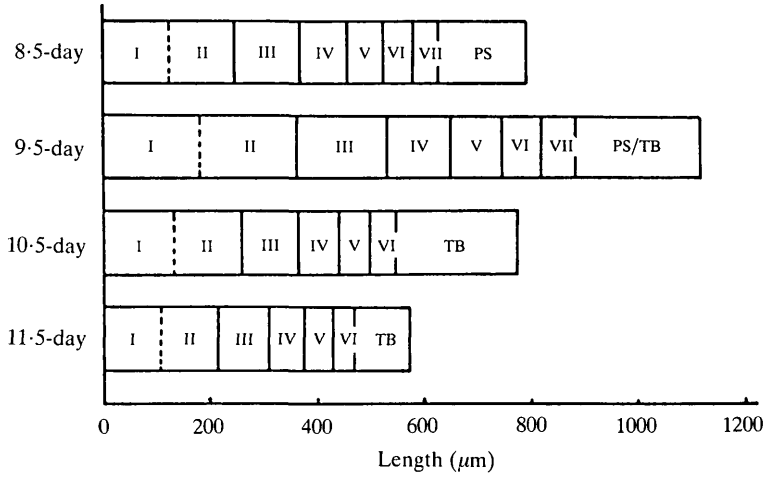


Fig. 18. A map of prospective somites in the presomitic mesoderm of 8·5-day to 11·5-day mouse embryos. The prospective somites were labelled I to VII from the cranial end of the presomitic mesoderm. The position of the boundary between prospective somites was derived from the regressional equations in Table 4, but that between I and II was estimated by an equal partition of the domain occupied by the two units. In the caudal tissue, PS refers to the primitive streak and TB refers to the tail bud.

structures normally associated with the segmental plate (Packard & Jacobson, 1976, 1979; Stern & Bellairs, 1984*a*). However, the size and shape of the somites were altered under *in vitro* conditions because of changes in the physical force exerted by the surrounding epithelia and neural tube (Menkes & Sandor, 1977; Packard & Jacobson, 1979; Stern & Bellairs, 1984*a*; the present study).

The other compelling evidence for a prepattern of somites is the demonstration of a developmental relationship between somitomeres initially present in the presomitic mesoderm and the somites formed in the explants. The presence of distinctive clusters of mesenchymal cells which show a unique concentric orientation about a centre was first described in the paraxial mesoderm of the chick embryo (Meier, 1979). Subsequently similar cellular patterns are also found in the apparently unsegmented cranial mesoderm of newt (Jacobson & Meier, 1984), snapping turtle (Meier & Packard, 1984), chick, quail (Meier, 1981, 1982) and mouse (Meier & Tam, 1982) and in the presomitic mesoderm and segmental plate of snapping turtle (Packard & Meier, 1984), birds (Packard & Meier, 1983) and mouse (Tam *et al.* 1982). It has been shown in the present study on mouse embryos and in other studies on avian (Packard & Meier, 1983) and snapping turtle embryos (Packard & Meier, 1984) that the number of somitomeres identified in the presomitic mesoderm or segmental plate correlates very well with the number of prospective somites contained in the same tissues. The formation of a somite in the explants results in the corresponding disappearance of one somitomere. The somitomeric organization in the presomitic mesoderm is therefore a morphological manifestation of the metameric pattern found in the embryonic axis.

It becomes apparent from the map shown in Fig. 18 that in order to accommodate all six to seven prospective somites in presomitic mesoderm of different sizes, each and every prospective somite undergoes a proportional change in its dimensions. The more caudal members occupy smaller domains than the cranial counterparts. Since the series of prospective somites represents progressively more mature entities culminating in somite segmentation, an expansion of the prospective somites after their inception might be an essential step in the differentiative process of becoming an epithelial somite. This expansion in size may be coincidental to the burst of cellular proliferation observed in the cranial portion of the segmental plate (Stern & Bellairs, 1984*b*). Such a transition from a closely packed to an expanded state of prospective somites has recently been described in the tail bud of the *Xenopus* embryo (Elsdale & Davidson, 1983). The so-called packing zone in the tail bud of *Xenopus* may be equivalent to the primitive streak of the chick (Meier & Jacobson, 1982; Stern & Bellairs, 1984*a*) and the caudal tissue of the mouse (Tam, 1984). The zone of extension and zone of prepattern are perhaps analogous to the caudal and cranial portions, respectively, of the presomitic mesoderm in the mouse embryo.

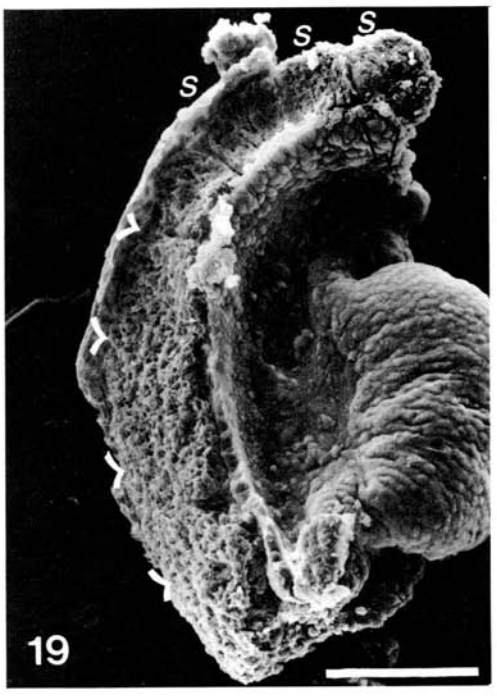
The present study also showed that somite formation stopped abruptly in explants without the caudal tissue when the entire presomitic mesoderm has been segmented. A similar truncation of the somitic pattern occurred after the removal of the tail bud in amphibians (Cooke, 1975; Elsdale & Davidson, 1983), and the primitive streak in birds (Packard & Jacobson, 1976; Packard, 1980*b*; Packard & Meier, 1983), snapping turtle (Packard, 1980*a*) and mouse (Smith, 1964). The caudal tissue which has extensive histogenetic potential (Tam, 1984) has been suggested as an important source of cells for somitogenesis (Tam, 1981). It has recently been postulated that in the chick embryo the process of somite formation depends upon the constant interaction of a small population of somitic progenitor cells with new cells generated from the primitive streak and the tail bud. The immediate product of such an interaction is the formation of the somitomere or the prospective somite (Bellairs & Veini, 1984). It has yet to be shown that there are

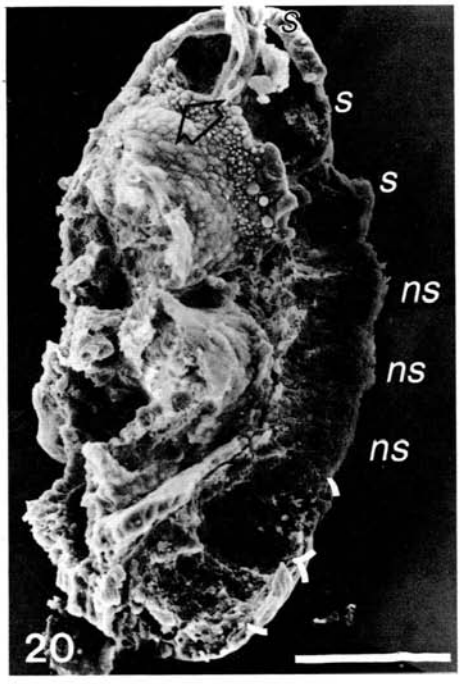
Fig. 19. Scanning electron micrograph of the bisected explant. The neural tube was dissected away to expose the presomitic mesoderm. Three somites (*s*) and five somitomeres were identified. The intersomitomeric boundary was marked by curved lines. Arrow points cranially. Bar, 100 μm.

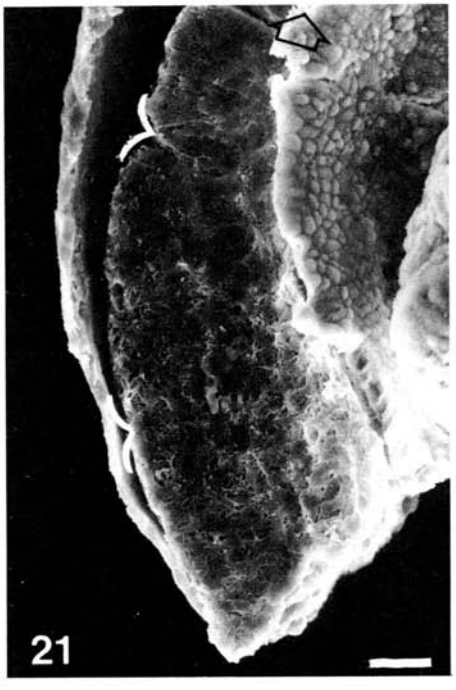
Fig. 20. Scanning electron micrograph of the corresponding half of the explant after 8 h *in vitro*. Three new somites (*ns*) were formed and two somitomeres were seen at the caudal part of the explants. The somitomeres were marked by curved lines and the three somites originally present were labelled (*s*). Arrow points cranially. Bar, 100 μm.

Fig. 21. Scanning electron micrograph showing the cellular pattern seen in three somitomeres in the presomitic mesoderm of an explant fixed before culture. The intersomitomeric boundary was marked by curved lines. Cells in the cranial-most somitomere were more closely packed. Arrow points cranially. Bar, 20 μm.

Fig. 22. Scanning electron micrograph showing the medial aspect of somites derived from the three somitomeres showed in Fig. 20. The somites were compressed craniocaudally because of the reduced elongation of the axial structures. Positions of intersomitic fissures were indicated by arrow heads. Arrow points cranially. Bar, 20 μm.


s
s
s
s
19


s
s
s
ns
ns
ns
20


21

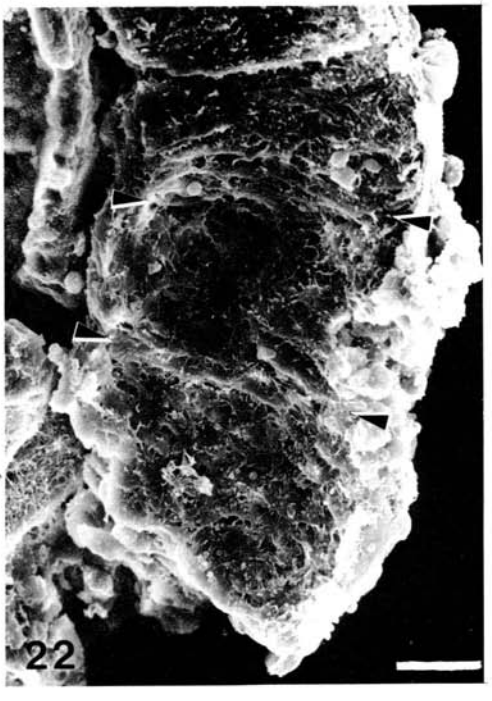

22

Table 5. *A comparison of the number of metameric segments in the presomitic mesoderm before and after culture*

	Original explant		Explants after culture		
	Somites	Somitomeres*	New somites†	Somitomeres	Total segments*
8·5-day	2	6	4	2	6
	1	6	3	3	6
	1	6	4	2	6
	3	5	5	0	5
	3	6	2	3	5
	2	6	5	2	7
		5·8 ± 0·2 (6)			
9·5-day	2	5	2	3	5
	3	6	2	4	6
	2	5	2	3	5
	3	4	4	0	4
	2	6	4	2	6
	2	6	4	2	6
	2	6	5	0	5
	1	6	5	2	7
	3	5	5	0	5
	4	4	1	2	3
	1	6	3	2	5
	3	5	5	0	5
	2	6	5	0	5
	4	5	5	0	5
	4	3	3	0	3
	4	4	3	1	4
	3	5	5	0	5
	2	6	6	0	5
	1	5	5	0	5
	1	5	5	1	6
	2	4	4	0	4
	0	6	6	0	6
		5·1 ± 0·2 (22)			

* Significant correlation between these two values ($r = 0{\cdot}82$, $P < 0{\cdot}001$, d.f. = 26 by linear correlation analysis and Student's t-test).

† Number of new somites = (total number of somites present in the explant at the end of culture) − (number of somites originally present before culture).

indeed two cell populations within a somitomere or later in a somite, but the primitive streak and the tail bud of the chick embryo have been shown to contribute cells to the somites (Meier & Jacobson, 1982; Schoenwolf, 1977). The precise role of the caudal tissue in the specification of somitomeres or prospective somites in the mouse embryo remains to be elucidated.

REFERENCES

ADELMAN, H. B. (1925). The development of neural folds and cranial ganglia of the rat. *J. comp. Neurol.* **39**, 19–171.

BELLAIRS, R. (1979). The mechanism of somite segmentation in the chick embryo. *J. Embryol. exp. Morph.* **51**, 227–243.

BELLAIRS, R. & VEINI, M. (1984). Experimental analysis of control mechanisms in somite segmentation in avian embryos. II. Reduction of materials in the gastrula stage of the chick. *J. Embryol. exp. Morph.* **79**, 183–200.

COOKE, J. (1975). Control of somite number during development of a vertebrate, *Xenopus laevis*. *Nature, Lond.* **254**, 196–199.

COOKE, J. (1977). The control of somite number during amphibian development: model and experiment. In *Vertebrate Limb and Somite Morphogenesis* (ed. D. A. Ede, J. R. Hinchliffe & M. Balls), pp. 433–448. Cambridge University Press.

DEUCHAR, E. M. & BURGESS, A. M. C. (1967). Somite segmentation in amphibian embryos: is there a transmitted mechanism? *J. Embryol. exp. Morph.* **17**, 349–358.

ELSDALE, T. & DAVIDSON, D. (1983). Somitogenesis in amphibian. IV. The dynamics of tail development. *J. Embryol. exp. Morph.* **76**, 157–176.

FLINT, O. P., EDE, D. A., WILBY, O. K. & PROCTOR, J. (1978). Control of somite number in normal and *amputated* mutant mouse embryos: an experimental and a theoretical analysis. *J. Embryol. exp. Morph.* **45**, 189–202.

JACOBSON, A. G. & MEIER, S. (1984). Morphogenesis of the head of a newt: mesodermal segments, neuromeres and distribution of neural crest. *Devl Biol.* **106**, 181–193.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin reagent. *J. biol. Chem.* **193**, 265–275.

MEIER, S. (1979). Development of the chick embryo mesoblast. Formation of the embryonic axis and establishment of the metameric pattern. *Devl Biol.* **73**, 25–45.

MEIER, S. (1981). Development of the chick mesoblast: morphogenesis of the prechordal plate and cranial segments. *Devl Biol.* **83**, 49–61.

MEIER, S. (1982). The development of segmentation in the cranial region of vertebrate embryos. *Scanning Electron Microscopy* **III**, 1269–1282.

MEIER, S. (1984). Somite formation and its relationship to metameric pattern in the mesoderm. *Cell Diff.* **14**, 235–243.

MEIER, S. & JACOBSON, A. G. (1982). Experimental studies of the origin and expression of metameric pattern in the chick embryo. *J. exp. Zool.* **219**, 217–232.

MEIER, S. & PACKARD, D. S. JR (1984). Morphogenesis of the cranial segments and distribution of the neural crest in the embryos of the snapping turtle, *Chelydra serpentina*. *Devl Biol.* **102**, 309–323.

MEIER, S. & TAM, P. P. L. (1982). Metameric pattern development in the embryonic axis of the mouse. I. Differentiation of the cranial segments. *Differentiation* **21**, 95–108.

MENKES, B. & SANDOR, S. (1977). Somitogenesis, regulation potencies, sequence determination and primordial interactions. In *Vertebrate Limb and Somite Morphogenesis* (ed. D. A Ede, J. R. Hinchliffe & M. Balls), pp. 405–419. Cambridge University Press.

NEAL, H. V. (1918). Neuromeres and metameres. *J. Morph.* **31**, 293–315.

PACKARD, D. S. JR (1978). Chick somite determination: the role of factors in young somites and the segmental plate. *J. exp. Zool.* **203**, 295–306.

PACKARD, D. S. JR (1980*a*). Somite formation in cultured embryos of the snapping turtle, *Chelydra serpentina*. *J. Embryol. exp. Morph.* **59**, 113–130.

PACKARD, D. S. JR (1980*b*). Somitogenesis in cultured embryos of the Japanese quail, *Coturnix coturnix japonica*. *Amer. J. Anat.* **158**, 83–91.

PACKARD, D. S. JR & JACOBSON, A. G. (1976). The influence of axial structures on chick somite formation. *Devl Biol.* **53**, 36–48.

PACKARD, D. S. JR & JACOBSON, A. G. (1979). Analysis of the physical forces that influence the shape of the chick somites. *J. exp. Zool.* **207**, 81–92.

PACKARD, D. S. JR & MEIER, S. (1983). An experimental study of the somitomeric organization of the avian segmental plate. *Devl Biol.* **97**, 191–202.

PACKARD, D. S. JR & MEIER, S. (1984). Morphological and experimental studies of the somitomeric organization of the segmental plate in snapping turtle embryos. *J. Embryol. exp. Morph.* **84**, 35–48.

SCHOENWOLF, G. C. (1977). Tail (end) bud contributions to the posterior region of the chick embryo. *J. exp. Zool.* **201**, 227–246.

SMITH, L. J. (1964). The effects of transection and extirpation on axis formation and elongation in the young mouse embryo. *J. Embryol. exp. Morph.* **12**, 787–803.

STERN, C. D. & BELLAIRS, R. (1984*a*). The roles of node regression and elongation of the area pellucida in the formation of somites in avian embryos. *J. Embryol. exp. Morph.* **81**, 75–92.

STERN, C. D. & BELLAIRS, R. (1984*b*). Mitotic activity during somite segmentation in the early chick embryo. *Anat. Embryol.* **169**, 97–102.

TAM, P. P. L. (1981). The control of somitogenesis in mouse embryos. *J. Embryol. exp. Morph.* **65**, **Supplement**, 103–128.

TAM, P. P. L. (1984). The histogenetic capacity of tissues at the caudal end of the embryonic axis in the mouse. *J. Embryol. exp. Morph.* **82**, 253–266.

TAM, P. P. L., MEIER, S. & JACOBSON, A. G. (1982). Differentiation of the metameric pattern in the embryonic axis of the mouse. II. Somitomeric organization of the presomitic mesoderm. *Differentiation* **21**, 109–122.

(*Accepted 23 September 1985*)