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# Morphological and experimental studies of the somitomeric organization of the segmental plate in snapping turtle embryos

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## SUMMARY

The segmental plate mesoderm of snapping turtle embryos *(Chelydra serpentina)* was examined with stereoscanning electron microscopy imaging. A metameric pattern was detected along the entire length of the segmental plates. This pattern consisted of a tandem sequence of mesodermal units, called somitomeres. Each somitomere was oval to cubic in shape and the processes of the constituent mesodermal cells tended to be arranged in concentric rings about the centre of the somitomere. Several experiments from a previous study (Packard, 1980b) of snapping turtle segmental plates were repeated, but, instead of culturing the explants and observing the numbers of somites that formed, the explants were fixed immediately for scanning electron microscopy and the number of somitomeres was counted. The segmental plates were found to contain an average of 6·5 ± 0·7 somitomeres, which is almost identical to the average number of somites formed by such segmental plates when cultured (6·6 ± 1·2). Furthermore, the number of somitomeres was identical in right and left explants removed from the same embryo, and the number of somitomeres was consistent regardless of the length of the segmental plate. Both of these observations are identical to those made previously for somite formation in culture. This association between numbers of somitomeres and somites strongly suggests that one gives rise to the other. Finally, it was demonstrated that for each somite formed by a segmental plate in culture, the segmental plate contained one less somitomere. This showed in a direct manner that turtle somitomeres become somites. It was concluded that the segmental plate mesoderm of snapping turtle embryos is already segmented, and that the 'segmentation' seen under a dissecting microscope is actually the final stage of somitomere differentiation into an epithelial somite.

## INTRODUCTION

Between the last-formed pair of somites and Hensen's node, the paraxial mesoderm of amniote embryos consists of a pair of mesodermal bars lying on either side of the neural tube; these bars are known as the segmental plates. Somites appear to form from the cranial ends of the segmental plates. Meier

[1]This work is dedicated to the memory of Professor Chester L. Yntema.

(1979) analysed chicken embryo segmental plates in stereo with the scanning electron microscope (SEM) and showed that the segmental plates, rather than being unsegmented, actually contained a subtle, but discernable segmental pattern. Each segment of the pattern appeared to originate at Hensen's node as a domain of cells whose somas and processes formed concentric circular arrangements when observed from the dorsal perspective. Meier counted 10 or 11 of the segments, or 'somitomeres', along each segmental plate undergoing changes culminating in somite formation. Somitomeres have now been observed in the segmental plates of mouse embryos as well, although the number varies between 5 and 7 (Tam & Meier, 1982).

The presence of somitomeres in chick segmental plates and the numbers of them that were observed proved very interesting since it had been shown experimentally that the segmental plates of chicken (Packard & Jacobson, 1976; Packard, 1978) and Japanese quail (Packard, 1980a) embryos are already developmentally committed to forming 10 to 12 somites. It seemed possible that somitomeres were the physical manifestation of the commitment to form somites. We re-examined segmental plates of chicken and Japanese quail embryos with the SEM (Packard & Meier, 1983) and made segmental plate-containing tissue explants identical to those used earlier by Packard & Jacobson (1976) and Packard (1978, 1980a). Explants were examined in stereo with the SEM and were found to contain an average of 10·0 ± 1·5 somitomeres; this number is similar to the number of somites formed by such explants when they were allowed to develop in culture (11·9 ± 1·1 – Packard & Jacobson, 1976; 10·7 ± 2·1 – Packard, 1978; 11·3 ± 2·9 – Packard, 1980a). The bilateral symmetry and independence from segmental plate length shown earlier for the prospective somite pattern, was also observed with respect to somitomeres. We provided direct evidence that somitomeres become somites by first removing both segmental plates, as tissue explants. Left explants were fixed immediately and the number of somitomeres they contained was determined by analysis with the SEM. Right explants were cultured *in vitro*. Since paired explants form the same number of somites when cultured, it was reasoned that if somitomeres become somites, the number of somitomeres identified in each left, uncultured, explant would equal the sum of the number of somitomeres and somites in the corresponding right, cultured, explant. This expected result was, in fact, obtained and was interpreted to demonstrate that avian somitomeres are prospective somites.

The study of turtle embryos can provide an interesting confirmation of the above results on avian embryos because the segmental plates of snapping turtle embryos (Packard, 1980b) like those of mouse embryos (Tam, Meier, Jacobson, 1982) form only 5 to 7 somites when allowed to develop in culture. If somitomeres do represent an early stage in somite differentiation, then turtle segmental plates should contain only 5 to 7 somitomeres. Moreover, as previously shown for turtle prospective somites, the number of somitomeres

should be bilaterally symmetrical and consistent regardless of segmental plate length. This study will demonstrate that turtle somitomeres are present in the expected numbers and that they do, in fact, develop into somites.

## MATERIALS AND METHODS

From 1980 to 1983, fertile eggs of the snapping turtle, *Chelydra serpentina*, were obtained and incubated as described previously (Yntema, 1964; Packard, 1980b). Embryos having between 3 and 19 somite pairs were removed from the eggs and segmental plate-containing explants were cut from the embryos as described in a previous study (Packard, 1980b). The explants contained the full length of the segmental plate between the last-formed somite and the node, one-half of the adjacent neural tube, the notochord, the adjacent lateral plate mesoderm, the underlying endoderm, and the overlying ectoderm. When paired explants were removed, the notochord remained with the right explant (see Fig. 3 in Packard, 1980b). Explants were cultured according to the method described by Packard (1980b).

Embryos and explants to be examined with the scanning electron microscope were washed with Tyrode's (1910) saline, fixed in half-strength Karnovsky's fixative (1965) and dissected. Specimens were post fixed in 1% osmium tetroxide for 1 h, dehydrated through graded alcohols, and critical-point dried and sputter coated as described by Packard & Meier (1983). The tissue samples were observed with an ISI Super IIIA SEM at 15 kV. All stereo pairs were taken at a tilt angle of 10° and are shown for viewing with a standard stereopticon (divergent viewing).

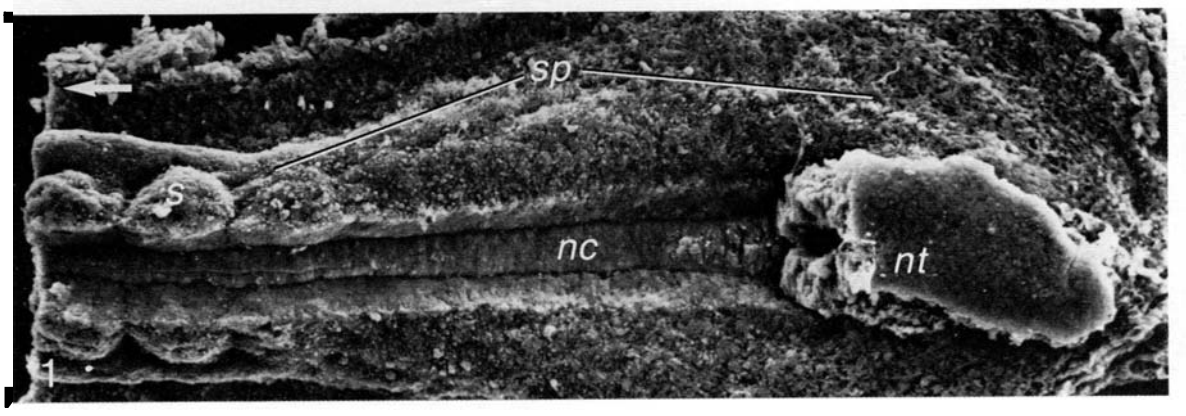
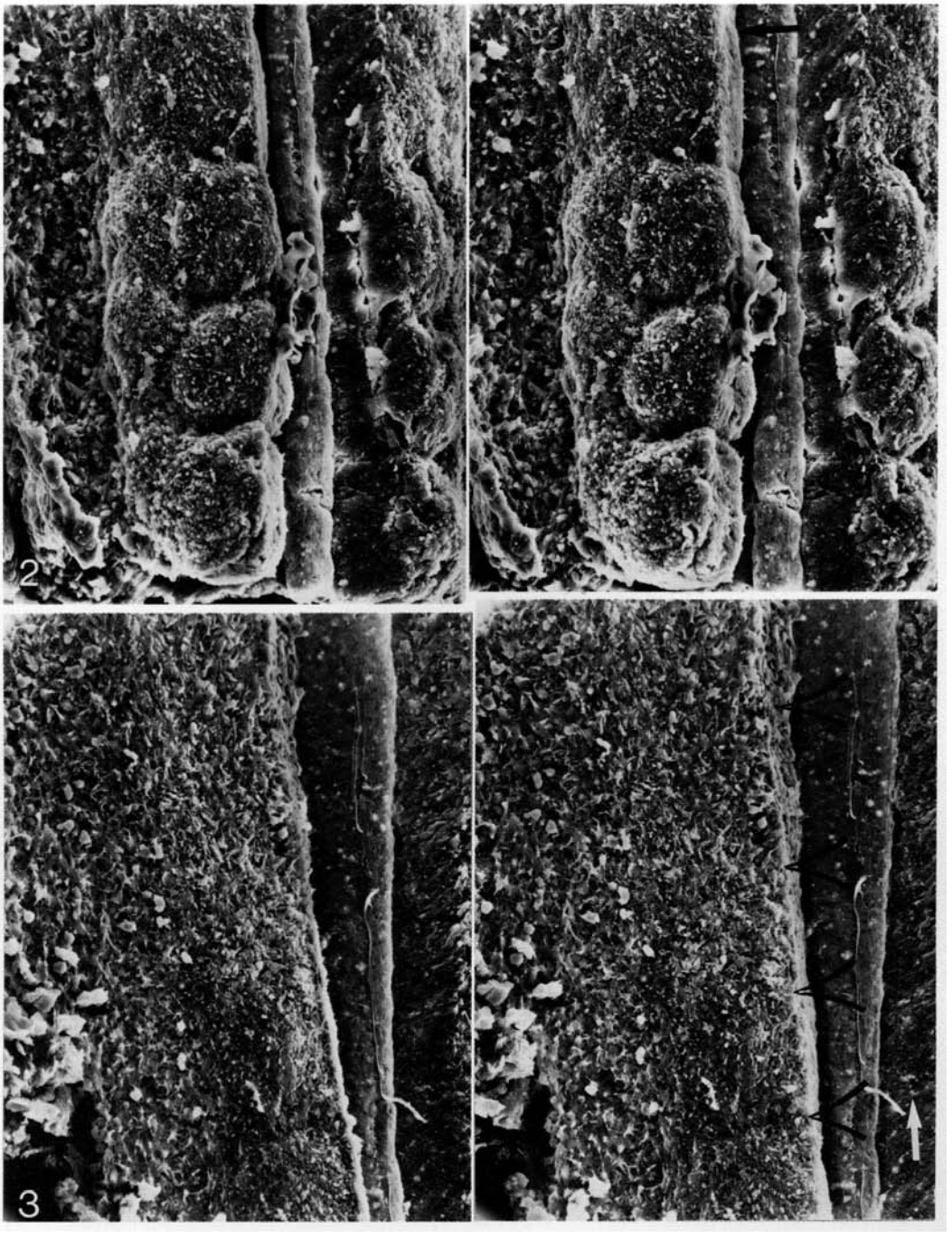



Fig. 1. Dorsal view of the caudal end of a 9-somite snapping turtle embryo from which the neural tube and surface ectoderm have been removed (white arrow points craniad). Overt somites (*s*) precede the segmental plate (*sp*) mesoderm along the axis. *nt*, neural tube; *nc*, notochord. × 175.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*Results*

Somite formation in snapping turtle embryos is very similar to that in chicken embryos. Figure 1 is an overview of the mesoderm of the caudal portion of a 9-somite turtle embryo that was bisected transversely between the seventh and eighth pairs of somites. When the surface ectoderm and neural tube are removed, the notochord can be seen lying in the bottom of the groove formerly occupied by the neural tube. At the most caudal end of the axis, a portion of the neural tube remains in the node region. At the cranial end of the explant, the tenth pair of somites is nearly completely formed so that the expanse of mesoderm caudal to this somite pair represents the segmental plate. At first glance, the segmental plate mesoderm appears unsegmented. However, careful examination in stereo with the SEM indicates that this is not the case, and that the segmental plate is, in fact, composed of a tandem sequence of mesodermal units. At the cranial end of the segmental plate (Fig. 2), the mesodermal units are roughly cubic in shape and are separated cranially and caudally from one another by pronounced intersegmental gaps that permit their identification as somites. The mesodermal cells that compose each of these units are difficult to recognize as individuals because they are closely applied to one another and are enshrouded by extracellular matrix. The dorsal surface of the units bulge convexly whereas the medial surface, facing the neural tube (removed) is somewhat concave. More caudally (Fig. 3), the mesodermal units are less compact and there are no pronounced intersegmental grooves along their cranial–caudal interface. In fact, units are less cubic and their dorsal surface is usually concave, causing the intersegmental interfaces to appear as raised cellular ridges. The processes of individual mesodermal cells are more easily distinguished and often convey the impression that they are arranged in concentric rings. Further caudally (Fig. 4) near the tail bud, the segmental units become more distended and less compact. There is little matrix to obscure the cells and the radial arrangement of the mesoderm can be seen more easily. Since the mesodermal units nearest the node region strongly resemble those found in the same location in chick embryos, they will be referred to hereafter as 'somitomeres' for ease of discussion.

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Fig. 2. Stereo SEM (tilt angle, 10°) of the cranial end of the right segmental plate prepared from an embryo similar to that in Fig. 1. Three overt somites precede a somitomere. The intersegmental region delimiting the first somitomere from the second is indicated by the black arrow. × 235.

Fig.3 Stereo SEM (tilt angle, 10°) of a more posterior portion of the segmental plate shown in Fig. 2 (white arrow points caudally). A portion of the first, all of the second, third, and fourth somitomeres, and a portion of the fifth somitomere are indicated by the black carets at intersegmental borders. × 235.

The impression that tandemly aligned somitomeres undergo morphogenesis culminating in somite formation, suggested by observing the dorsal surface of the segmental plate, is further suggested by analysis of cross sections. Transverse sections taken through somitomeres near the somitic end of the segmental plate (Fig. 5) show that the units are nearly cubic, since they are nearly as tall as they are wide. Individual mesodermal cells are columnar and closely applied to each other along their lateral surfaces. Cells are aimed about the central core of the somitomere and convey the appearance of a simple epithelium. In more caudal regions of segmental plate, near the tail bud (Fig. 5B), somitomeres are dorsoventrally flattened discs. Individual mesodermal cells are shaped heterogeneously, mostly cubic, and are not as closely applied to one another. Cells of these somitomeres are aligned in two layers, an upper, dorsal layer associated with the neural plate and surface ectoderm, and a ventral layer associated with endoderm. These layers encase a thin space that later becomes the myocoel of definitive somites.

In order to establish whether somitomeres are the morphological manifestation of the prospective somites that were detected in turtle segmental plates in a previous study, we repeated some of the experiments of that study and monitored somitomere development. The number of somitomeres along the segmental plates of ten randomly aged snapping turtle embryos was counted. The mean value was 6·6 somites with a standard deviation of 0·7. One of the striking observations made previously was that while the length of the segmental plate varied widely with embryo age, the number of somites formed by an explanted segmental plate was consistently 5 to 7. In order to document better the variation of segmental plate length with age, measurements were made on embryos of various ages and these measurements were added to those reported previously. Measurements for 183 embryos are shown in Fig. 6. While there was significant variation among the embryos of a given stage of development, a general trend was evident in which the shortest segmental plates tended to occur in the oldest embryos and the longest segmental plates occurred in embryos having about 9 pairs of somites.

Explants identical to those used in the previous study were taken from embryos of known age and fixed immediately for SEM analysis. We were able

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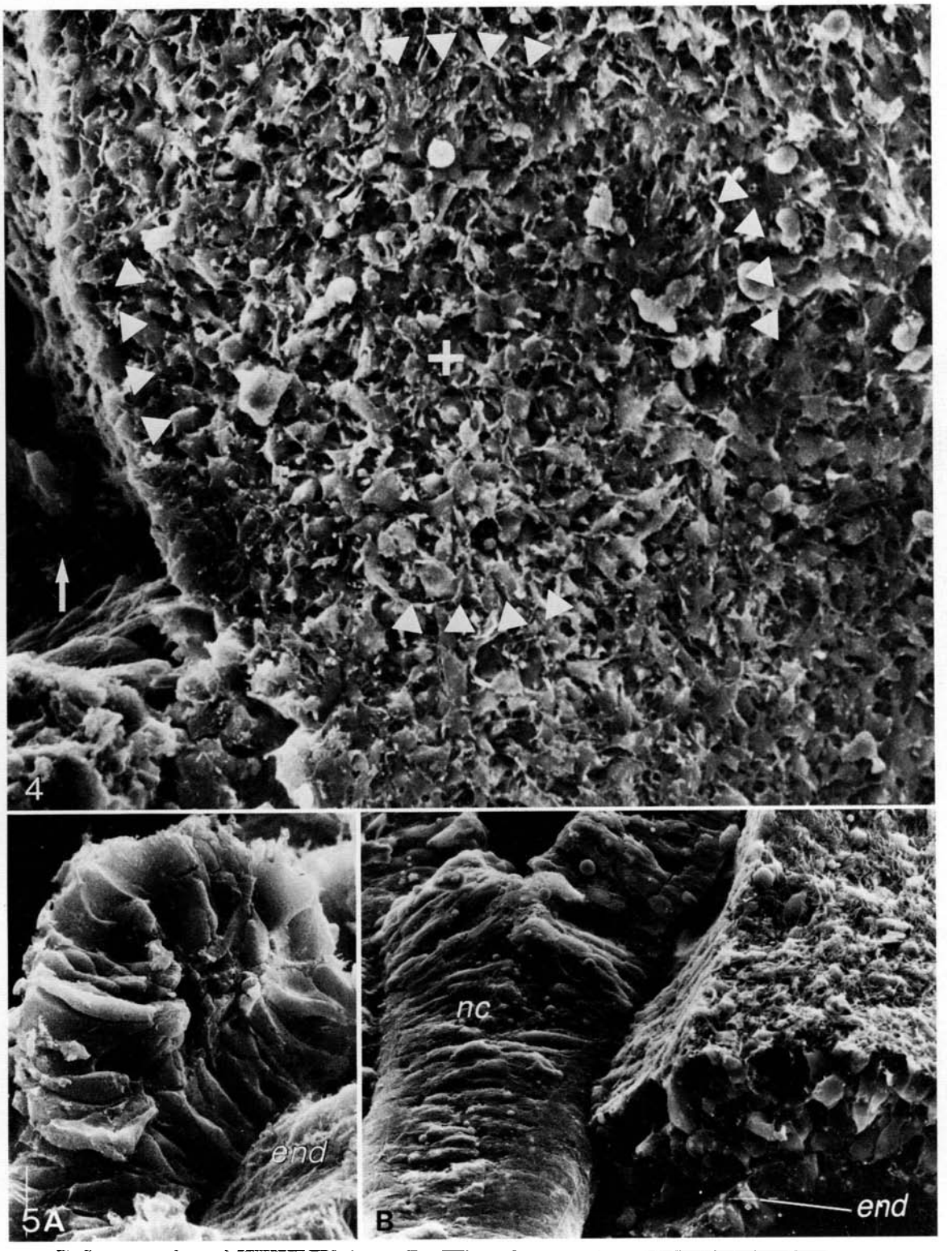
Fig. 4. A dorsal view of a single somitomere in a more posterior portion of the segmental plate shown in Fig. 3 (white arrow lies on the midline and points cranially). The outer boundary of the unit is indicated by the white triangles, whereas the centre of the unit is marked with a white plus sign. × 610.

Fig. 5. Cross sections taken through somitomeres in various stages of morphogenesis culminating in somite formation. (A) At the cranial end of the segmental plate, somitomeres are almost cube shaped; (B) At the posterior end of the segmental plate, somitomeres have a bilayered conformation. *end*, endoderm; *nc*, notochord. (A) × 90; (B) × 490.


4
5A
end
nc
B
end

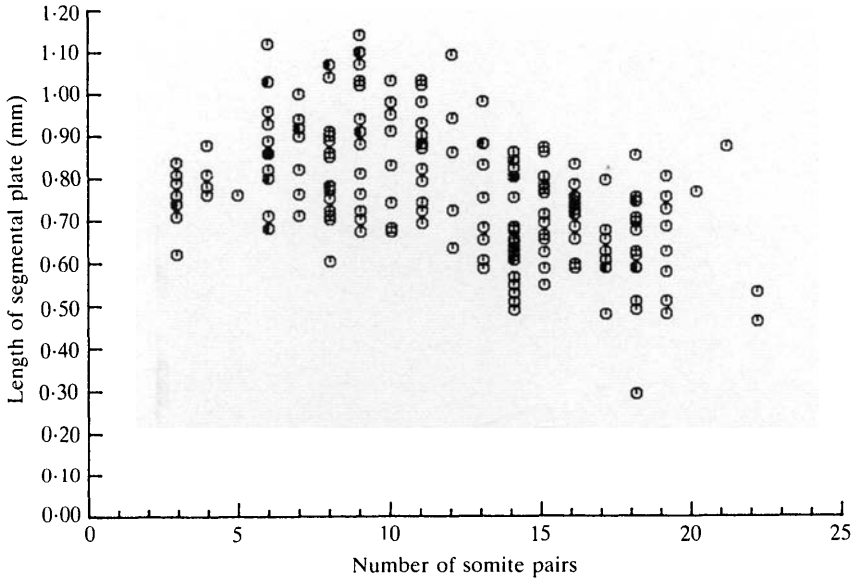



Fig. 6. Graph of segmental plate length versus age of embryo. Age was recorded as the number of somite pairs in each embryo. The shortest segmental plates tended to occur in the oldest embryos. Some of these data were reported by Packard (1980b). Open circles represent single datum, half-filled circles represent two coincident data, and solid circles represent three coincident data.

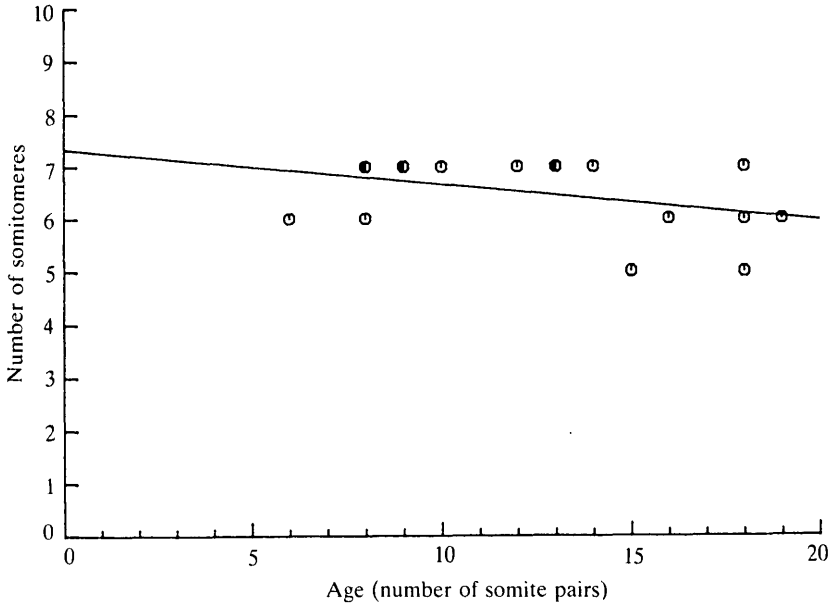



Fig. 7. Graph to show that the number of somitomeres contained in the segmental plate is relatively independent of embryo age. Open circles represent single datum, and half-filled circles represent two coincident data.

to count somitomeres in 17 explants (others were lost or broken in preparation or were obscured by yolk particles or excessive fibrous material). The results, presented in Fig. 7, show that only a slight variation in the number of somitomeres occurred (5 to 7 somitomeres) in segmental plate explants taken

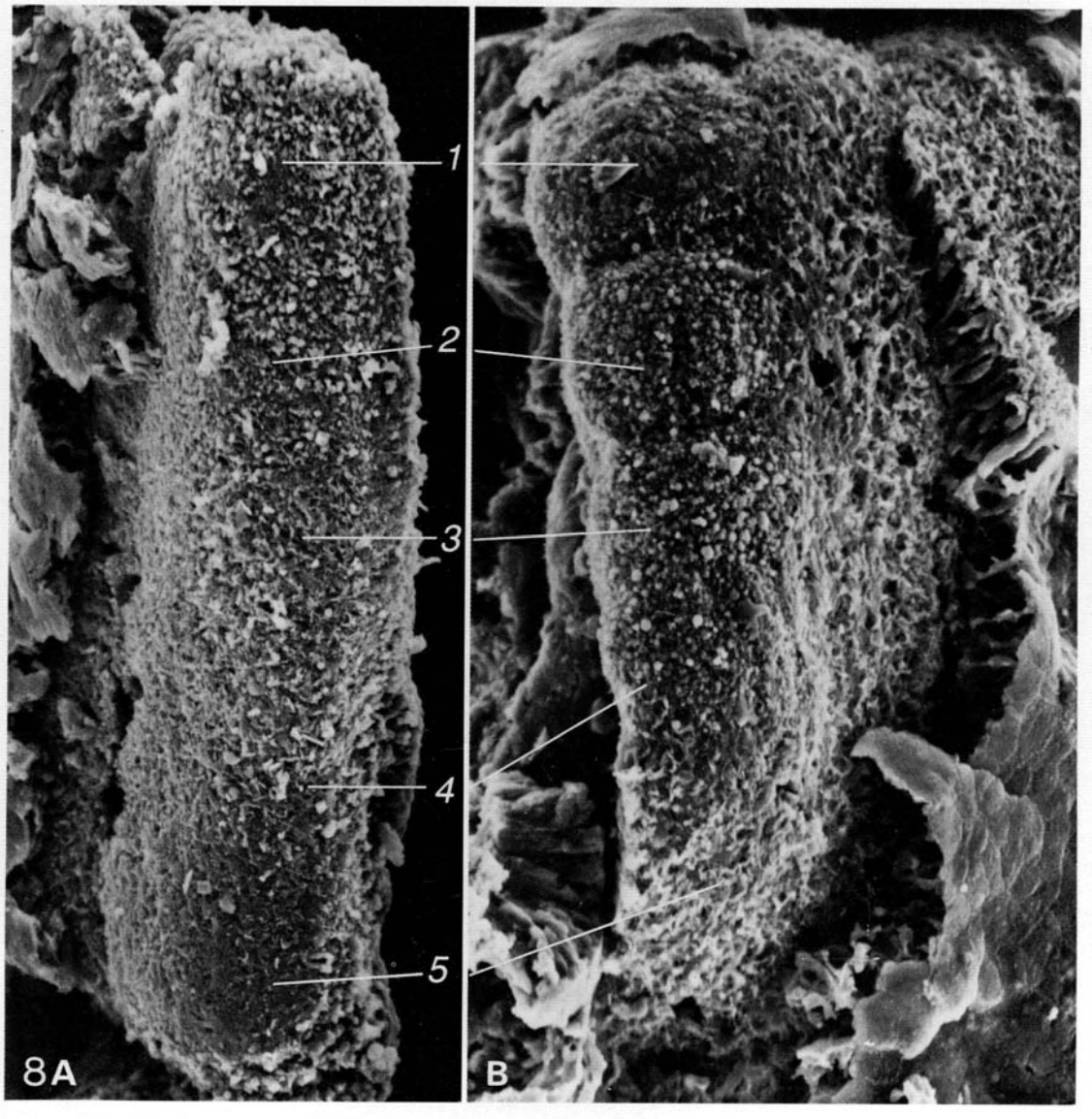



Fig. 8. Dorsal view of the segmental plates isolated as explants from a snapping turtle embryo (surface and neural ectoderm removed). (A) The left explant was examined in stereo and 5 somitomeres were identified (cranial end *1*, numbered near somitomere centres). (B) The right explant, cultured for 20 h, formed two somites at the cranial end (*1* and *2*), with three somitomeres remaining in the more caudal end (*3, 4, 5*). (A) × 400; (B) × 435.

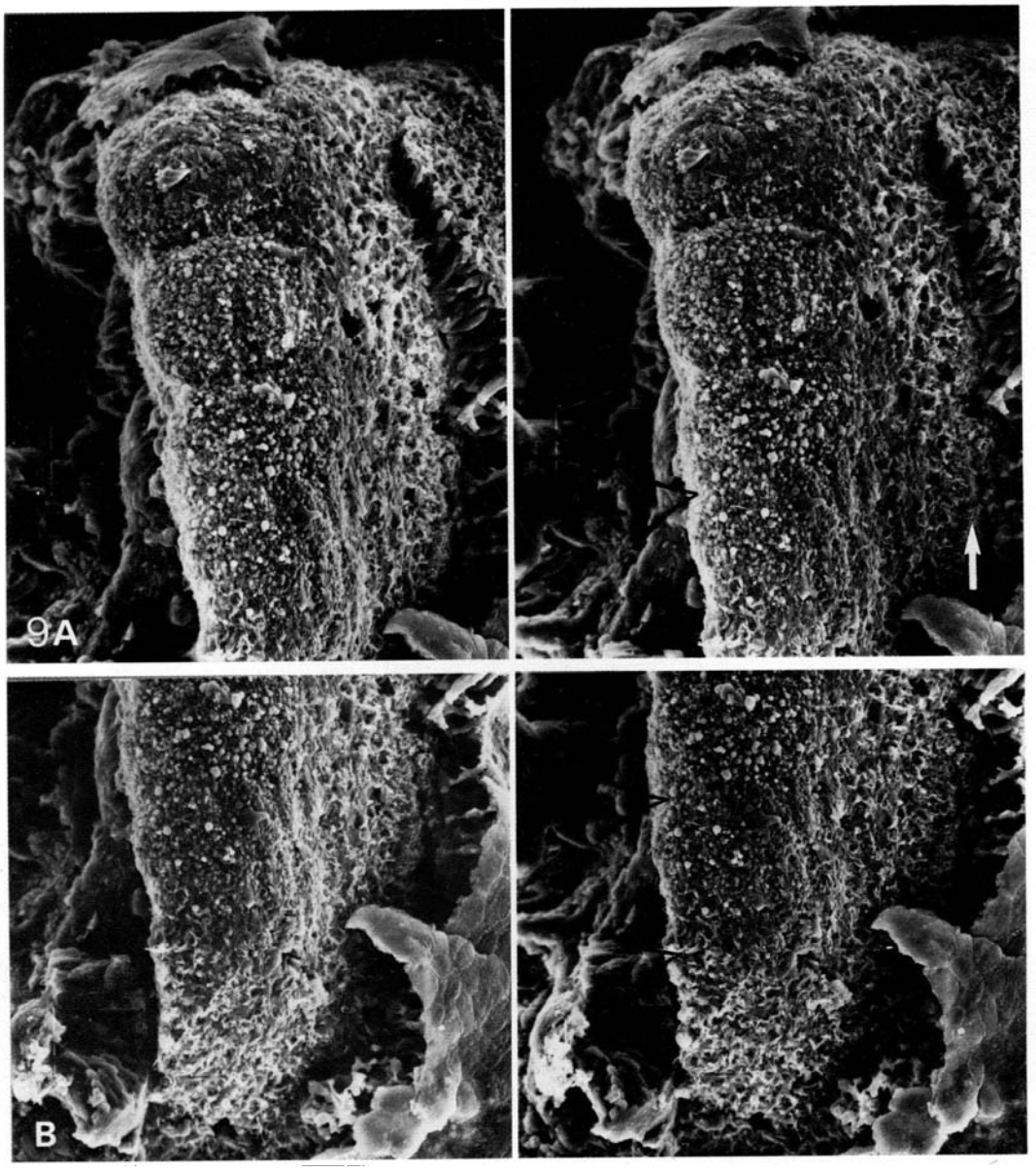



Fig. 9. Stereo SEM (tilt angle 10°) of the explant shown in Fig. 8B (white arrow points cranially). (A) At the cranial end, two somitomeres have undergone morphogenesis into somites that can be recognized at the light level. (B) At the caudal end of the explant, only the most posterior somitomere (the fifth) has retained the primitive somitomere morphology of distended, radially arranged cells. Black carets mark intersegmental borders between somitomeres. (A) × 315. (B) × 315.

Table 1

| Uncultured Segmental Plate | | | Cultured Segmental Plate | | |
|---|---|---|---|---|---|
| No. of Somitomeres | No. of Somites | Total Segments | Total Segments | No. of Somites | No. of Somitomeres |
| 6 | 0 | 6 | 6 | 2 | 4 |
| 7 | 0 | 7 | 7 | 4 | 3 |
| 7 | 0 | 7 | 7 | 2 | 5 |
| 6 | 0 | 6 | 6 | 2 | 4 |
| 5 | 0 | 5 | 5 | 2 | 3 |

from embryos varying widely in age (8 to 19 pairs of somites). The length of nine of these explants was measured prior to making the explant. In these nine cases, the correlation between the length of each segmental plate and the number of somitomeres counted in it was very low (0·123). In order to get an estimate of the average number of somitomeres in each segmental plate, the number of somitomeres in 19 segmental plate explants was counted. The result was an average of 6·5 ± 0·7 somitomeres.

Finally, in order to show that somitomeres become somites, we cultured segmental plate explants *in vitro*. It is known that the right and left segmental plates, removed from a particular snapping turtle embryo, will nearly always form identical numbers of somites when cultured (Packard, 1980b). We removed tissue explants containing segmental plates from turtle embryos. In each case the left explant was fixed immediately for SEM analysis (Fig. 8A). The right explant was placed in culture at 30 °C for 16 to 20 h; during this time 2 to 4 somites usually formed (Fig. 8B). The right explant was fixed for SEM analysis and the number of somites and somitomeres was counted (Fig. 9). In five cases we were able to count somitomeres and somites in paired explants. As shown in Table 1, each pair of explants contained an identical number of segments and, for each somite formed in a cultured explant, there was one less somitomere.

## DISCUSSION

One important question that must be answered before we can begin to understand the problem of metameric pattern formation, is when and where within the embryo the future somite cells become committed to form somites and to form them in a particular pattern. The present study ties together a large body of evidence on the state of commitment of amniote segmental plate mesodermal cells to form somites. It has been shown that the intact segmental plate mesoderm of chicken, Japanese quail, and snapping turtle embryos can segment completely into a predetermined number of somites when cultured in the absence of tissues that normally surround them (Packard & Jacobson, 1976; Packard, 1980a; Packard, 1980b). These observations were interpreted to mean that the segmental plate mesoderm of these animals already contains a

complete complement of prospective somites that will subsequently become the definitive somites seen at the light microscopic level (Packard & Jacobson, 1976; Packard, 1980a; Packard, 1980b). Subsequently, the morphological basis for this prepattern of segmentation was demonstrated in avian embryos by Meier (1979) in the form of somitomeres. It was later shown that the paraxial mesoderm cranial to the first-formed somites of chick (Meier, 1981), mouse (Meier & Tam, 1982), and snapping turtle (Meier & Packard, 1984) embryos contains several more somitomeres that did not undergo morphogenesis into overt somites. These embryos are therefore viewed as forming their body axis by the cranial to caudal addition of initially identical mesodermal segments that undergo differential morphogenesis dependent on their position along the axis.

The present study has provided evidence that the segmental plates of snapping turtle embryos contain an average of 6·5 ± 0·7 somitomeres. This number is almost identical to the number of prospective somites estimated to be in turtle segmental plates (6·6 ± 1·2 somites; Packard, 1980b). Therefore, whereas avian segmental plates contain about 10 to 12 prospective somites and about 10 somitomeres, turtle segmental plates are more like those of mouse embryos because they both contain only 5 to 7 prospective somites and 5 to 7 somitomeres. This association between numbers of prospective somites and somitomeres strongly suggests that prospective somites and somitomeres are identical. Other support for this conclusion comes from the fact that like prospective somites, the numbers of somitomeres are bilaterally symmetrical and nearly constant despite variations in total segmental plate length. Finally, the demonstration that for each somite formed by a cultured segmental plate, there is one less somitomere, shows in a direct manner that turtle somites form at the expense of somitomeres.

Packard (1980a; 1980b) has suggested that the consistency in the number of somitomeres found in segmental plates of various lengths may be due to the fact that the rate of somitomere specification in the node region is similar to the rate of somite formation. This situation would result in a relatively constant number of somitomeres being in each segmental plate at any particular time during somitogenesis despite variations in segmental plate length. This hypothesis can also account for species-specific numbers of somitomeres in segmental plates: the process of somite formation may begin at a species-specific interval after the initiation of somitomere formation. For instance, if somitomeres and somites form at similar rates in chick and quail embryos and somite formation begins at somitomere 8 when there are 19 pairs of somitomeres (the first 7 somitomere pairs do not become somites; Meier, 1981), then there will be 11 pairs of somitomeres caudal to the forming somite. As both processes continue to create somitomeres and somites, the number of somitomeres in the segmental plate will remain at about 11. A similar situation may occur in the snapping turtle (which also forms 7 pairs of head segments; Meier

& Packard, 1984) except that the first somites would form from the eighth pair of somitomeres when there is a total of only 14 pairs of somitomeres.

We believe there is now sufficient evidence to state unequivocally that the mesodermal cells found in the segmental plates of amniote embryos, including those of the snapping turtle, chicken, Japanese quail, and mouse, are developmentally committed to form somites in a particular spatial pattern by virtue of the fact that the segmental plates of these animals are already segmented into somitomeres. We suggest that the 'segmentation' of the segmental plate into somites, which is visible under a dissecting microscope, is actually the final stage of differentiation of the somitomere into a somite (Meier, 1979; Packard & Meier, 1983; Tam, *et al.* 1982). The presence of somitomeres in the paraxial mesoderm of an anamniote, the newt (Jacobson & Meier, 1984), further suggests that for many vertebrate embryos the paraxial mesoderm, once established by the morphogenetic movements that occur during gastrulation, is already segmented. It is likely that changes in mesoderm organization (Lipton & Jacobson, 1974; Beloussov & Naumidi, 1983), cell adhesivity (Bellairs, Sanders & Portch, 1980; Poole & Steinberg, 1982; Cheney & Lash, 1984), and in other factors associated with somite morphogenesis (Chernoff & Lash, 1981; Ostrovsky, Cheney, Seitz & Lash, 1983; Ostrovsky, Sanger & Lash, 1983) are actually aspects of the final stage of somitomere differentiation. The fact that the paraxial mesoderm of amniote embryos is committed to somite formation as far caudally as the node (Packard, 1976; 1980a; 1980b; Meier & Jacobson, 1982; Triplett & Meier, 1982; Meier & Tam, 1982) suggests that the specification of the segmental pattern may occur in the region of the node and the cranial primitive streak rather than in the 'unsegmented' paraxial mesoderm (Meier, 1979; Triplett & Meier, 1982; Packard & Meier, 1983). If we are to learn more about when and where, in the embryo, cells become committed to somite formation, we must look in the region of the primary organizer (Hornbruch, Summerbell & Wolpert, 1979; Fazakas-Todea & Sandor, 1984).

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