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Experimental analysis of control mechanisms in somite segmentation in avian embryos

II. Reduction of material in the gastrula stages of the chick

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SUMMARY

A new theory of control of somite segmentation in chick embryos is proposed. This supposes that tiny clusters of already programmed cells are present throughout the presumptive somite area at stage 4, but that in order to fulfill their destiny they probably depend on the addition of further cells from the primitive streak. Evidence is based on the two groups of experiments:

a) Experiments involving transection across the primitive streak at various stages, (which results in a 'tail' which possesses mesodermal derivatives) and across the segmental plate (which results in a 'tail' lacking mesodermal derivatives).

b) Experiments in which parts of embryos have been explanted with or without their primitive streak.

It is suggested that the initial clusters of pre-programmed cells move further and further posteriorly, developing into somitomeres (the precursors of true somites) only as they receive re-inforcements from the primitive streak or, ultimately, from the tail bud.

INTRODUCTION

In vertebrate embryos the somites are the first segmented structures to form and the pattern which they establish influences that of all the other segmental tissues which subsequently develop, especially the vertebrae, the spinal nerves and the intersegmental arteries. In particular, the number of somites which form in the initial stages is important in determining the number of vertebrae even though it may become modified later in development.

The way in which an embryo controls the number of somites remains one of the major problems of embryology, though a number of seminal hypotheses have been put forward. One of the most critical experiments was carried out by Cooke (1975), who showed that if the amount of material was reduced in an *amphibian* blastula, so that the embryo was reduced in length, it nevertheless developed the appropriate number of somites though each somite was smaller than normal. Thus in some way the embryo had utilized the available material to make the

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correct number of (small) somites, rather than a reduced number of normal-sized somites. As a result of this experiment, Cooke & Zeeman (1976) put forward their 'Clock and Wavefront Model' for amphibian embryos, the purpose of which was to explain how a fixed length of tissue becomes divided into the correct number of somites. Cooke (1977) suggested moreover that the situation was very similar in the chick embryo, since miniaturized somites have sometimes been reported in the literature after experimental interference (Spratt, 1955; 1957).

In a recent paper however, Veini & Bellairs (1983) showed that when the

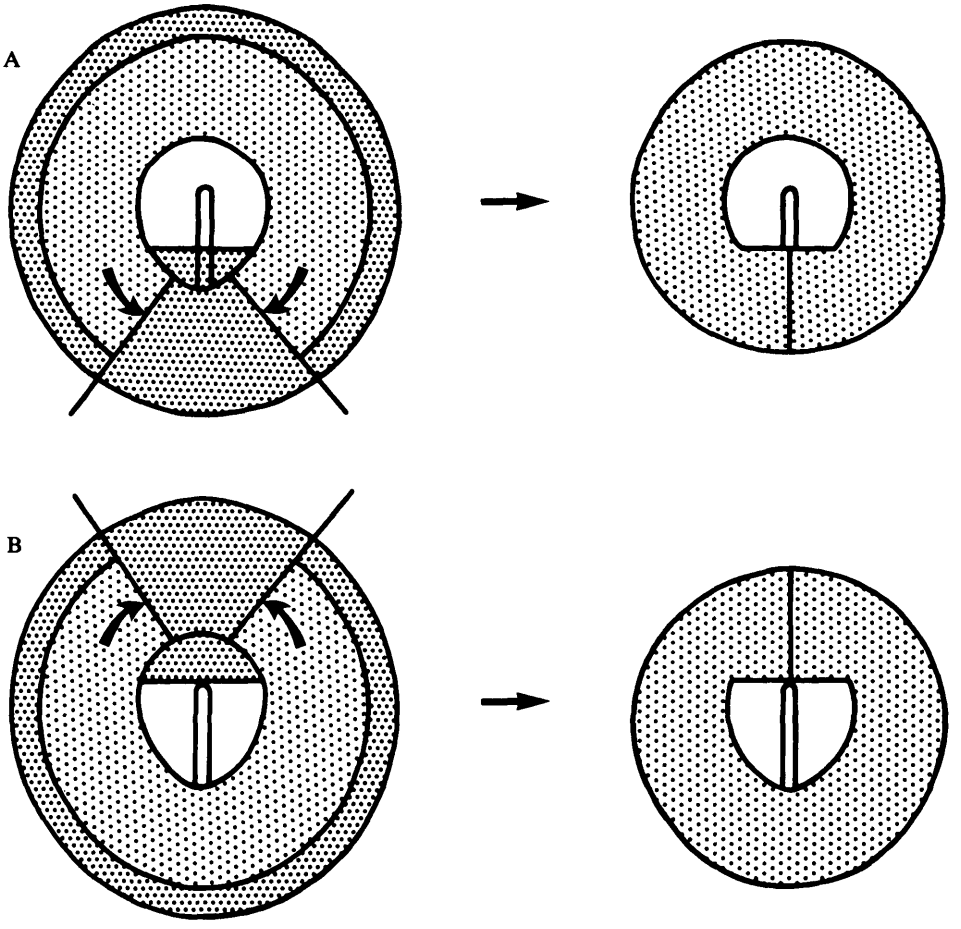


Fig. 1. *Experiment a*

Reduction of area pellucida. About one third or one quarter of the area pellucida was extirpated, together with a segment of area opaca, and the rim of the area opaca. The remaining area opaca was then pulled medially (curved arrows) into contact with the cut edge of the area pellucida. A. Removal of posterior part of area pellucida. B. Removal of anterior part of area pellucida (control experiment). Heavy stipple: tissue extirpated. Light stipple: area opaca remaining.

amount of tissue was reduced in an *avian blastula*, the somites which developed were of normal size.

In the present paper, we describe experiments performed on chick embryos at the *gastrula* stage which result in the formation of miniaturized somites. These and other results are used as the basis for a new theory on somite formation.

MATERIALS AND METHODS

Hens' eggs were incubated to early or late gastrula stages (between about stages 3 and 10 of Hamburger & Hamilton, 1951). The embryos were dissected from the yolk and explanted according to the technique of New (1955). The operation was then performed and the culture dish was returned to a 37·5 °C incubator.Embryos were examined at intervals and usually fixed after 18–24 h although it was sometimes necessary to fix at an earlier stage to prevent dissociation of formed somites (see Discussion).

Operations were carried out using either tungsten needles or finely sharpened irridectomy knives. In all experiments which involved cutting alongside the primitive streak, the cut was made immediately lateral to it. In every case, including the controls, the periphery of the area opaca was removed in order to reduce the degree of tension in the blastoderm which normally occurs in New culture (New, 1959; Bellairs, Bromham & Wylie, 1967). This was particularly

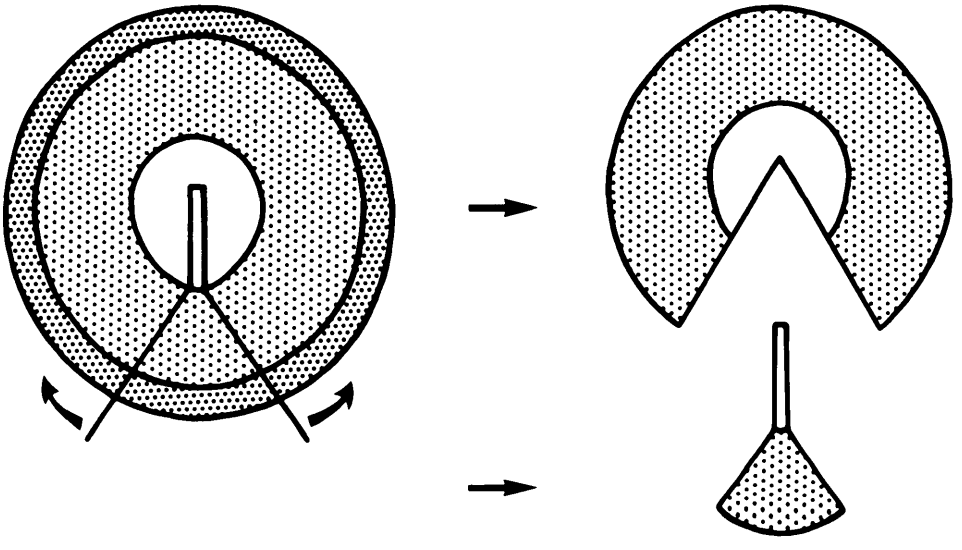


Fig. 2. *Experiment b*

Removal of primitive streak. The primitive streak, together with a segment of area opaca was extirpated and explanted separately. The cut edges of the remainder of the area pellucida were pulled apart to prevent healing, and the rim of the area opaca removed (heavy stipple).

necessary to prevent distortion of the fragments of the embryo which retained area opaca on one side only (e.g. experiments b, c, d and e, below).

I *Experiments on the early gastrula* (Stages 3+ or 4)

Experiment a (Fig. 1)

This experiment led to a reduction in size of the area pellucida. the amount removed was either about $\frac{1}{3}$ or $\frac{1}{4}$ of the posterior end. In control experiments, the anterior $\frac{1}{3}$rd of the area pellucida was removed.

Experiment b (Fig. 2)

The primitive streak was extirpated and the cut edges of the area pellucida pulled apart to prevent healing. The primitive streak, together with its associated piece of area opaca, was explanted separately.

Experiment c (Fig. 3)

The embryo was cut longitudinally immediately to one side of the primitive

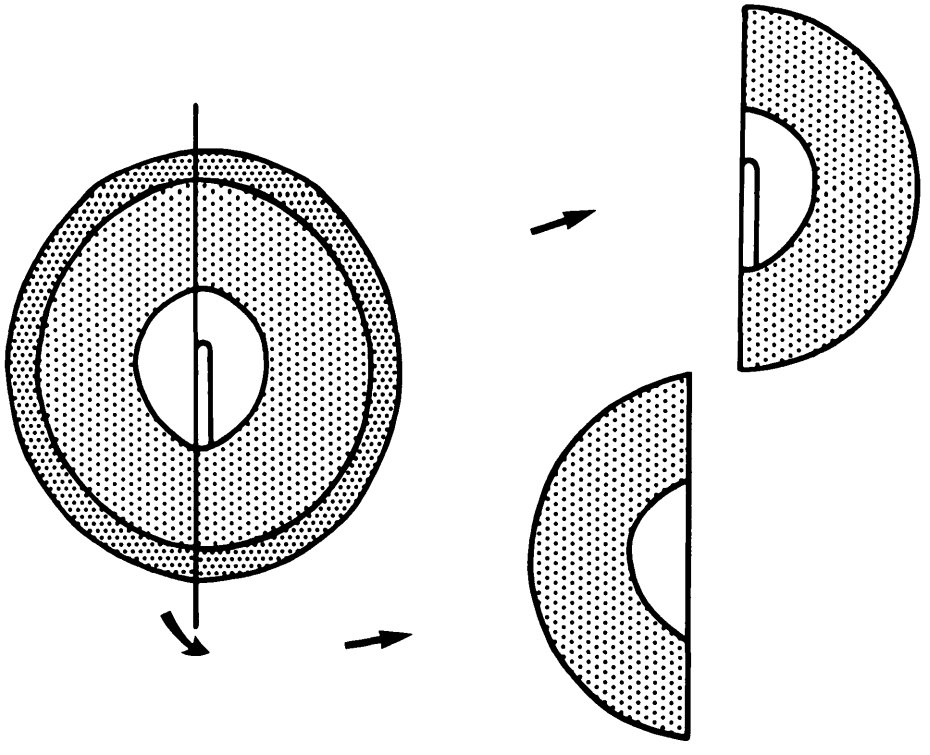


Fig. 3. *Experiment c*
Comparison of presence or absence of primitive streak. The embryo was cut into two pieces as indicated. The rim of the area opaca was removed, (heavy stipple).

streak. In some specimens the cut was made to the left, in others to the right. The two pieces were explanted separately.

Experiment d (Fig. 4)

The primitive streak was extirpated together with a piece of area opaca. The remainder of the area pellucida was cut as indicated into two sections, one possessing the entire anterior part of the ara pellucida. The cuts were made so that in some cases the left side of the area pellucida was bigger, in other cases the right.

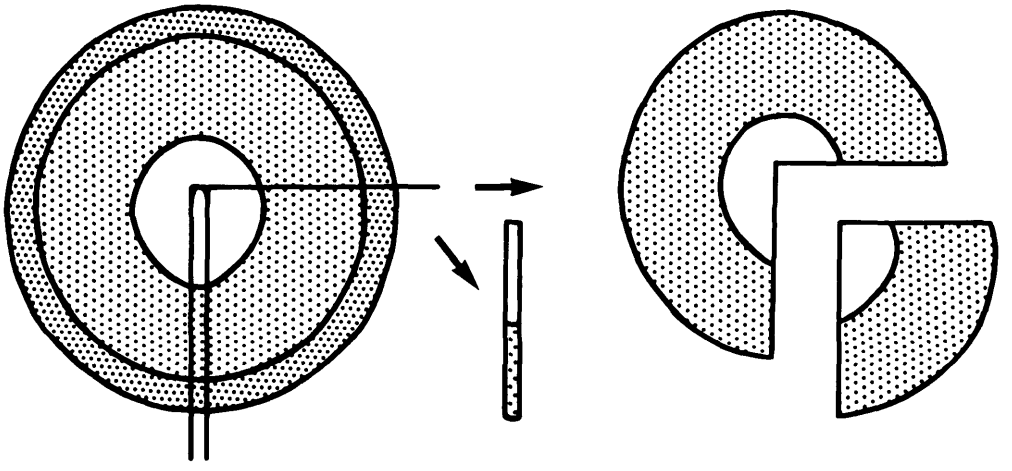


Fig. 4. *Experiment d*
Difference in amount of area pellucida. The embryo was cut into three pieces as indicated. The rim of the area opaca was removed (heavy stipple).

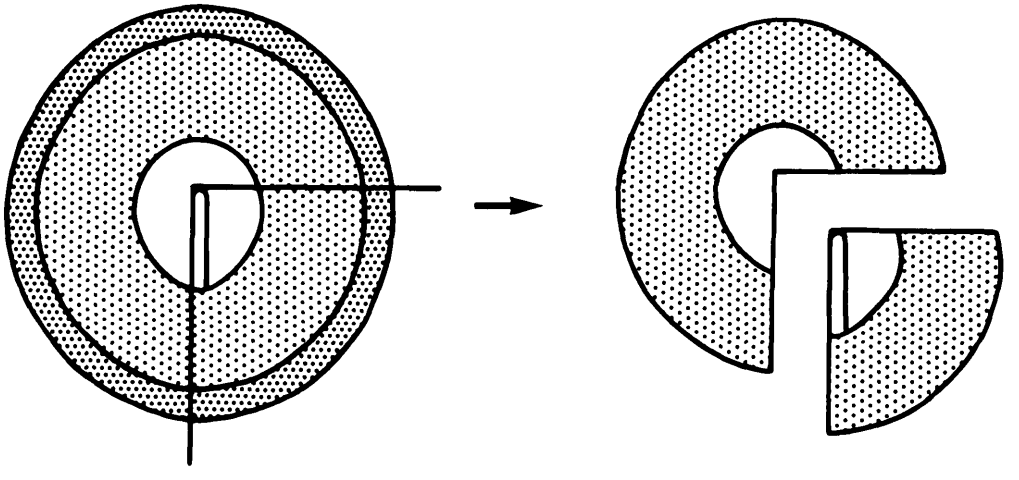


Fig. 5. *Experiment e*
This experiment differed from that in Fig. 4 in that the primitive streak was retained with the larger piece of area pellucida.

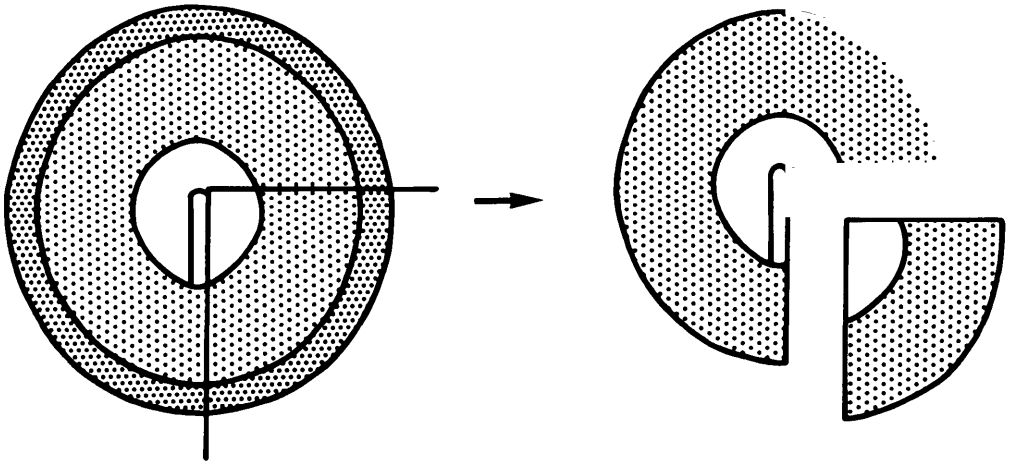


Fig. 6. *Experiment f*
This experiment differed from that in Fig. 4 in that the primitive streak was retained with the smaller piece of area pellucida.

Experiment e (Fig. 5)

This experiment differed from experiment *d* in that the primitive streak was retained with the larger piece of area pellucida.

Experiment f (Fig. 6)

This experiment differed from experiment *d* in that the primitive streak was retained with the smaller piece of area pellucida.

II *Experiments on the late gastrula* (posterior end of neurula stages).

Exeriment g

By stages 6–9 the anterior part of the embryo has started to undergo differentiation and can no longer be considered a gastrula. The posterior part however is still present as a primitive streak and this may therefore be considered to be still in the gastrula stage.

The experiment involved cutting across the area pellucida in the primitive streak region.

The control experiment however involved cutting across the embryo at the posterior border of the segmental plate.

Histology

Specimens were fixed in formal saline, stained with alcoholic cochineal (De Haan, 1967), dehydrated and cleared. They were then either made into whole mount preparations or were embedded in fibrowax, serially sectioned at 8 μm and stained with Harris' haematoxylin.

Measurements

The sizes of the somites were measured at ×40 using an eyepiece graticule in a Nikon dissecting binocular microscope. Measurements were made of the area of the dorsal surface of the most recently formed somites as seen in whole mounts, previous work (Veini & Bellairs, 1983) having already indicated that this may be used as a reliable measure of somite size. The sizes of somites were compared in various groups of embryos, using a 't' test corrected to allow for the different number of specimens in each group:

$$t = \frac{\bar{y} - \bar{z}}{\sqrt{\frac{sy^2}{ny} + \frac{SZ^2}{nz}}}$$

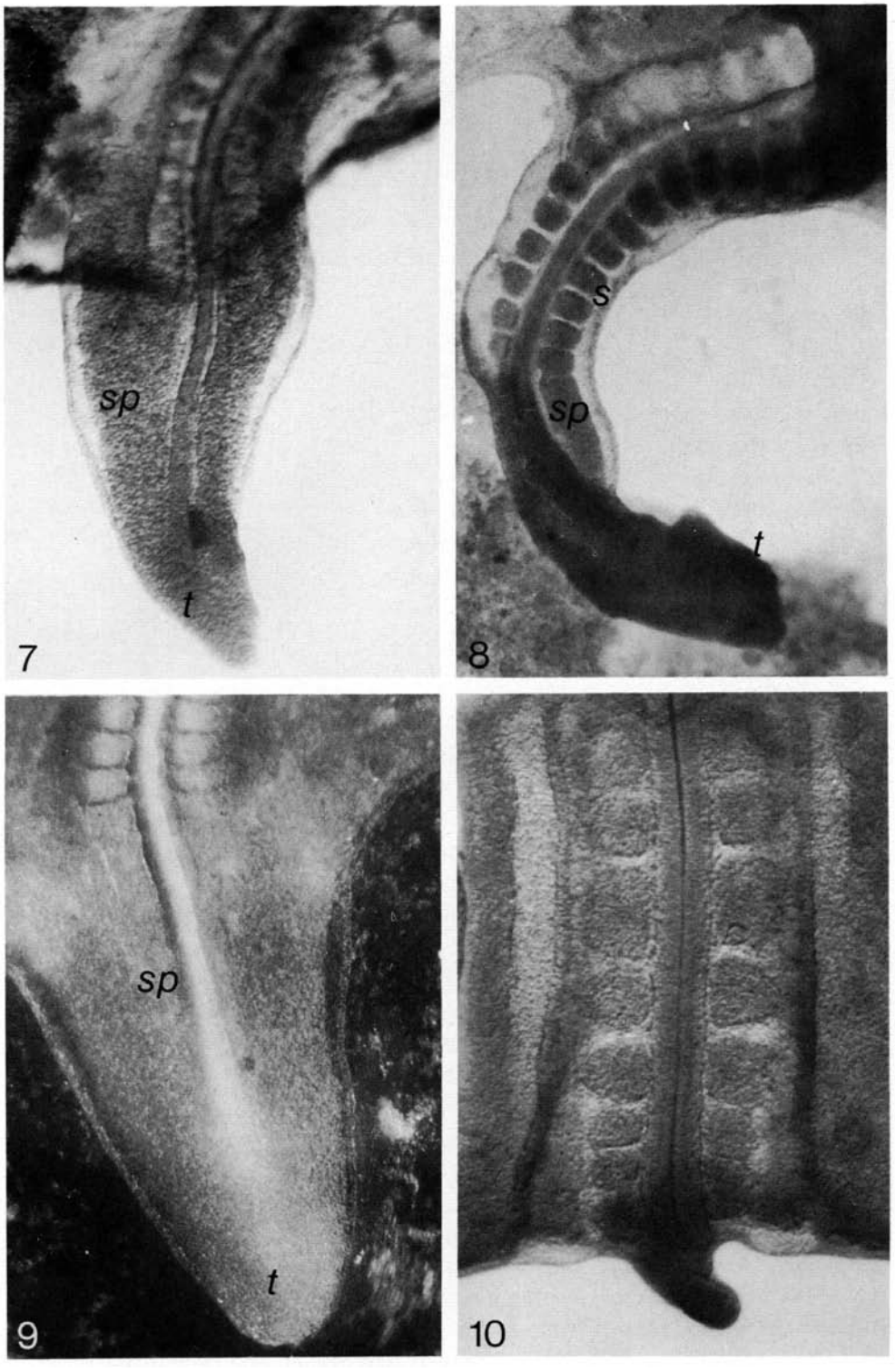
where $\bar{y}$ = the mean for one group
sy = standard deviation for mean $\bar{y}$
ny = numbers of specimens in group y
and $\bar{z}$, sz and nz are comparable values for the second group.
The significance level was taken as $t < 0{\cdot}01$.

RESULTS

Experiment a (Fig. 1)

The posterior third of the area pellucida was removed from 29 embryos; this had the additional effect of removing about $\frac{1}{4}$–$\frac{1}{3}$ of the primitive streak. In seven of these embryos the anterior edge of the area opaca healed completely to the area pellucida, whilst in the remainder it either healed incompletely or not at all. Irrespective of whether healing had occurred or not, a 'tail' formed from the cut edge of the primitive streak and extended out either beneath the ventral surface of the area opaca (healed specimens) or into the hole in the blastoderm (unhealed specimens). The tail varied in size and length, but in all cases it contained two thick bands of segmental plate, and a tail bud at its tip (Fig. 7).

Paired somites of normal size had formed in the main part of the embryo anterior to the tail, and in many cases somites had also differentiated in the anterior part of the tail (Fig. 8). The number of somites was usually between about 8 and 12 pairs. The size of the last-formed pair of somites in the tail was measured in whole mounts. In 12 specimens which were transected so that only $\frac{1}{4}$ of the posterior end of the primitive streak was removed, the mean dorsal area was 3975 sq. μm ± 933. By contrast, in eight specimens where over half of the primitive streak was removed, the somites were less than half that size, the mean dorsal area being 1563 sq. μm ± 988 which is significantly different. ($t = 5{\cdot}44$ d.f. = 18). In each of the specimens which were included in the above measurements, the more anterior somites in the tail were only slightly bigger than those at the tip. In three specimens however the size of the somites was less orderly,



Figs 7–10

large and small ones being present in each tail. In these embryos the tail was bent so that large somites were present on the outer curves and smaller ones on the inner curves, the two sides therefore not being matched.

In 16 specimens the posterior piece of area pellucida which had been removed was cultured separately. In no case did somites form, nor was segmental plate ever seen. In eight embryos (controls) the anterior one third of the area pellucida was removed. After 18 h futher incubation each embryo was found to possess anomalies of the head region, irrespective of whether or not the cut end of the area pellucida had healed to the area opaca. None showed abnormalities of the somites however, and the mean size of the last-formed somite was 4375 sq. μm $\pm$ 827. This was comparable to that in nine unoperated controls grown under the same conditions in New culture, in which the mean size of the last formed somite was 5046 sq. μm $\pm$ 1658 ($t = 1{\cdot}07$ not significant d.f. = 15).

Experiment b

This experiment which was carried out on 12 embryos, involved total removal of the primitive streak. Somites formed down both cut sides in five specimens and down one side only in four others. No somites had apparently formed at all in three specimens though it is possible they had dispersed. When somites formed they were usually small. The mean size of the last formed somite was 2916 sq. μm $\pm$ 833 ($n = 9$) which was significantly different from that of the nine unoperated controls cited above ($t = 3{\cdot}45$. d.f. = 16). In all embryos in experiment *b*, a band of tissue resembling segmental plate formed alongside the cut.

The isolated primitive streak elongated (see Fig. 11). In some specimens condensations of mesenchyme could be seen within the explants both in the whole mounts and the sections, but true somites were never seen. This was confirmed by examination of serial sections (Fig. 16).

Experiment c

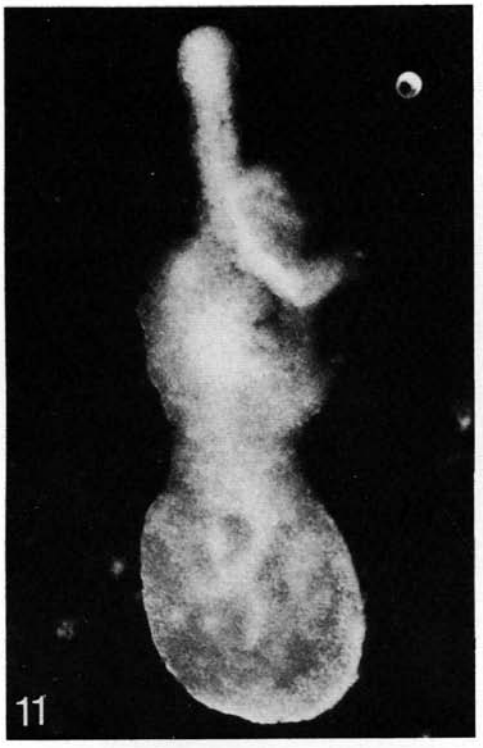
In this experiment which was performed on 20 embryos, somites frequently formed in both pieces of tissue, though fewer formed on the side which lacked a primitive streak. Indeed, no specimen developed more than six somites on the

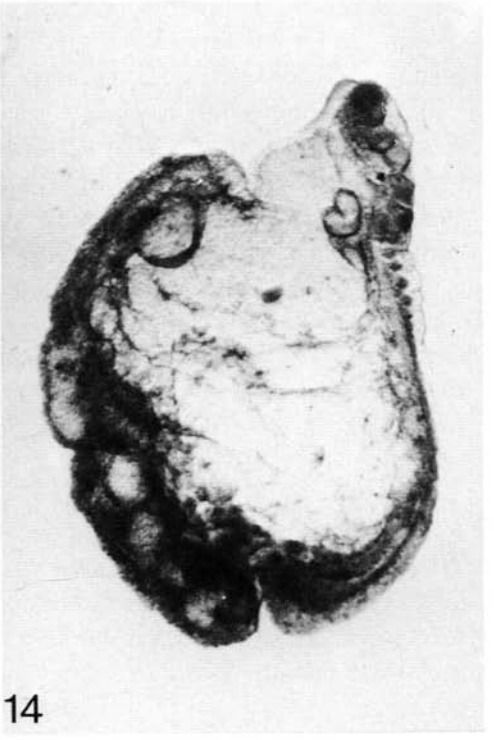
Fig. 7. 'Tail' formed after removing the posterior part of the area pellucida of a stage-4 embryo (experiment *a*). Fixed at 30 h and stained with cochineal. sp: segmental plate, t: tail bud. ×73.

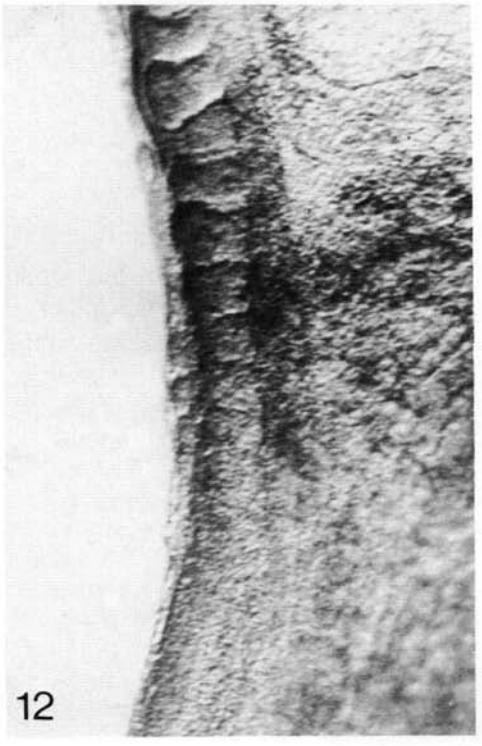
Fig. 8. 'Tail' formed after removing the posterior part of the area pellucida of a stage 4 embryo (experiment *a*). Fixed at 28 h and stained with cochineal. s: somites; sp: segmental plate; t: tail bud. ×80.

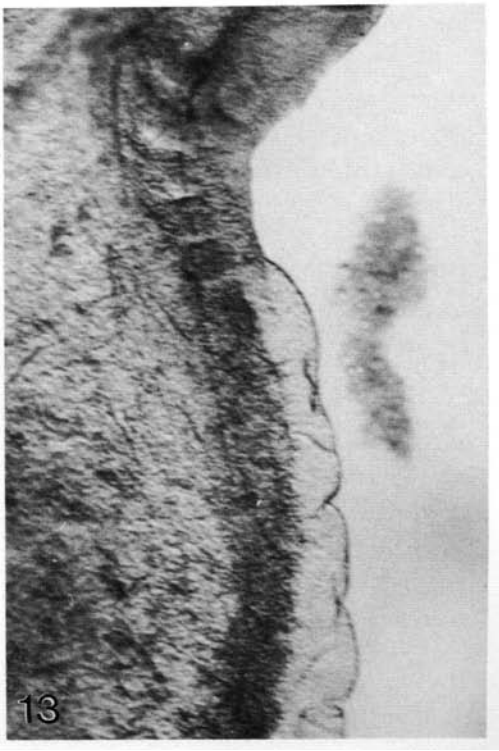
Fig. 9. 'Tail' formed after cutting across the primitive streak and removing the posterior part of the area pellucida at stage 7 (experiment *g*). Photographed at 18 h. sp: segmental plate; t: tail bud. ×65.

Fig. 10. 'Tail' formed after cutting across caudal end of segmental plate and removing posterior part of primitive streak at stage 10 (experiment *g*). Fixed at 20 h and stained with cochineal. Note that 'tail' lacks segmental plate or somites. ×122.









Figs 11–14

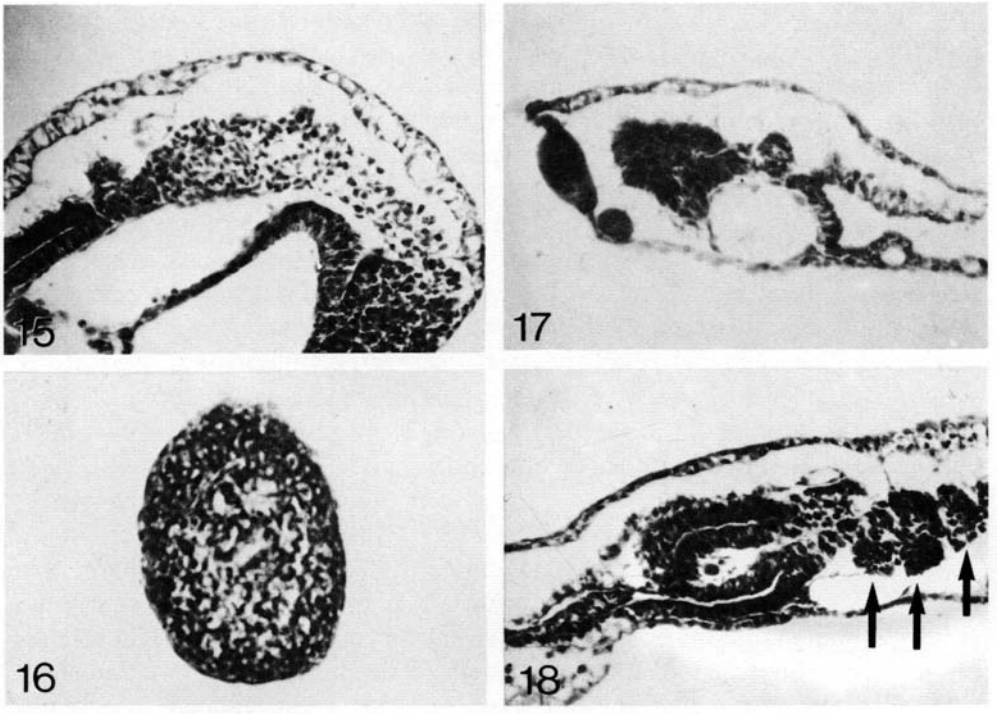


Fig. 15. T.S. through the 'immature' segmental plate of the same explant as the one illustrated in Fig. 13. ×115.

Fig. 16. Section across the explant shown in Fig. 11 ×164.

Figs 17 & 18. Sections through two explants obtained by cutting a stage-4 embryo into two pieces, as in experiment *e*. Fixed after 26 h. Fig. 17. T.S. through last formed somite of explant with primitive streak. Fig. 18. Oblique section through the three last formed somites (arrows) of explant lacking primitive streak. ×100.

side lacking a primitive streak (Fig. 14). During the same time, a row of between 6 and 12 somites appeared on the side which possessed the primitive streak. Indeed, it was possible to obtain a double row of somites for part or all of the distance on that side, and still get somites on the primitive streakless side. The somites which developed on the side lacking the primitive streak were usually, though not invariably, smaller (Figs 12, 13 and 17, 18). Thus on the streakless side the mean area of the dorsal sides of the somites was 2270 sq. μm $\pm$ 1248 (n = 15) whilst on the side with the primitive streak, the mean area was 4375 sq.

Fig. 11. Explant derived from the extirpated primitive streak of a stage-4 embryo (experiment *b*). Fixed after 26 h. ×57.

Figs 12 & 13. Two explants obtained by cutting a stage-4 embryo into two pieces. As in experiment *c*. Fixed after 16 h and stained with cochineal. Fig. 12: side which retained primitive streak. ×95. Fig. 13: side which lacked primitive streak. ×105.

Fig. 14. Explant obtained by culturing a piece of area pellucida lacking a primitive streak (experiment c). Note the four somites on the right. Fixed after 48 h ×36.

μm ± 1082 (n = 10). (N.B. for purposes of comparison we have included here only those measurements derived from specimens which had developed no more than six somites at the time of fixation. Many of the explants which possessed primitive streaks had formed larger numbers of somites and are not included). The difference between the two sides was significant (t = 4·48 d.f. = 23). In four specimens fixed after 12 h of postoperative incubation (i.e. earlier than usual) the same number of somites was present on each side at the time of fixation, and in three of these cases the somites which had developed in the absence of primitive streak were about half the size of the corresponding somites on the side which possessed the primitive streak. In embryos fixed later than about 18 h the somites on the streakless side had usually begun to disintegrate unless some neural tissue remained. In pieces of embryo possessing a primitive streak, segmental plate lay caudal to the somites. In those pieces lacking a primitive streak a similar band of mesoderm was often visible (Figs 13 and 15).

Experiment d

Somites formed in ten specimens operated in this way, and failed to form in three others. In 8 specimens more somites formed in the larger piece of area pellucida than in the smaller one, e.g. two specimens each formed eight somites on the larger side and five on the smaller. No specimen formed more than eight somites on either side however long it remained in culture. Indeed, the somites began to distintegrate after about 15–18 h postoperative culture.

The somites were small in size, the mean dorsal area of the last somite from the ten specimens being 2017 sq. μm ± 947. This is significantly less than the size of control somites (see p. 191) t = 4·8 d.f. = 17. There was no indication that somites in this experiment tended to be bigger on one side than the other. The isolated primitive streaks behaved in the same way as those in experiment *b*.

Experiments e and f

These experiments were similar to experiment *b* in that one side of the area pellucida retained the primitive streak; whilst the other was deprived of it. In experiments e and f however, the anterior part of the area pellucida either remained attached to the side which possessed the primitive streak (experiment e) or to the streakless side (experiment f). Similar results were obtained to those in experiment b, in that fewer and smaller somites developed on the streakless side. Thus on the side which possessed a streak the mean area of the dorsal side of the last-formed somite was 4211 sq. μm ± 962 (n = 11) whilst on the streakless side the mean dorsal area was 1543 sq. μm ± 870 (n = 7). This difference was significant (t = 6·09 d.f. = 16). Similarly segmental plate was visible caudal to the somites, irrespective of the presence or absence of primitive streak.

Experiment g

This experiment resembled experiment *a* in that the embryo was transected

across the primitive streak. The difference however was that experiment *g* was carried out on older embryos. Fourteen embryos were transected in this way and in each case a 'tail' was formed at the cut border of the anterior piece, whilst a small v-shaped indentation appeared in the posterior piece. The 'tail' contained somites, segmental plate and a tail bud (Fig. 9).

By contrast, in the control experiments when a cut was made across the segmental plate itself, somites formed in the anterior piece up to the level of the cut only (Fig. 10). A 'tail' formed but this consisted principally of neural tissue and lacked either somites or segmental plate.

DISCUSSION

1. Miniaturized somites

In a previous paper (Veini & Bellairs, 1983) we showed that if the amount of material was reduced in avian blastulae (pre-primitive streak stage) the embryos which developed were smaller than normal but nevertheless possessed somites of normal size. In the present paper however we have shown that if the amount of material is reduced in avian gastrulae (primitive streak stages), then miniaturized somites often develop. In our opinion, the basic difference between the two stages is that none of the tissues are yet determined in the blastula stages so that total regulation is still possible, whereas many tissues are at least partly programmed by the time that the primitive streak has formed so that the possibilities for regulation are reduced.

2. Source of material for somites

In the pre-primitive streak stages, the future embryonic tissues are distributed in a crescent at the posterior border of the area pellucida (Malan, 1953; Spratt & Haas, 1961, Vakaet, 1970). If this area (the marginal zone) is removed, then the embryonic axis fails to form (Azar & Eyal-Giladi, 1979). But if this area is merely disturbed during the formation of the primitive streak, total regulation can occur. As the primitive streak forms this material moves anteriorly until by stage 4 (Hamburger & Hamilton, 1951) it comes to lie along and to either side of the primitive streak. The presumptive somite area is then considered to be located on either side of Hensen's node and extending posteriorly to it for about $\frac{1}{4}$–$\frac{1}{3}$ of the distance along the primitive streak (Rosenquist, 1966; Nicolet, 1971). This material gives rise not only to the somites in the anterior end of the body, but also to those in the posterior end. At stage 4 the presumptive lateral plate mesoderm occupies the posterior part of the area pellucida though it later shifts from this region. The evidence that presumptive somite cells move posteriorly is derived primarily from marking experiments (Pasteels, 1937; Rosenquist, 1966), but it is also indicated by the results of experiment *a* in this paper. When the embryo was cut across the primitive streak posterior to the presumptive

somite area, somites never formed in the posterior piece; similar results have been reported many times in the literature (Waddington, 1932; Butros, 1962; Bellairs, 1963). However, segmental plate and somites almost always formed in the 'tail' which extended out from the cut end of the anterior piece of area pellucida. It appears therefore that the mesoderm in this 'tail' corresponds with the material which is normally shifted posteriorly in the intact embryo.

This displacement of presumptive somite cells towards the posterior end, corresponds in time with the regression of Hensen's node and with the general stretching and anteroposterior extension of the embryo which takes place at this stage. As the node regresses, new material is added to the segmental plate at its posterior end, whilst new somites are periodically segmented off the anterior end of the segmental plate, (see Bellairs, 1979, 1980).

We shall suggest below that although other types of mesoderm migrate from the primitive streak as it forms, many of the future somite cells are still present in the streak at later stages, and that these leave only as the node passes. We should add here that the physical presence of the node is not essential for this process (Bellairs, 1963). We merely emphasise here the correlation in time (see discussion by Stern & Bellairs, 1984).

Migration of cells from the primitive streak

We may now ask, 'what is the relationship between those cells which already lie in the presumptive somite area at stage 4, and those which we suggest migrate out later from the primitive streak?'

One possibility is that the presumptive somite cells become programmed as they leave the primitive streak. If this is so, then the presumptive somite area at stage 4 should contain material already programmed for some, if not all, somites since it has already left the primitive streak. Evidence from experiments, *b, c, d, e* and *f*, supports this idea, since somites frequently formed from pieces of tissue which had been removed from all contact with the primitive streak and Hensen's node. (The fact that these somites often did not survive after about 18 h was probably due to mechanical problems; Lipton & Jacobson, 1974*b*, have already demonstrated that good axial structures are usually necessary to maintain somites). The fact that these somites were frequently significantly smaller than normal suggests that groups of committed cells had already become associated together to form a somite at the time of the operation, but that the cell population of each group was inadequate to form a full-sized somite. We suggest that these small groups of cells had become cut off from re-inforcements of cells which would normally be arriving from the primitive streak. (The role played by localized bursts of mitosis in somite formation will be discussed by Stern and Bellairs, in preparation).

Programming of anterior somites

The idea that some of the future mesoderm cells are already programmed as

early as stage 4, is not a new one (see Lipton & Jacobson, 1974*b*). More recently, Meier & Jacobson (1982) and Triplett & Meier (1982) have claimed that 'somitomeres' are present on either side of Hensen's node at this stage. 'Somitomeres' are aggregations of radially arranged cells which will later condense into somites (Packard & Meier, 1983) and which have been clearly illustrated in stereoscanning electron microscopy of the segmental plates at later stages (e.g. Meier, 1979, Fig. 6). The SEM evidence that somitomeres are already present even at stage 4 is however suggestive rather than totally convincing.

In a previous paper, Veini & Bellairs (1983) showed that total regulation was possible prior to primitive streak formation. We conclude therefore that the most anterior somites become determined during primitive streak formation.

Programming of posterior somites

We may now enquire, 'when do the posterior somites become determined, and what influences, if any, are necessary?'

In the normal embryo, the somite material appears to be determined to form somites once it lies in the segmental plate. Evidence comes from experiments where segmental plates have been isolated from other axial structures, each giving rise to about 11–12 somites (Packard & Jacobson, 1976). Some morphological regulation is still possible, since if the segmental plate is cut longitudinally into two pieces both the medial and the lateral part can each give rise to a row of somites (Sandor, 1971; Lanot, 1971), but it is significant that each of these somites is miniaturized and has apparently been unable to restore its bulk by the addition of further cells to the segmental plate. It seems likely therefore that new cells enter the segmental plate only at its posterior end, and nowhere else along its length. This conclusion receives support from those embryos in experient *g*. If they were transected across the segmental plate, the 'tails' which developed consisted only of neural tissue and notochord. If they were transected across the primitive streak however, the 'tails' contained mesoderm as well.

We suggest therefore that the programming of the somite cells coincides with their migration out of the primitive streak. Certainly, segmental plate which becomes laid down in the absence of the primitive streak does not form somites (we will call this *immature* segmental plate). The presence of either the node and/or the primitive streak therefore seems to be necessary. If they are present, then not only are the cells programmed to form somites *per se*, but apparently to form specific somites. (See grafting experiments of Kieny, Mauger & Sengel, 1972).

The role of Hensen's node in somite formation has often been discussed. Many authors have suggested that it acts as an inductor of somites, but there is extensive evidence that if it is carefully removed, somites can still form (see Bellairs, 1963, 1980). It therefore seems unlikely that it is an inductor. Lipton & Jacobson (1974*a* and *b*) stated that the function of the regressing node is to cleave mechanically through the presumptive somite material, cutting it into right and left sides

and provoking segmentation. According to these authors, cutting down the primitive streak has the same effect, but since the cutting takes place simultaneously along the axis, it results in simultaneous segmentation of several pairs of somites. Stern & Bellairs (1984) who have repeated the experiment have however never found somites to segment other than sequentially. It may be noted too that somites apparently segmented sequentially in all the experiments reported in this paper. The idea therefore that cleaving of the tissues provokes segmentation does not seem likely to us.

We suggest that it is not the presence of the node itself (whose destiny is mainly notochord, some neural tissue and some endoderm), which is important if cells in the segmental plate are to form somites. We propose instead that it is the primitive streak that is important, and that cells gradually leave the primitive streak at levels which are further and further posterior and that they then interact with tissue drawn backwards from the original presumptive somite area. This tissue has already become formed into an immature segmental plate, but is perhaps unable to segment until it receives other cells from the primitive streak. Similarly, the isolated primitive streak is unable to form somites alone. Both the immature segmental plate and the primitive streak cells seem to be required.

A new theory

We suggest that:

1) A population of somite precursor cells is generated as the primitive streak forms, and that these become located in the presumptive somite regions by stage 4. As cells continue to leave the primitive streak they interact with the precursor cells to form somites. Thus, some of the somites which formed in experiments *b* and *c*, may have already received their full quota of cells and therefore became normal sized, whilst in others the full number was not present at the time of operation, so that smaller than normal somites developed.

During regression, the precursor cells become arranged along the antero-posterior axis, and more and more cells leave the primitive streak to join with them to form mature segmental plate. Each small group of precursor cells acts as a focus for these incoming cells, so that gradually somitomeres form. Eventually, each somitomere segments off completely to form a somite.

The remaining precursor cells become drawn further and further posteriorly, the last ones coming to lie in the tail bud. By this stage, less and less material is available in the streak or tail bud so that the newly formed tail somites are relatively small. Nevertheless, Schoenwolf (1977; 1978) has shown that even at stage 17, material is still passing from the tail bud to the somites.

This new theory should be distinguished from that of Spratt (1955). He suggested that morphologically invisible 'somite centers' were present on either side of the node region in the anterior part of the presumptive somite area. He considered that these 'somite centers' did not contain permanent cells but that they

moved along inducing any mesoderm with which they came into contact to form somites. Bellairs (1963) however showed that if the 'somite centers' were extirpated, somites could still form from the posterior part of the presumptive somite area.

The emphasis of the present theory is, on the contrary, on the fact that tiny clusters of already programmed cells are present throughout the presumptive area at stage 4 but that in order to fulfill their destiny they probably depend on the addition of further cells from the primitive streak. This aspect of the theory has some similarities with ideas proposed by Gearhart & Mintz (1972) as well as by Tam (1981) for mouse embryos.

In future papers we will describe experiments which will test this new hypothesis.

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