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Abnormalities in somite segmentation following heat shock to *Xenopus* embryos

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SUMMARY

The typical abnormality induced by a 15 min shock at 37 °C is a single discrete length along the somite file within which segmental boundaries are absent or irregular. The two sides of the same embryo present a similar but not necessarily identical appearance. Usually all the embryos in a treated batch show abnormalities of similar severity. Survival of treated embryos, the details of the visible malformations, and temporal aspects of the phenomenon have been studied.

The results indicate a temperature sensitive period that traverses the neurula, from head to tail at about the same rate as the somites form, but some hours beforehand. The temperature sensitive process is not associated with cell determination and differentiation, and there are reasons for thinking that the specification of the normal somite number occurs independently.

The results are discussed in relation to Cooke & Zeeman's model of a wave front interacting with an oscillator.

INTRODUCTION

Somitogenesis is attracting the interest of experimentalists and theoreticians alike. One good reason for this is the fact that the species typical number of somites is developed under a variety of abnormal conditions. Thus, in the case of artificially small embryos, obtained after extruding material from the early blastula, Cooke (1975) has shown that the somites are smaller than normal but the usual number are developed. Again, haploid embryos develop the usual number of somites, although Hamilton (1969) has shown that the number of cells per somite at the time of their segmentation is greater than normal. These observations point to a mechanism of somite specification with interesting regulative properties, and this mechanism is not understood.

The phenomenon we document is of interest in relation to the periodic nature of somite formation, and the processes leading up to segmentation.

The phenomenon does not appear to have been described before, although

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there are sporadic references to mesodermal abnormalities following temperature shocks. Hoadley (1938) noted abnormalities somewhat similar to those we describe, in the somites of *Rana pipiens* embryos part-reared at supra-maximal temperatures.

MATERIALS AND METHODS

Xenopus embryos were obtained from the Genetics Department, Edinburgh University, from spawnings induced by chorionic gonadotrophin injection. Embryos were reared in tapwater dechlorinated by aeration, and were sorted into batches of the same stage (Nieuwkoop & Faber, 1956) using the dissecting microscope. To deliver a heat shock, a batch of around 50 embryos, still in their jelly coats, were pipetted into bottles containing 150 ml of water, that had been standing in a 37 °C water-bath for at least 1 h. At the end of a measured period the embryos were pipetted back into an excess of water at room temperature. Embryos were fixed in Smith's fixative immediately before shock and after stage 32, for paraffin sectioning and staining in haematoxylin. All the data on the axial position and extent of segmental abnormalities was obtained in the following way: larvae at stages 22 and 36 were placed in Smith's fixative, after 5 min, but before the tissue had become brittle after about 15 min, the epidermis could be easily stripped off both sides of an embryo to reveal the length of the somite file. Stripped embryos were examined under the dissecting microscope using oblique illumination to show the segmental boundaries in relief. The first somite seen in stage-22 embryos was taken to be the first somite developed. The first somite posterior to the otic vesicle on stage-36 larvae was taken to be the fifth somite developed, the first four somites to be developed having been obliterated by this stage (Nieuwkoop & Faber, 1956).

Description of somitogenesis in Xenopus

The chorda mesoderm of the late gastrula is a continuous sheet a few cells thick. The notochord forms at stage 13 immediately following the closure of the blastopore. Some hours later at stage 17 (late neural folds) the first somite is formed. The first few somites segment rapidly, thereafter somites are formed at a constant rate down the trunk. The first four somites are obliterated by stage 25 as the result of the growth of the otic capsule. Cooke (1975) refers to those as 'head somites', but they are not homologous with the head somites of classical morphology.

The details of somite formation vary within the Amphibia and even among Anurans. The mode of formation in *Xenopus* has been described by Hamilton (1969). Pre-somite cells elongate and align perpendicular to the notochord. Following 2 or 3 h after this preparation, the visible formation of a somite involves the concerted turning through 90° of a group of those cells. Each somite, therefore, is a bundle of elongated cells now lying parallel to the noto-

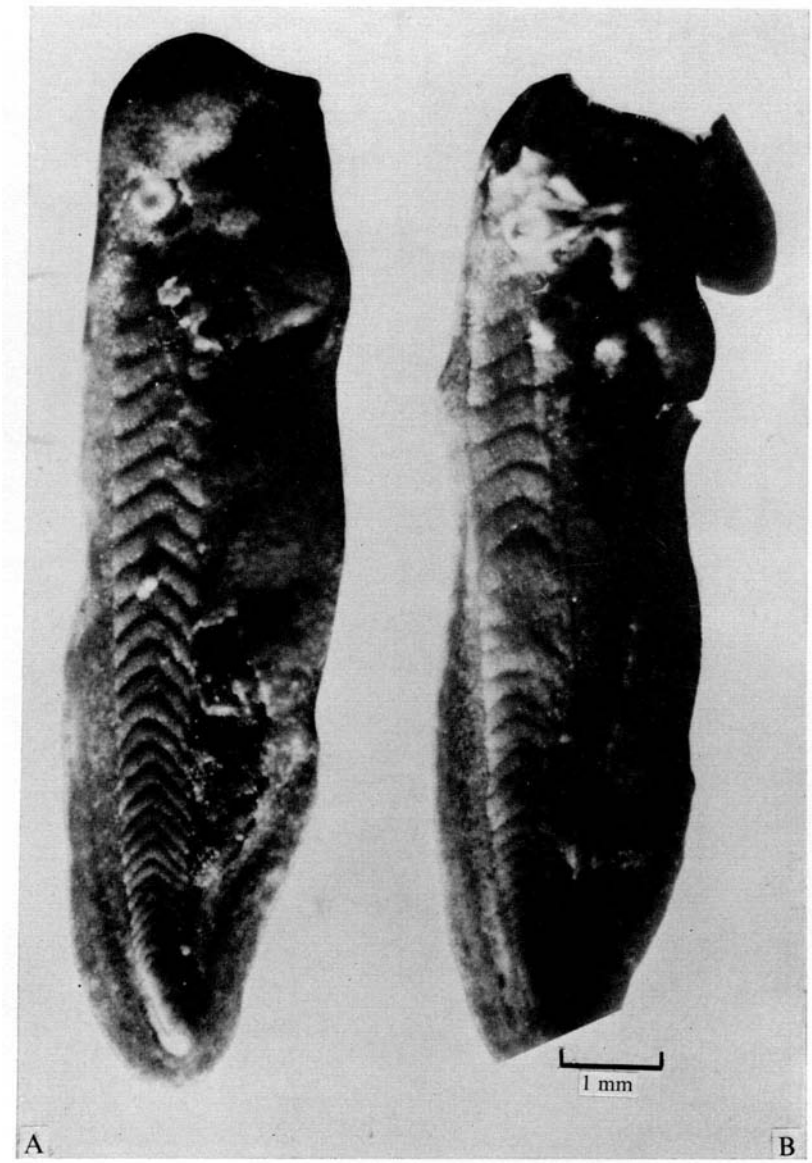

Fig. 1. Stripped embryos, stage 36. (A) Control. (B) Embryo given a 15 min shock at stage 19. Somites 1–8 (as they appear on specimen, see Materials and Methods) normal, somites 9–*c*15 abnormal, return to normal posteriorly.

chord, each cell stretching the length of the bundle. Trunk somites are about 10 cells in width. The dermatome appears to remain unsegmented beneath the epidermis: the sclerotome is not prominent at this stage. The cells differentiate into myofibrillar elements and remain mononucleate.

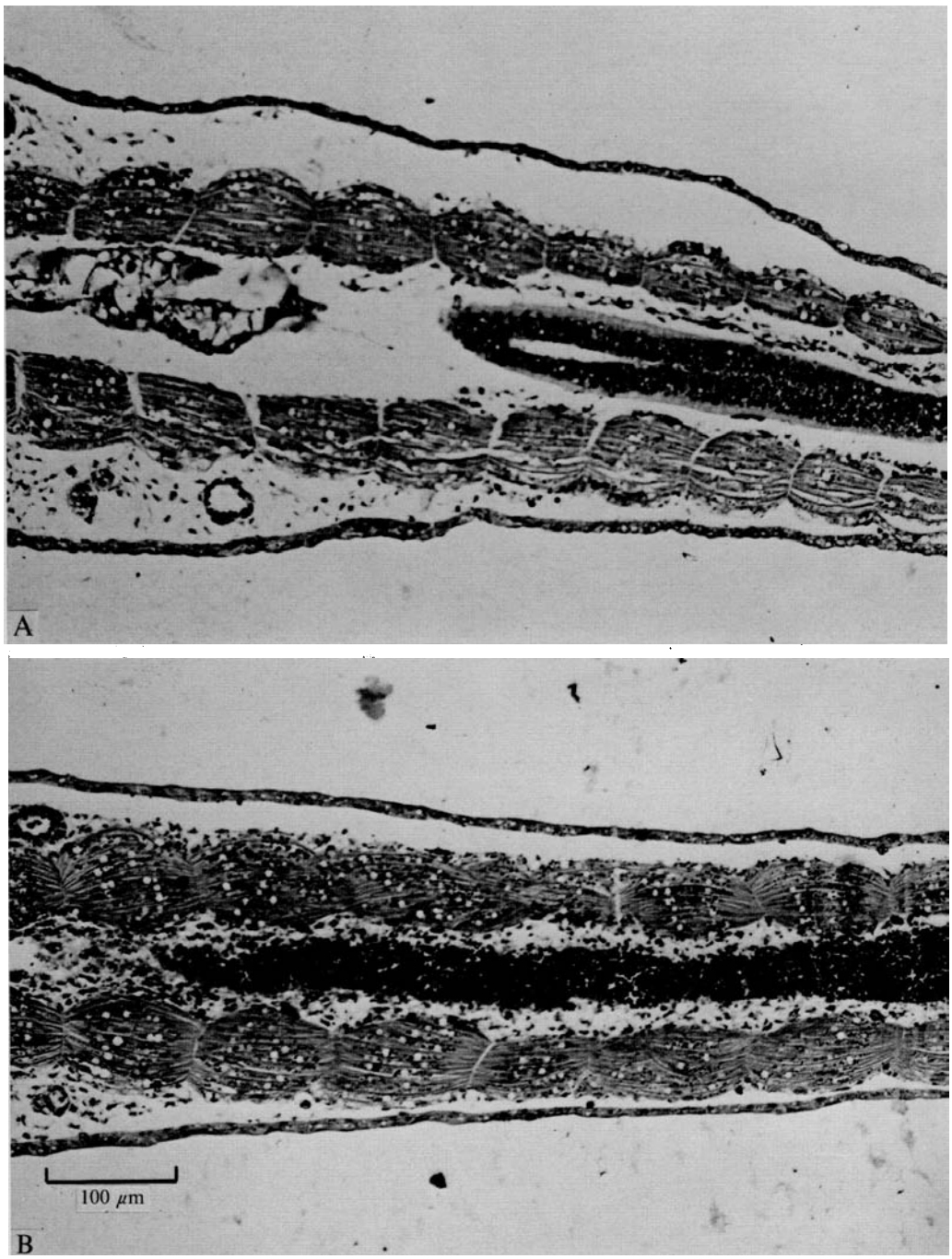

Fig. 2. Longitudinal horizontal sections stage 36. Part of trunk. (A) Control. (B) Embryo given a 15 min shock at stage 19. Irregular arrangement of myoblasts, absent or irregular intersomitic boundaries.

RESULTS

Visible malformations induced by heat shock

Examination of stripped, stage-35 larvae following heat shock usually reveals a single, limited abnormal zone along each somite file (Fig. 1). Anteriorly one or several intersomitic fissures may be absent altogether, followed posteriorly by a confused length characterized by rudimentary and branching fissures, and a gradual return to normal somite boundaries. Only among very severely stunted embryos is it not possible to see a return to normal segmentation posterior to an irregular region. Rarely, there may be two abnormal zones along the same somite file separated by a region of normal segmentation.

A more intimate comparison between a control and a treated animal is provided by the pair of horizontal longitudinal sections in Fig. 2. The orderly sequence of intersomitic boundaries is apparent in the control (Fig. 2A). Myoblasts with normally striated fibres visible under higher magnification are also present in the experimental animal (Fig. 2B), but the organization of the somite cells is grossly abnormal over part of the picture. One sees a number of myoblasts converging as at an intersomitic boundary while their neighbours pass to other points of attachment. In this example the orientation of the cells is still predominantly anterior–posterior; in severely affected cases myoblasts may lie at various angles to the long axis without obvious terminal attachments.

Survival of embryos given a heat shock

We found a wide variation in tolerance between different ovulations. Early blastula stages are especially susceptible, even a 10 min shock is lethal. In contrast after the beginning of gastrulation, the great majority of embryos survive a 15 min shock. The tolerance of neurulae is only a little greater than gastrulae. Mortality increases with shocks of longer than 15 min duration.

The first abnormal somite

Because a shock usually results in a single disturbed region, and the disturbance is greatest anteriorly, the first abnormal somite can be used to indicate the position of the disturbance. On this basis comparisons can be made between the results of applying a shock to different stages, and also the result of applying shocks of different duration to the same stage, and to different ovulations. Representative data relating to the result of shocks to different stages is displayed in the histograms of Fig. 3, and in Tables 2 and 3.

Because of their lower toleration stage-9/10 embryos received only a 5 min shock instead of the standard 15 min shock given to older embryos. This difference in treatment is responsible for the fact that 70 % of stage-9/10 embryos developed normally after shock, whereas fewer than 10 % of older embryos treated for a longer duration developed normally.

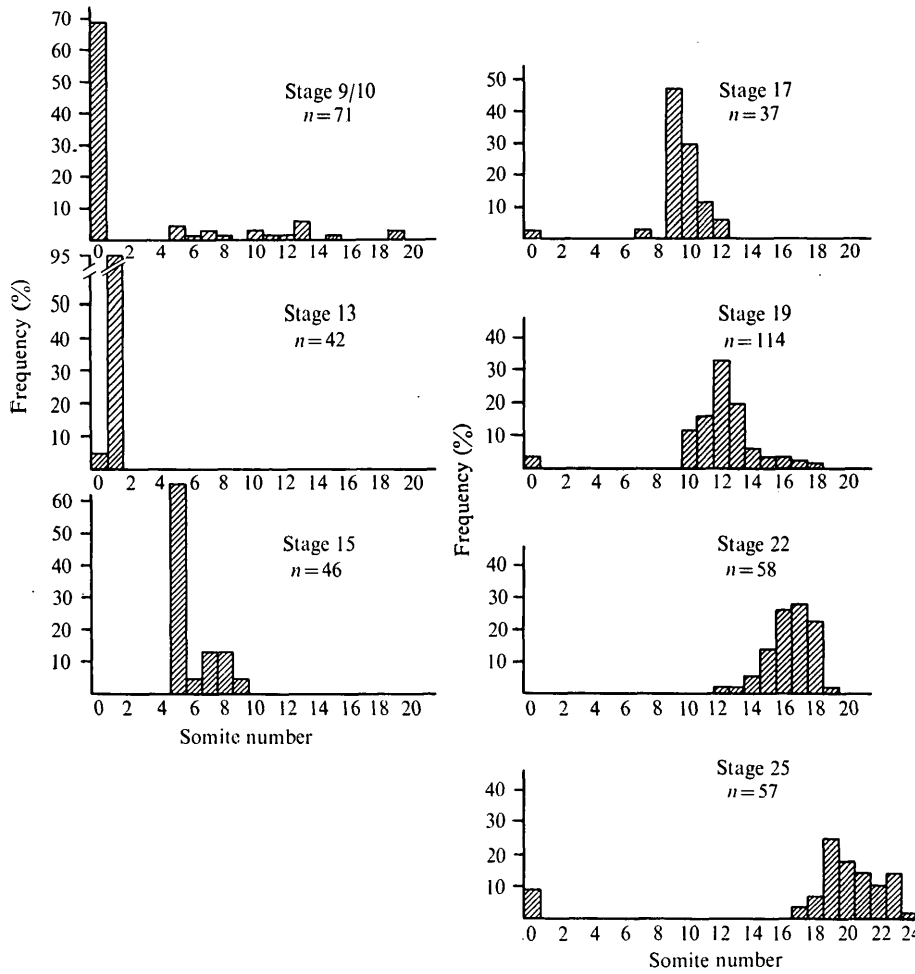

Fig. 3. Distribution of first abnormal somite among *Xenopus* embryos given heat shock. *n* refers to number of somite files scored. Stage refers to stage at shock. Abcissa scale refers to somites in chronological order of development, the '0' column is used to indicate normal files. Embryos shocked at stage 13 were stripped and scored at stage 22 prior to obliteration of anterior somites, the remainder at stage 36 after obliteration.

Reference to the histogram shows that the abnormal region may commence anywhere along the trunk file following shock to stage-9/10 embryos. This result is distinctly different from that obtained following shocks to older embryos. Most striking is the comparison of the fore-mentioned result with that obtained following shock to stage-13 embryos where the abnormal region invariably commences with the first somite developed. It appears that some change concomitant with the processes of gastrulation is responsible for a striking switch from a less to a more predictable result following heat shock.

The histogram shows that from stage 13 onwards the position of the first

Table 1. *First abnormal somite following heat shock to* Xenopus *embryos at different stages*

Details of embryos at time of shock			Data from scoring stripping embryos		Assessment of lag	
Stage	Age (h)	No. of somites developed	Mean first abnormal somite	S.D. ±	Somite difference	Lag (h) = $x-y$
13	14¾	0	1·0	0·0	—	4
15	17½	0	5·9	1·0	—	3¾
17	18¾	1	9·6	1·0	8·6	5
18	19¾	3–4	—	—	—	—
19	20¾	4–6	12·4	1·8	7·4	4
20	21¾	6–7	—	—	—	—
21	22½	8–9	—	—	—	—
22	24	9–10	16·4	1·4	7·0	4
23	24¾	12	—	—	—	—
24	26	15	—	—	—	—
25	27½	16	20·0	2·3	4·0	4½
27	31	19				
28	32½	20–22				

Age in hours (column 2) and number of somites developed (column 3) corrected to development at 22–23 °C according to Nieuwkoop & Faber (1956). Somite difference = mean first abnormal somite (column 4) minus number of somites developed at shock (column 3). x = developmental age in hours according to Nieuwkoop & Faber of a normal embryo at the time it had developed the same number of somites as appear under mean first abnormal somite (column 4). y = age in hours at time of shock (column 2).

abnormal somite advances down the trunk in a series of embryos that underwent shock at progressively later stages. In the case of embryos shocked later than stage-17, this result is to be expected, for somite segmentation has commenced before the shock is given.

Table 1 shows that the first few somites formed immediately after a shock are normal, and that a lag occurs between the giving of a shock and the registration of its effect in segmentation abnormalities. Thus a shock to stage-17 embryos, forming the first somite, results in abnormalities commencing around the ninth somite, and a shock to stage-24 embryos with about ten somites formed results in abnormalities commencing around the sixteenth somite. The length of this lag has been assessed in hours according to a procedure explained in the legend to Table 1. The lag is about 4½ h for development at 22–23 °C. The lag, furthermore, is the same in the case of a shock to stage-13 embryos, prior to somite formation, and stage-25 embryos with 16 somites already formed. The data suggests therefore that the lag is constant and independent of the stage of treatment from stage 13 onwards. The lag can also be assessed in terms of the difference between the ordinal number of the first affected somite and the number of somites already formed at the time of the shock. Table 1,

Table 2. *Position of first abnormal somite: influence of duration of shock, and variation between ovulations*

Shock duration (min)	Ovulation date	Somite first affected (mean), $\bar{X}$	Standard deviation, s	No. of embryos, n
		(*a*) Same ovulation		
10	7/75	12·6	1·69	19
15	7/75	12·6	2·33	20
20	7/75	12·3	1·45	21
		(*b*) Different ovulation		
15	9/74	12·95	2·30	37
15	10/74	11·58	0·78	24
15	2/75	13·06	1·92	17
15	5/75	12·37	1·34	21

Stage-19 embryos (*a*) from the same ovulation batch subjected to 37 °C for different periods; and (*b*) from different ovulation batches subjected to the same 15 min shock at 37 °C.

column 6, shows that the somite difference is around eight in the case of shock to stage-17 embryos reducing to around four in the case of a shock to stage-25 embryos. In the light of a constant temporal lag, column 6 suggests a slowing down of somite formation from stage 22. Pearson's (unpublished) observations show no such thing, and the apparent reduction in somite difference probably reflects sampling errors in the Nieuwkoop & Faber Normal Table.

It was of interest to determine the extent of any variation in the position of the first affected somite on the two sides of the same embryo subjected to shock. Embryos between stages 17 and 20 from the same ovulation were subjected to a 10 min shock, and later stripped. About two thirds of these embryos were selected as favourable for scoring. Of the 20 embryos scored, 18 showed the first affected somites to have the same ordinal number on the two sides, two embryos showed a difference of one somite between the two sides.

In order to confirm that the stage of treatment was the overriding variable determining the position of the first affected somite, stage-19 embryos from the same ovulation were subjected to shocks of different durations. In addition data were collected on the first affected somite observed on embryos from four different ovulations subjected to a standard 15 min shock at stage 19. The data are presented in Table 2. The results indicate that neither of the two variables investigated here significantly influences the determination of the first affected somite.

Table 3. *Number of abnormal somites: duration of shock, and variation between ovulations*

Time at 37 °C (min)	Ovulation	Mean no. of somites affected $\bar{X}$	S.D.	No. of embryos
		(*a*) Same ovulation		
10		2·31	0·48	17
15	3/7/75	3·40	1·90	20
20		5·14	2·35	21
		(*b*) Different ovulation		
—	9/74	6·73	2·38	37
—	10/74	6·42	1·67	24
—	2/75	3·47	0·80	17
—	5/75	3·29	1·01	21

(*a*) Groups of embryos taken from the same ovulation and developing synchronously were subjected to 37 °C shocks for different periods. The mean number of affected somites varies with the length of the shock. (*b*) Embryos from different ovulation show great variability in the extent of the abnormality following a standard 15 min shock. All embryos were stage 19.

The number of abnormal somites

Table 3 shows that the two variables, which were found to have little effect on the determination of the first affected somite, are both important determinants of the number of abnormal somites following heat shock, measuring the extent of the disturbance along the somite file. Within an ovulation [Part (*a*)], the extent of the disturbance is roughly proportional to the duration of shock. Part (*b*) of Table 3 shows that different ovulations vary significantly in their response to a standard treatment. A two-fold difference in the mean number of abnormal somites, with overlapping ranges, has been observed between ovulations.

DISCUSSION

Abnormalities in the arrangement of myofibrillar elements in the somites result from the delivery of a temperature shock at any stage from the blastula to the post-neurula. The overall effects of shocks to different stages are not, however, the same. Shocks delivered prior to around stage 12 have, in the main, unpredictable effects in so far as the resulting irregularities may occur anywhere along the somite file. After stage 12 the effects are more predictable and irregularities are restricted to those somites first in the file, or, when these are already segmented at the time of treatment, to those segmenting a few hours after the shock. Most of our remarks concern the treatment of neurula stages when the predictability of the effect is greatest.

There are both qualitative and quantitative aspects to the phenomenon. Qualitatively, the first affected somite depends on the stage shocked and is independent of the duration of the shock. Quantitatively, the number of affected somites depends crucially on the duration of the shock, and stage differences (after stage 12) are not significant. There is a spatial asymmetry in connexion with the disturbance; the anterior somites in a disturbed region are the most irregular, posteriorly there is a gradual return to normal. Usually there is a single disturbed region along the file, occasionally two separate disturbed regions are observed.

The segmentation of the first somites following a shock is normal, and there is invariably a lag of several hours (or about five somites) before irregularities occur. We conclude that the processes immediately responsible for segmentation are not themselves susceptible to heat shock, they register a prior invisible disturbance.

Heat shock does not impair the ability of somitic cells to differentiate into striated myofibrillar elements. There is no evidence that extra-somitic cells are caused to participate in somite formation. We conclude that the processes of determination and differentiation are not affected by a heat shock delivered later than stage 12.

The region posterior to an irregular region along the file segments normally. There is no 'jump' in the specification of somites to larger and fewer than normal or smaller and more numerous. We conclude that shocks delivered after stage 12 do not upset the specification of the normal somite number.

It appears then that independent of cell differentiation and overall pattern specification, heat shocks after stage 12 have a restricted effect on the processes leading up to segmentation.

An interesting recent addition to ideas on somite specification and formation is provided by Cooke & Zeeman (1975). They consider a model with two interacting components. First, a wavefront of competence for some rapid cellular change – in locomotor or adhesive properties for example, that traverses the paraxial mesoderm from head to tail in a fixed time. This provides for the maturation of the cells for somite formation in a fairly smooth progression down the axis, starting from the head end. The second component is an oscillator present in all pre-somitic cells, and it is assumed that the individual oscillators become entrained in some phase-coordinated manner. It is convenient in picturing the model to think of a temperature-dependent oscillation with a period of around 1 h commensurate with the time interval between the formation of one somite and the next. The interaction between the wavefront and oscillator is conceived as follows: the cells that have matured to the point of readiness to undertake segmentation can only in fact embark on these changes when they are in a particular stage of their oscillatory cycle. As the oscillators are phase-entrained, neighbouring cells will enter this stage at about the same time. The situation will be like a continuous file of people approaching a gate that opens

to admit them for only a short period every hour. People will arrive at the gate in ones and twos, and issue from it in bunches. This then is the way Cooke & Zeeman seek to account for the periodic formation of discontinuous somites.

The reasons for thinking that shocks delivered after stage 12 create a disturbance independent of differentiation and pattern specification have already been discussed. Cooke & Zeeman's model is apposite because it suggests how such a restricted disturbance might arise. The cellular oscillator, on their model, functions solely as a modulator. The idea that shocks to later stages disturb the oscillator alone, suggests expectations about the limited effects of the treatment, that seem consonant with the experimental observations. The anterior–posterior asymmetry in the observed segmental anomalies might reflect the gradual restoration of the original oscillations following a disturbance. The lag between the delivery of a shock and its visible registration suggests that the interaction between wavefront and oscillator takes place some hours before segmentation, and this is consistent with the model as Cooke & Zeeman point out. The increase in the predictability of the effects with treatment of later stages might reflect the gradual phase-entrainment of the individual oscillators. Whether heat shock engenders a change in amplitude of the oscillations, or results in local incoherence with or without a change in frequency are matters suggesting further experimentation.

Whatever the heuristic value of these speculations, we do not yet conclude that the observations here presented have confirmed Cooke & Zeeman's model to the exclusion of others. Different theories of somitogenesis are discussed in Cooke & Zeeman's paper.

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