

The formation of mesodermal tissues in the mouse embryo during gastrulation and early organogenesis

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Summary

Orthotopic grafts of [³H]thymidine-labelled cells have been used to demonstrate differences in the normal fate of tissue located adjacent to and in different regions of the primitive streak of 8th day mouse embryos developing *in vitro*. The posterior streak produces predominantly extraembryonic mesoderm, while the middle portion gives rise to lateral mesoderm and the anterior region generates mostly paraxial mesoderm, gut and notochord. Embryonic ectoderm adjacent to the anterior part of the streak contributes mainly to paraxial mesoderm and neur ectoderm. This pattern of colonization is similar to the fate map constructed in primitive-streak-stage chick embryos. Similar grafts between early-somite-stage (9th day) embryos have established that the older primitive

streak continues to generate embryonic mesoderm and endoderm, but ceases to make a substantial contribution to extraembryonic mesoderm.

Orthotopic grafts and specific labelling of ectodermal cells with wheat germ agglutinin conjugated to colloidal gold (WGA–Au) have been used to analyse the recruitment of cells into the paraxial mesoderm of 8th and 9th day embryos. The continuous addition of primitive-streak-derived cells to the paraxial mesoderm is confirmed and the distribution of labelled cells along the craniocaudal sequence of somites is consistent with some cell mixing occurring within the presomitic mesoderm.

Key words: gastrulation, primitive streak, paraxial mesoderm, somitogenesis, mouse embryo.

Introduction

The process of gastrulation in the mouse establishes not only the basic body plan of the animal but also the orderly distribution and differentiation of the definitive fetal tissues. Primarily, this is achieved by the ingression, and the subsequent differentiation, of embryonic ectoderm through the primitive streak (Snell & Stevens, 1966). That embryonic ectoderm is the sole founder tissue of the fetus is strongly indicated by the consensus of a wide variety of studies, employing very different experimental strategies (for review, see Beddington, 1983a). Therefore, one of the keys to the initial organization of the fetus, both in terms of cellular differentiation and morphogenetic rearrangements, must lie in the temporal and spatial pattern of tissue diversification within the embryonic ectoderm. This study extends previous work on the fate of tissue within the embryonic ectoderm (Beddington, 1981, 1982; Snow, 1981;

Copp, Roberts & Polani, 1986) by examining the fate of cells adjacent to and in different regions of the primitive streak in 8th day mouse embryos. In addition, the fate of cells from the early-somite-stage primitive streak has been examined, in order to determine whether or not there is a change with age in the repertoire of tissues emerging from the streak.

Morphological studies in the rat and mouse suggest that mesoderm and definitive endoderm are the principal tissues produced by the primitive streak (Jolly & Ferester-Tadie, 1937; Snell & Stevens, 1966; Poelmann, 1981a,b). In this paper, the pattern of cell recruitment into mesodermal tissues has been examined, using both orthotopic primitive streak grafts of [³H]thymidine-labelled cells and by specifically labelling the entire ectoderm population with wheat germ agglutinin conjugated to colloidal gold (WGA–Au) and determining the distribution of labelled cells after subsequent development in culture. The distribution of cells in the definitive

endoderm and associated primordia will be described elsewhere (Beddington & Tam, in preparation).

The initial allocation of cells to the embryonic mesoderm has been analysed with particular reference to the paraxial mesoderm. By virtue of the precise craniocaudal segmentation of somites (Flint, Ede, Wilby & Proctor, 1978), which provides a convenient measure of position along the embryonic axis, the paraxial mesoderm is the most suitable mesodermal tissue in which to study the initial deployment of cells leaving the primitive streak. There is tissue contiguity between the primitive streak and the presomitic mesoderm, as well as between the streak and the gut, neural tube and notochord, indicative of continuous cell recruitment from the streak during elongation of the embryonic axis (Snell & Stevens, 1966; Tam, 1981; Tam, Meier & Jacobson, 1982; Schoenwolf, 1984; Švajger, Kostović-Knežević, Bradamante & Wircher, 1985). Removal of the posterior part of the embryo, containing the primitive streak and/or tail bud, interrupts elongation of the embryonic axis and curtails somitogenesis (Smith, 1964; Criley, 1969; Packard & Jacobson, 1976; Schoenwolf, 1977; Tam, 1986). If maintenance of the paraxial mesoderm is indeed dependent upon an influx of cells from the primitive streak (Tam, 1981; Tam & Beddington, 1986; Ooi, Sanders & Bellairs, 1986) it is of interest to know how these cells are distributed in the presomitic mesoderm. Morphologically discrete aggregates of mesodermal cells, somitomeres, have been described in the cranial and presomitic mesoderm (Tam *et al.* 1982; Meier & Tam, 1982), and it has been shown that the number of somitomeres present in the presomitic mesoderm corresponds to the number of somites generated by explants of the presomitic region (Tam, 1986). Therefore, somitomeres may be the morphological manifestation of cell allocation into metameric units, in which case one would expect cell mixing to be minimal within the presomitic mesoderm. The temporal and spatial pattern of recruitment of primitive-streak-derived cells into newly formed somites has been examined in order to assess the extent of cell mixing within the presomitic mesoderm.

Materials and methods

The experimental approach

Two different experimental strategies were used to examine the origin and initial distribution of certain mesodermal tissues during gastrulation and early organogenesis. The first was to make synchronous grafts of [^3H]thymidine-labelled cell clumps orthotopically into (i) different regions of the primitive streak of 8th day embryos, (ii) into the primitive streak of 9th day embryos and (iii) into the presomitic mesoderm of 9th day embryos. The distribution

of labelled cells in chimaeras after 20–22 h of further development *in vitro* was analysed autoradiographically. The second approach, applied to embryos of both stages, was to label all the ectoderm cells lining the amniotic cavity by injecting a solution of wheat germ agglutinin conjugated to colloidal gold (WGA–Au) into the cavity. This enabled the distribution of any mesoderm which originated from ectoderm during the subsequent culture period to be determined. In both studies, particular attention was paid to the distribution of labelled cells in the paraxial mesoderm (the somites and presomitic mesoderm) in the expectation that this might clarify the pattern of allocation of cells to the embryonic axis.

Recovery and culture of embryos

Embryos were obtained from a closed colony of outbred albino PO strain (Pathology, Oxford) mice maintained on a cycle of 14 h light/10 h dark, the midpoint of the dark cycle being 19.00 h. The day on which a vaginal plug was detected was designated the 1st day of gestation. On the 8th and the 9th day of gestation, embryos were dissected from the uterus in PB1 medium (Whittingham & Wales, 1969) containing 10% fetal calf serum (FCS, Gibco). The parietal yolk sac was removed and the embryos were washed in several changes of fresh PB1+FCS medium.

After manipulation or labelling, embryos were cultured in rotating (30 revs min^{-1}) 30 ml universal tubes (Sterilin) containing 3 ml of culture medium made up of equal parts of Dulbecco's modified Eagle's medium (DMEM, Flow Laboratories) and rat serum (Steele & New, 1974; New, Coppola & Cockcroft, 1976; Tam & Snow, 1980). The DMEM was supplemented with L-glutamine (58.4 mg 100 ml^{-1}) and, for grafting experiments, thymidine was added ($8 \times 10^{-6}\text{ M}$). The culture medium was sterilized by filtration (Sartorius, pore size $0.45\text{ }\mu\text{m}$) and equilibrated with 5% CO_2 in air overnight. Four to five embryos were cultured in each tube and at the start of culture the medium was gassed with a mixture of 5% CO_2 , 5% O_2 and 90% N_2 . Cultures were regassed after 7–8 h.

After culture (20–22 h) embryos were examined for the presence or absence of heart beats and visceral yolk sac circulation before being transferred to phosphate-buffered saline (PBS). Various developmental features, such as the formation of cranial neural folds, the invagination of the gut portal and the fusion of the allantois to the chorion in cultured 8th day embryos, and the closure of the cephalic neural tube, the formation of structures such as the fore-limb buds, otic capsules and pharyngeal arches, and the degree of axial rotation in cultured 9th day embryos, were noted. The somite number was also recorded. Grossly abnormal and developmentally retarded embryos were discarded. The remainder were processed for histology. After autoradiography or silver impregnation a preliminary histological examination was made and embryos exhibiting excessive cell death or disorganized development were excluded from further analysis.

Production of in vitro chimaeras

(1) Preparation of labelled grafts

Following the removal of the parietal yolk sac, 8th day embryos were transferred to medium containing the alpha

modification of Eagle's medium (Flow Laboratories) supplemented with 30 μM each of adenosine, guanosine, cytidine and uridine, 10% FCS and 10 $\mu\text{Ci ml}^{-1}$ of [^3H]thymidine (specific activity 12.5 Ci mm^{-1} ; Radiochemicals, Amersham). The embryos were labelled for 2 h under conditions described previously (Beddington, 1981). 9th day embryos were labelled for 3 h in rotating tubes containing rat serum + DMEM + 10 $\mu\text{Ci ml}^{-1}$ [^3H]thymidine (specific activity 12.5 Ci mm^{-1}).

After labelling, embryos were washed for 10–15 min in three changes of PB1 + FCS containing an additional 8×10^{-6} M of thymidine. Some embryos were then fixed in Carnoy's fluid to serve as controls for the degree of [^3H]thymidine incorporation (*uptake controls*). In every experiment, some labelled embryos (*labelled controls*) were cultured for the same duration as experimental embryos. *Labelled controls* serve to ensure the compatibility of [^3H]thymidine incorporation with normal development and also provide an important measure of the expected dilution of label in different tissues during the culture period (Beddington, 1981).

The remaining labelled embryos were dissected with siliconized (Repelcote) fine glass needles to isolate the embryonic ectoderm required for grafting. Fig. 1 illustrates the four regions (B, C, D and E) dissected for orthotopic

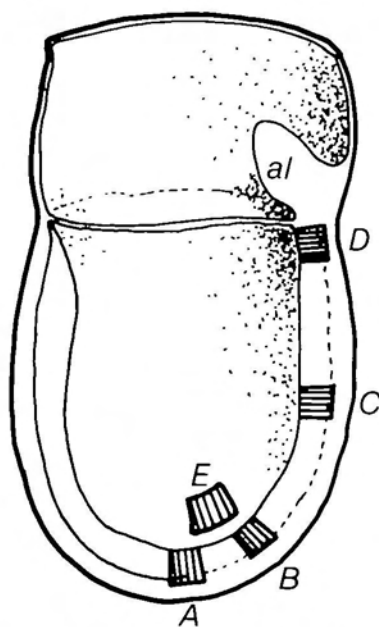


Fig. 1. A diagram of the right half of an 8th day primitive-streak-stage embryo showing the sites of orthotopic grafting of [^3H]thymidine-labelled cells. A is a region at the anterior extreme of the primitive streak which corresponds to the 'node' region. B (anterior streak) lies in the anterior third portion and C (midstreak) corresponds to the middle portion of the streak. D (posterior streak) is at the posterior end of the streak close to the base of the allantoic bud (al). E marks an area of the embryonic ectoderm immediately lateral to the anterior region of the streak. Although area E is marked on the right side of the embryo, orthotopic grafts were always made to a similar area on the left side of the embryo.

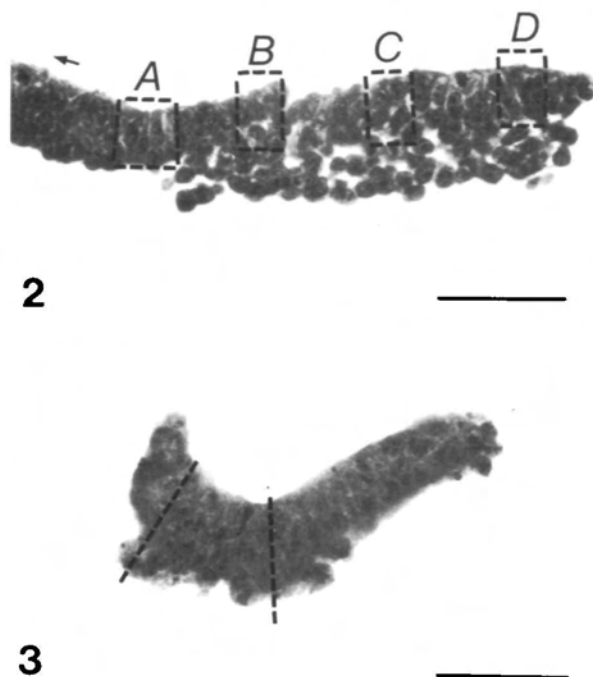


Fig. 2. A longitudinal section through the fragment of tissue isolated from an 8th day embryo containing the primitive streak and the 'node' region. The area from which cells were isolated for grafting is marked with boxes and these correspond to the regions marked in Fig. 1. The epithelial (ectodermal) portion of the primitive streak was always used in the grafting experiments described in this study. The arrow points cranially. Haematoxylin and eosin (H&E). Bar, 50 μm .

Fig. 3. A transverse section through the midregion (C) of fragment containing the primitive streak. The dashed lines demarcate the region of primitive streak from which clumps of cells were isolated for grafting. H&E. Bar, 50 μm .

grafting in 8th day embryos. Region A, corresponding to the most anterior extreme of the primitive streak, represents the area receiving orthotopic and heterotopic grafts in previous experiments (Beddington, 1982) and is included because colonization of the paraxial mesoderm in these chimaeras has been further analysed in this study. The mechanical isolation of the tissue for grafting (Figs 2, 3) was similar to that described elsewhere (Beddington, 1982), the final graft used for injection containing approximately 15–30 cells.

In order to isolate the primitive streak fragment from the 9th day embryo, the posterior region of the embryo was first separated from the trunk by a transverse cut made at the level of the last-formed somite. A wedge of tissue was obtained from the posterior region of the neuropore by making two oblique cuts as shown in Fig. 4. The primitive streak tissue was then isolated by a horizontal cut through the fragment (Fig. 5), followed by further dissection to remove as much of the adhering mesenchyme as possible (Fig. 6). Presomitic mesoderm was mechanically isolated

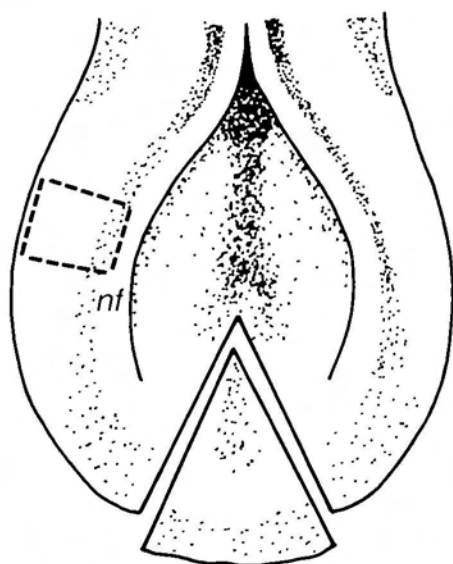


Fig. 4. A diagram of the posterior region (dorsal view) of the 9th day early-somite-stage embryo showing the position of two oblique cuts made medial to the neural folds (*nf*) at the posterior neuropore in order to isolate a wedge of tissue containing the primitive streak. This was further dissected as shown in Fig. 5. The box on the lateral side of the embryo marks the region from which labelled presomitic mesoderm tissue was obtained for orthotopic grafting.

from the region shown in Fig. 4. The potential graft tissue was further subdivided with needles into clumps of a suitable size for injection, each consisting of about 40 cells (see below).

(2) Grafting of labelled tissues

The developmental stage of the recipient embryos was always matched as closely as possible with that of donors to minimize any asynchrony between graft and host. Primitive-streak-stage embryos were matched according to size and certain morphological features such as the extent of amnion formation and the appearance of an allantoic bud. Somite number in conjunction with the extent of cephalic neurulation (Jacobson & Tam, 1982) were used to compare 9th day embryos.

The clumps of labelled cells and the recipient embryos were transferred to a drop of PB1+FCS containing 8×10^{-6} M of thymidine. The manipulation of the 8th day embryos was carried out in a hanging drop of the medium in a Leitz manipulation chamber filled with liquid paraffin (Boots UK Ltd) mounted on the fixed stage of a Zeiss (Ergoval) binocular microscope. 9th day embryos were injected in a drop of the medium located on the lid of a culture dish (Sterilin) and covered with paraffin. The preparation of holding and injection pipettes and the basic grafting procedure have been described before (Beddington, 1981). In more advanced 9th day embryos (6–8 somites), it was not possible to directly observe the injection pipette in the primitive streak. The pipette was directed through the primitive streak *via* the hindgut portal so that its tip protruded from the posterior region of the

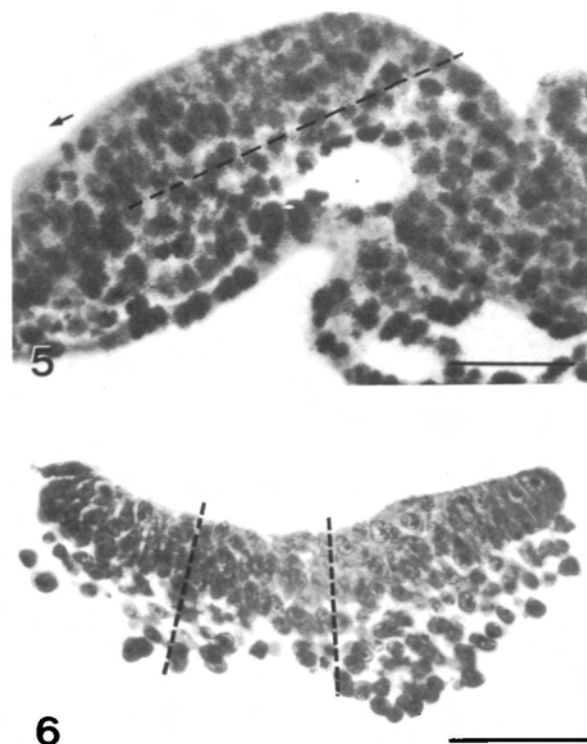


Fig. 5. An autoradiograph of the posterior region of a 9th day embryo after labelling for 3 h with [3 H]thymidine. Virtually all cells in the streak and the underlying mesenchyme are labelled. The dashed line indicates the plane of a horizontal cut that is made to obtain a fragment of the primitive streak. Arrow points cranially. H&E. Bar, 50 μ m.

Fig. 6. A transverse section of an explant of the primitive streak of a 9th day embryo. The fragment contains the epithelial (ectodermal) portion of the streak and some mesenchyme found at the basal aspect of the streak. The dashed lines demarcate the region from which clumps of cells were isolated for grafting. H&E. Bar, 50 μ m.

embryo into the amniotic cavity. The pipette was then slowly withdrawn and the graft expelled when its tip disappeared into embryonic tissue.

Fig. 7 shows a clump of labelled cells in the primitive streak region of a 9th day embryo fixed immediately after grafting. Most of the graft is located in the deep aspect of the primitive streak and is in intimate contact with the overlying endoderm. In a series of fifteen 9th day embryos which were examined autoradiographically immediately after grafting, a mean number of $38.7 (\pm 4.8)$ cells were found. In most cases, the graft was located in the midline but in four the labelled clump was found slightly (approximately 100 μ m) to one side of the streak.

Autoradiography

The preparation of autoradiographs and the criteria used for identifying and counting colonizing donor cells were the same as those described by Beddington (1981).

Lectin-conjugate-labelling experiments

Preparation of the WGA-Au label was as described by Horisberger & Rosset (1977) and Smits-van Prooije, Poelmann, Dubbeldam, Mentink & Vermeij-Keers (1986). The WGA molecules (Sigma) were first cross linked to bovine serum albumin (BSA, Miles Laboratory) by reacting with glutaraldehyde. The WGA-BSA complex was then conjugated in the presence of polyethylene glycol to colloidal gold particles [10–15 nm; 0.005 % solution in citrate buffer pH 5.5 (Polysciences)]. The WGA-BSA-Au conjugate was spun at 120 000 *g* for 30 min at 4–10°C in a Beckman L5-50 ultracentrifuge. The pellet was re-suspended in PBS solution A (Oxoid), recentrifuged and washed three times. A concentrated WGA-Au label was obtained by suspending the final pellet in about 50 µl of citrate buffer pH 5.5. The final preparation has an equivalent of 1–2 mg WGA ml⁻¹ and has a shelf life of at least a

month if kept at 4°C. The concentration of free WGA used for injection was 1 mg ml⁻¹ in PBS. Injection of free WGA was undertaken to eliminate the possibility of spurious silver impregnation (see below) in the presence of lectin alone.

The apparatus and procedure for injecting a small quantity of the label into the amniotic cavity were similar to those for grafting of labelled cells. Prior to injection, the micropipettes were calibrated with an ocular micrometer on the microforge so that the volume of solution delivered was standardized to about 0.2 nl (for 8th day) and 1.0 nl (for 9th day) for injection of free WGA. A larger volume of about 2 nl (for 8th day) and 10 nl (for 9th day) of WGA-Au label was injected. Based on the estimation by Burgoyne, Tam & Evans (1983), the 8th day embryo contains about 15 nl fluid in the amniotic cavity, whereas the volume of amniotic fluid in the 9th day embryo is about 500 nl (Cockroft, personal communication). To reduce the risk of forced diffusion of

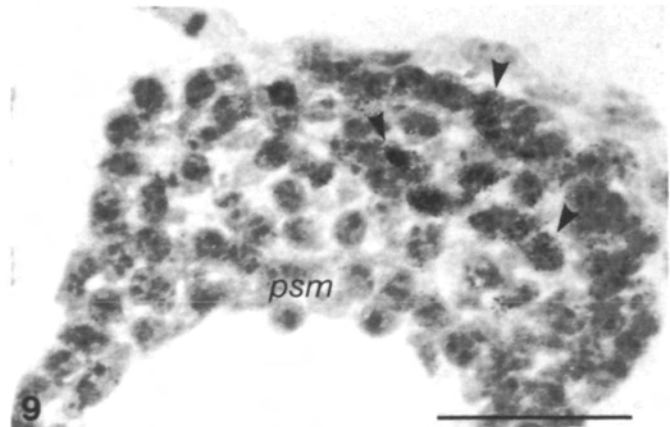
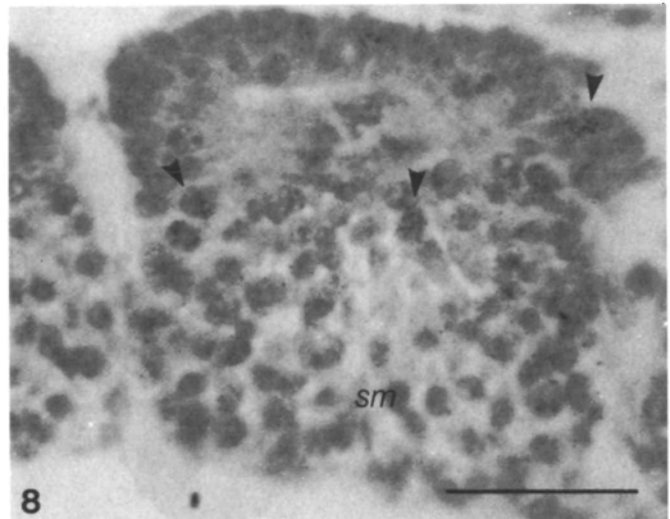


Fig. 7. An autoradiograph of a 9th day embryo fixed immediately after grafting of [³H]thymidine-labelled cells. In this embryo, about 43 cells were placed in the primitive streak. The indentation in the endoderm (*en*) is caused by the passage of the micropipette during the course of grafting. *al*, allantois. H&E. Bar, 100 µm.

Fig. 8. A longitudinal section through the somite (*sm*) of a chimaeric embryo produced by orthotopic grafting of primitive streak cells to a 9th day embryo. Both the dermamyotome and the sclerotome are colonized by labelled cells (arrowheads). H&E. Bar, 50 µm.

Fig. 9. A transverse section of the presomitic mesoderm (*psm*) of a chimaeric embryo produced by orthotopic grafting of labelled primitive streak cells to a 9th day embryo. Arrowheads mark a group of labelled cells in the dorsolateral region of the presomitic mesoderm. H&E. Bar, 50 µm.

the label through the ectodermal epithelium caused by excessive hydrostatic pressure, injection of label was usually preceded by the withdrawal of an equivalent volume of the amniotic cavity fluid from the embryo using a second micropipette (internal diameter 5–10 μm). This pipette was mounted adjacent to the injection pipette. The injection pipette was always inserted through the visceral yolk sac and the amnion to prevent damage to the embryonic region. After trimming the ectoplacental cone, for identification purposes, uninjected embryos (*unlabelled controls*) were placed in the manipulation drop during the injection of operated embryos and were subsequently cultured in the same tube as labelled embryos.

Embryos injected with WGA–Au or free WGA and the *unlabelled controls* were fixed in Carnoy fluid, embedded in paraffin wax and serially sectioned at 8 μm . Gold particles were visualized by a silver enhancement procedure (Snow & Springall, personal communication). The dewaxed and hydrated sections were treated with a silver developer (2.35 g trisodium citrate dihydrate (BDH), 2.55 g citric acid (BDH), 0.85 g hydroquinone (BDH) and 0.11 g silver lactate (Fluka) dissolved in 100 ml milli-Q water) at pH 3.9 for 5–7 min, followed by fixing in a photographic fixer (Unifix, Kodak). The treatment resulted in the formation of silver grains of 100–200 nm around the gold particles which can be readily seen by light microscopy. The sections were counterstained with 0.25 % aqueous solution of fast green for better contrast of the silver grains. Analysis of *unlabelled controls* revealed that individual silver grains were occasionally present in cells but that the occurrence of two or more silver grains in a single cell was extremely rare. Cells in injected embryos were regarded as positively labelled when two or more silver grains were found embedded in their cytoplasm.

Results

Embryonic development in vitro

Over 80 % of 8th day *unlabelled control* embryos and *labelled controls* developed normally during 20–22 h in culture (Table 1). They formed 4–5 pairs of somites and showed no significant difference from embryos of

the same age recovered from *in vivo* with respect to the various morphological features assessed (Table 1). Similarly, embryos receiving [^3H]thymidine grafts or those injected with WGA–Au or free WGA showed development comparable to controls, although those labelled with lectin or lectin-conjugate had a slightly elevated somite number (Table 1). The 9th day early-somite-stage embryos also underwent extensive growth and morphogenesis in culture (Table 2). Again, over 80 % of *labelled controls*, grafted and injected embryos developed normally and showed no consistent deviation from the pattern of development seen in *unlabelled* and *in vivo controls* (Table 2). A somewhat reduced incidence of axial rotation was observed in WGA–Au-injected embryos and of those labelled with unconjugated WGA only 50 % developed a visceral yolk sac circulation (Table 2). It is unlikely that these minor anomalies were a specific result of lectin binding because WGA–Au- and WGA-injected embryos differed in the particular characteristics affected.

The results in Tables 1 and 2 show that, in general, development of 8th and 9th day embryos was not adversely affected by the labels used or by grafting, and that the extent of differentiation and morphogenesis permitted a detailed analysis of the normal fate of grafted or labelled tissue. Specific assessment of embryonic growth, by measurement of total protein content, was not undertaken because previous studies using similar culture conditions have established that growth during the first 24 h *in vitro* parallels that *in vivo* (Beddington, 1981; Tam & Snow, 1980; Tam, 1986). Most embryos appeared normal histologically although approximately 10 % of both control and experimental 8th day embryos were excluded from further analysis due to a marked deficiency of cranial mesenchyme and severely attenuated neural epithelium. Excessive cellular necrosis was seen in two macroscopically normal 9th day

Table 1. The development of 8th day embryos in vitro following grafting, labelling and culture

	No. of embryos	No. developed normally	No. (%) of embryos with				Somite no. (n)
			Neural folds	Gut	Heart beat	Fused allantois	
<i>In vitro</i> chimaeras							
Labelled control	11	9 (82 %)	9 (100)	8 (89)	7 (78)	7 (78)	5.1 \pm 0.6 (9)
Grafted	58	46 (79 %)	46 (100)	42 (91)	32 (70)	36 (78)	5.0 \pm 0.3 (41)
Lectin-conjugate labelling							
Control	24	21 (88 %)	21 (100)	15 (71)	19 (90)	21 (100)	4.2 \pm 0.5 (18)
WGA–gold	56	44 (79 %)	40 (91)	37 (84)	34 (71)	38 (86)	6.2 \pm 0.3 (42)*
WGA	6	6 (100 %)	6 (100)	6 (100)	4 (67)	6 (100)	6.3 \pm 0.2 (6)†
<i>In vivo</i> 9th day embryo (3 litters)	19	16 (84 %)	15 (94)	13 (81)	15 (94)	12 (75)	5.0 \pm 0.3 (16)

Significantly different from control at *, $P < 0.001$; †, $P < 0.05$ by Student's unpaired *t*-test.

Table 2. The development of 9th day embryos following grafting, labelling and culture

	No. of embryos	Initial somite no. (n)	No. embryos developed normally	No. (%) of embryos showing					Somite no. (n)
				Yolk sac circulation	Neural tube closure	Beating heart	Forelimb buds	Axis rotation	
<i>In vitro</i> chimaeras									
Labelled control	26	4.5 ± 0.3 (22)	24 (92 %)	23 (96)	16 (61)	24 (100)	13 (54)	23 (96)	18.6 ± 0.8 (22)
Grafted	73	5.1 ± 0.2 (44)	61 (84 %)	49 (80)	42 (69)	56 (92)	38 (62)	52 (85)	18.0 ± 0.5 (58)
Lectin-conjugate labelling									
Control	46	4.7 ± 0.2 (38)	35 (76 %)	29 (83)	16 (46)	33 (94)	20 (57)	29 (83)	16.9 ± 0.7 (35)
WGA-gold	77	4.5 ± 0.3 (57)	65 (84 %)	49 (75)	49 (75)†	58 (89)	30 (46)	38 (58)*	16.8 ± 0.5 (65)
WGA	8	5.8 ± 0.3 (8)	8 (100 %)	4 (50)*	3 (38)	8 (100)	5 (63)	6 (75)	19.0 ± 1.0 (8)
<i>In vivo</i> 10th day embryos (3 litters)	21	—	18 (86 %)	17 (94)	10 (56)	18 (100)	12 (67)	16 (89)	17.2 ± 0.5 (18)

Significantly different from the control at *, $P < 0.05$; †, $P < 0.01$ by chi-square test.

embryos that had received grafts, and these were also excluded from further analysis.

The distribution of [³H]thymidine-labelled cells in chimaeras

(1) Uptake and labelled controls

After 2 h in labelling medium dense grains were seen over all nuclei visible in every tissue of the primitive-streak-stage embryos (see Beddington, 1981). The primitive streak region of 9th day embryos appeared to be similarly labelled following incubation in [³H]thymidine for 3 h (Fig. 5). The density of labelling in other tissues of 9th day *uptake controls* was more variable but over 95 % of nuclei were labelled. After 20–22 h in culture silver grains could still be detected over at least 90 % of nuclei in 8th and 9th day *labelled controls*. In cultured 8th day embryos gut endoderm, notochord and paraxial mesoderm exhibited the highest density of grains, whereas neurectoderm and surface ectoderm appeared least labelled. Labelling was equally widespread but generally less dense in 9th day *labelled controls*. Nuclei in gut endoderm, notochord and cardiac mesoderm were more heavily labelled than those in the cranial mesenchyme or brain. The spinal cord, paraxial mesoderm and primitive streak exhibited a more patchy distribution of both densely and lightly labelled nuclei. Both stages of *labelled controls* had fairly uniformly labelled visceral yolk sac endoderm whilst the mesodermal component showed more variable levels of labelling. These results indicate that all the cells in a graft would be labelled and that most, if not all, of their progeny should be detectable autoradiographically after 20–22 h of further development in culture.

(2) Orthotopic grafts to 8th day embryos

Table 3 shows the distribution of [³H]thymidine-labelled cells in chimaeric embryos obtained from

orthotopic grafts to regions *B*, *C*, *D* and *E* (Fig. 1). Of 46 embryos analysed, 35 proved to be chimaeric (76 %). Grafts to the posterior extreme of the streak (*D*) generated chimaerism predominantly in extra-embryonic mesoderm (mesoderm of the amnion, visceral yolk sac and allantois). There was no contribution to paraxial mesoderm but some to lateral mesoderm (here defined as that embryonic mesoderm lateral to the paraxial mesoderm). Labelled tissue injected into the middle of the streak (*C*) colonized almost exclusively lateral mesoderm. More anterior grafts (*B*) contributed to paraxial mesoderm, the primitive streak, notochord and head process/notochordal plate. In addition, two of the chimaeras were colonized entirely in the cranial mesenchyme. Grafts lateral to the anterior part of the streak (*E*) gave rise largely to paraxial mesoderm although colonization of other mesodermal tissues was observed and three embryos were chimaeric in the neural tube. In these chimaeras, labelled cells were usually found on the same side of the embryo as the original graft (left side) but in four cases some labelled cells were also located contralaterally. Orthotopic grafts to all regions produced some chimaeras that were colonized in the primitive streak itself.

In the above series, chimaerism in the paraxial mesoderm was almost always confined to the presomitic mesoderm, incorporation into definitive somites probably requiring a longer period of development in culture. In a previous study (Beddington, 1981, 1982), where embryos were cultured for 36 h, orthotopic and heterotopic grafts of posterior streak tissue to the anterior extreme of the primitive streak (*A*) gave rise to chimaerism in definitive somites as well as in the presomitic mesoderm. From 20 orthotopic and 23 heterotopic grafts 25 chimaeras were obtained. Of these, 10 were chimaeric in the paraxial mesoderm. The distribution of labelled cells along the cranio-caudal axis of these chimaeras has now been mapped

and is shown in Fig. 10. The most anterior cells are found in the myelencephalon, which corresponds to the seventh somitomere of Meier & Tam (1982). Approximately 50–80% of the chimaeras have labelled cells in somites caudal to the third somite and they are all chimaeric in the presomitic mesoderm. The number of labelled cells in each somite was usually not more than two and not all the somites in a given chimaera were colonized. Labelled cells were found in somites on both sides of the embryo but only in 35% of cases (29/82) were both somites at the same level colonized. Four chimaeras had labelled cells in the primitive streak. No difference in the pattern of distribution was seen between embryos receiving orthotopic and heterotopic grafts.

(3) Orthotopic grafts to 9th day embryos

Autoradiographs were prepared from 61 9th day embryos that had received grafts in the primitive streak region. 41 (67%) were found to be chimaeric. Table 4 shows the distribution of labelled cells in 26 of

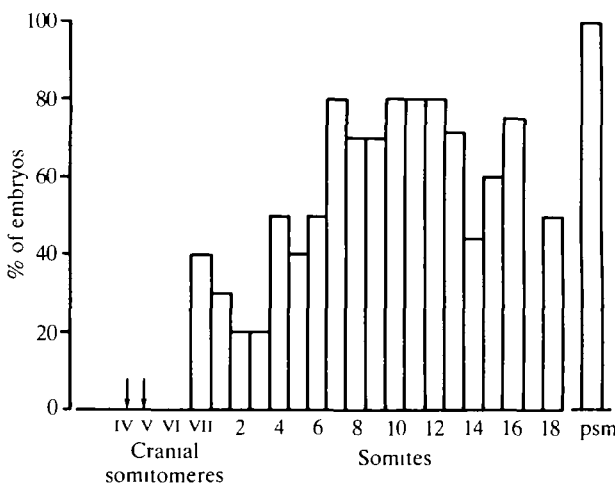


Fig. 10. The distribution of [^3H]thymidine-labelled cells to the paraxial mesoderm of chimaeras produced by grafting primitive streak cells to the 'node' region of the 8th day embryo followed by 36 h of culture. These chimaeras are part of a collection described by Beddington (1981, 1982). The arrows mark the stage of development of the embryo, at the time of grafting, in terms of the metameric units formed in the paraxial mesoderm, which correspond to the 4th and 5th somitomeres in the cranial mesenchyme at the late-primitive-streak stage. A total of 10 chimaeras were analysed and the histogram shows the percentage of chimaeras having a labelled somite at various segmental levels of the axis. It can be seen that only somitomeres or somites formed after grafting are colonized and that the presomitic mesoderm is always chimaeric. Because of the longer period of culture, these chimaeras formed more somites than those described in Table 3. A somite is considered as labelled if it contains one labelled cell. psm, presomitic mesoderm.

these chimaeras. The pattern of colonization in the remaining 15 embryos was similar but, to simplify the presentation of results, only embryos with more than 30 colonizing cells are included in Table 4.

The labelled cells in these chimaeras were mainly distributed to the paraxial mesoderm (Figs 8, 9), the primitive streak and the lateral mesoderm. Labelled cells were also found in the endoderm of the hindgut and the neighbouring notochord (Table 4). The majority of the labelled cells were found in the trunk and caudal regions of the embryo. The only cranial contribution was the presence of four labelled cells in the cranial mesenchyme (chimaeras 1, 3 and 18). Probably, as a result of the route adopted for grafting, unincorporated clumps of labelled cells were often (13/26) seen in the hindgut lumen.

Fig. 11 shows the distribution of labelled cells in the somites that were formed after grafting. It is apparent that the more posterior somites are the ones most frequently colonized and contain the most labelled cells. In some chimaeras labelled somites were confined to one side of the primitive streak. In seven chimaeras labelled cells were found in the first few somites formed immediately after the graft was injected. This occurred despite the fact that the initial location of the graft was at least three or four somite lengths away from the region of somite segmentation (see Tam, 1986). This might be accounted for by an anterior movement of cells in the presomitic mesoderm prior to somite segmentation. However, such movement might be overestimated if manipulation of the embryo caused a delay in somitogenesis.

Cell mixing was examined more directly by orthotopically grafting a clump of about 40 presomitic mesoderm cells. In all four of the resultant chimaeras, obtained from transplanting cells to 10 embryos, three or four contiguous somites were colonized in an otherwise nonchimaeric sequence of somites (Table 5). Labelled cells were found interspersed with host cells both in the sclerotome and in the dermamyotome of colonized somites. This suggests that some cell mixing must have occurred within the presomitic mesoderm because it is highly unlikely that donor cells could invade the epithelial component of a somite after segmentation has taken place. Very rarely, colonization of more anterior somites, which were already segmented at the time of grafting, was also seen: labelled cells were found in one somite in five chimaeras and in two somites in one chimaera. Each of these somites was colonized by only one or two labelled cells (see Discussion).

The distribution of WGA–Au-labelled cells

Injection of WGA–Au into the amniotic cavity resulted in extensive labelling of the embryonic ectoderm of 8th day embryos (Fig. 12). A similar

Table 4. The distribution of [^3H]thymidine-labelled primitive streak cells to embryonic tissue after grafting orthotopically on the 9th day and cultured for 20–22 h in vitro

Tissues	Chimaeras (n = 26)																									
	1	2	3	4	5	8	9	10	12	14	15	17	18	22	23	24	28	29	30	32	33	35	37	38	40	41
Neural tube	39			3	3	3	3		8		8	2		2			2	2	4						4	
Surface ectoderm	1		2						2				1			1	2	1	1							
Cranial mesenchyme	1		1										2													
Heart + endothelium						1														1	3					
Paraxial mesoderm	18	151	69	12	8	3	82	20	45	49	12	32	29	18	15	94	23	10	13	66	23	21	40	32	125	200
Lateral mesoderm		6	65	17	1	34	21	2	52	2		3	3	10			8	27	25		2	2		8	6	
Primitive streak	5	48	36	14	35	10	11	10	1	2	39				1	17	1	8	8	8	9	20	2		13	119
Gut endoderm	4	26	9	45	10	57	13	4	10			10		16	1		5	12	17	2	52	2		3	6	
Notochord				1			1				17			3		3			1	1		49	15	1		
Extraembryonic mesoderm	5		1								2															5
Total no. of labelled cells	73	231	183	92	57	108	131	36	118	53	78	47	35	50	33	99	48	60	69	70	89	45	91	58	149	330

□ denotes the tissue that was colonized by $\geq 40\%$ of the labelled population.

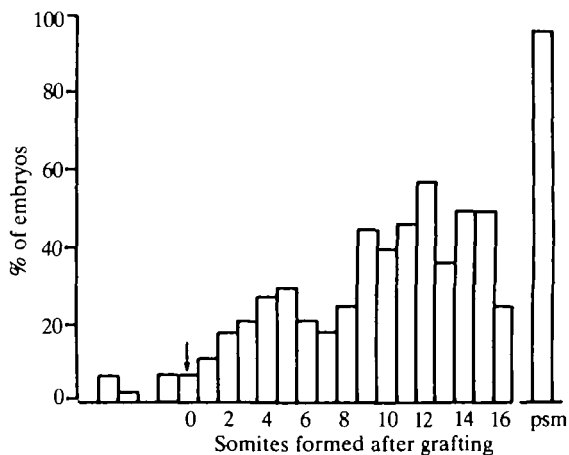


Fig. 11. The distribution of labelled cells to the paraxial mesoderm of the chimaeric embryos produced by orthotopic grafting of [^3H]thymidine-labelled cells to the primitive streak on the 9th day embryos followed by 20–22 h of culture. Because of the variation in the initial somite number of the embryos (3–7 pairs of somites), the somites formed after grafting were scored in each chimaera in a numerical order starting from the last-formed somite at the time of grafting (somite 0, marked by arrow). Somites 1 to 16 are therefore formed subsequently in culture and those to the left of the arrow are already segmented before the experiment. Altogether 41 chimaeras were scored. The histogram shows the percentage of embryos having a labelled somite at various segmental levels of the body axis. A somite is considered labelled if it contains one labelled cell. psm, presomitic mesoderm.

blanket labelling of the ectodermal tissue and the primitive streak was also achieved in 9th day embryos (Fig. 14). Embryos cultured for 3 h after injection showed positively labelled cells deep in the primitive streak (Figs 13, 14) but there was no sign of silver-enhanced gold particles elsewhere in the mesoderm or underlying endoderm. Uninjected embryos and embryos injected with free WGA did not contain silver deposits characteristic of gold particle labelling.

After 20–22 h in culture, 8th and 9th day embryos injected with WGA–Au contained numerous labelled cells in the neur ectoderm and surface ectoderm. In addition, some cranial mesenchyme was labelled: in early-somite-stage embryos, derived from cultured 8th day embryos, labelled cells were located in the lateral subectodermal region, whereas cultured 9th day embryos had positively labelled cells not only in this region but also in the mesenchyme of the pharyngeal arches and in cellular condensations at the prospective sites of cranial nerve ganglia. These labelled cells may have been of neural crest or placodal origin. The cranial mesenchyme around the base of the neural tube and notochord contained few, if any, labelled cells.

The distribution of gold-containing cells in the paraxial mesoderm is shown in Fig. 15. The pattern is similar to that obtained with [^3H]thymidine-labelled grafts: those somites or regions of cranial mesenchyme formed after the start of labelling were frequently labelled and the presomitic mesoderm and primitive streak almost invariably contained positive cells. Again, labelled cells were found in somites

Table 5. The distribution of [^3H]thymidine-labelled presomitic mesoderm cells to somites following orthotopic grafting to the presomitic mesoderm of 9th day embryos

Embryos			No. of [^3H]thymidine-labelled cells in							
	Initial somite no.	Final somite no.	Somite 6	Somite 7	Somite 8	Somite 9	Somite 10	Somite 11	Somite 12	Somite 13
(1)	6	20	0	0	0	0	12	28	18	0
(2)	5	14	0	5	4	2	0	0	0	0
(3)	5	18	0	0	0	2	1	4	17	0
(4)	5	14	0	0	0	2	5	2	0	0

formed immediately after the injection of label and in a very few cases somites present at the time of injection were also labelled. Anterior somites were only considered to be unequivocally labelled if more than 5% (approximately 5–10 cells) of the dermo-myotome contained gold particles. The sclerotome was ignored because of the possibility of scoring migrating neural crest cells. Examples of labelled tissues are shown in Figs 16–18.

In most embryos the number of cells containing gold particles was of the order of 5–15 per somite, 20–30 in the presomitic mesoderm and about 30 in the lateral mesoderm. Undoubtedly, if WGA–Au were behaving as a truly heritable marker, one would expect to see more positive cells. Furthermore, despite ubiquitous labelling of ectoderm cells initially, some negative cells were seen in the neuroepithelium and surface ectoderm after development in culture. However, the absence of obviously extracellular gold particles suggests that the relatively low number of labelled cells is probably due to unequal partition of particles at cell division rather than to the loss of label from the cytoplasm. Nonetheless, it is important to recognize that in this experiment only a fraction of the progeny of initially labelled cells is being identified.

Discussion

Both the developmental fate of cells in the primitive streak and the pattern of cell allocation within the paraxial mesoderm of intact late-primitive-streak- and early-somite-stage mouse embryos have been examined. Compelling evidence has been obtained for regionalization in the deployment of cells at different levels of the primitive streak during the later stages of gastrulation. However, the analysis of the distribution of cells in the presomitic mesoderm and their recruitment into somites proved somewhat less conclusive.

Regionalization of cell fate

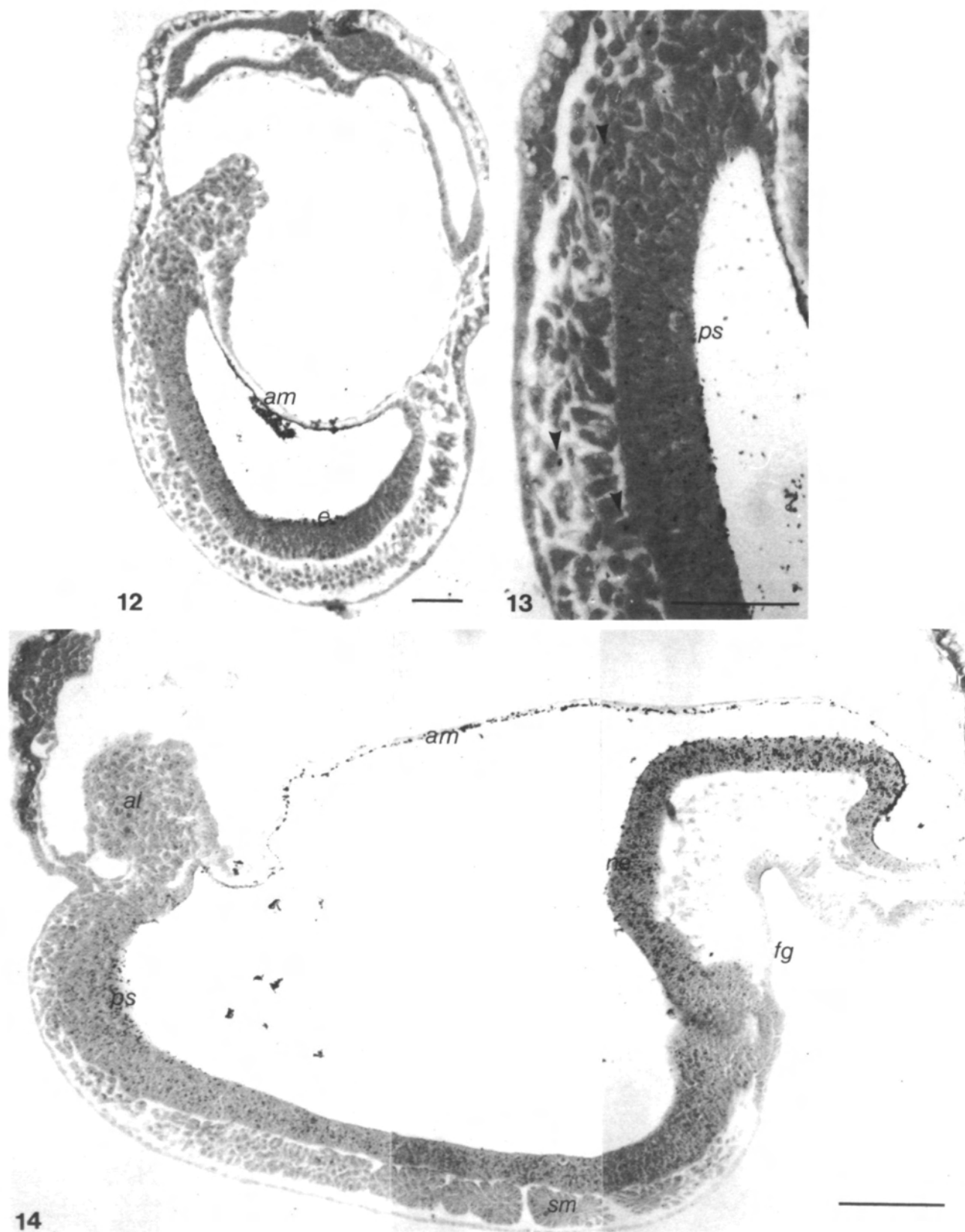
Previous studies in the mouse have indicated that cells invaginating at different points along the streak

may have distinct developmental fates. When specific fragments of the streak are isolated *in vitro*, without disturbing the relationship of the constituent germ layers, a difference is seen between different regions in their propensity to form allantois, tail bud structures and primordial germ cells (Snow, 1981). Orthotopic grafts, similar to those employed in this study, have demonstrated that the anterior extreme of the primitive streak, corresponding in location to Hensen's node in the avian embryo (Snell & Stevens, 1966; Rugh, 1968; Bellairs, 1986), generates gut endoderm and notochord, as suggested by several morphological studies (Jolly & Ferester-Tadie, 1936; Jurand, 1974; Poelmann, 1981a; Tam & Meier, 1982), in addition to lateral and paraxial mesoderm and vascular endothelium (Beddington, 1981). Grafts to the posterior part of the streak, on the other hand, produce exclusively mesodermal tissues (Beddington, 1982). In accordance with isolation experiments (Snow, 1981) and histochemical observations (Ożdżewski, 1967), primordial germ cells are also produced by orthotopic grafts to this region (Copp *et al.* 1986). The current study extends the map of the primitive streak and confirms that different prospective mesodermal tissues emerge from different regions of the streak (Table 3). Furthermore, grafts to early-somite-stage embryos demonstrate that the primitive streak remains an active source of mesodermal tissue at least during the early stages of organogenesis (Table 4). This is consistent with the wide variety of differentiated tissues observed in experimental teratomas derived from ectopic grafts of caudal 9th day tissues containing the primitive streak (Tam, 1984). The primitive streak may also remain regionalized, in terms of different developmental fates, in the 9th day embryo but the bulkiness of the posterior region and diminished size of the streak precluded accurate localization of grafts along its length at this stage.

The putative origin of the various embryonic and extraembryonic tissues along the craniocaudal axis of the 8th day primitive streak, and the predominant contribution to paraxial mesoderm by embryonic ectoderm located lateral to the anterior part of the

streak, is remarkably similar to the situation described for the chick embryo at a comparable developmental stage (stages 3–4; Pasteels, 1937; Spratt, 1955; Rosenquist, 1966; Nicolet, 1970, 1971; Vakaet,

1984). Thus, in both chick and mouse embryos, cells emerging from the anterior part of the streak contribute mainly to gut, notochord and paraxial mesoderm. Cells from the middle region give rise to lateral



mesoderm, whereas the caudal part of the streak is devoted largely to the provision of cells for extra-embryonic mesoderm. Interestingly, whereas grafts to the early-somite-stage primitive streak continue to colonize definitive embryonic fetal tissues, there was minimal chimaerism in the extraembryonic mesoderm suggesting that recruitment of cells into this tissue ceases during early organogenesis.

In all categories of graft, after 20–22 h some chimaeras retained labelled cells in the primitive streak (Tables 3, 4). These cells were interspersed with host cells, showed the same dilution of label as equivalent cells in *labelled controls* and did not resemble unincorporated graft tissue. This suggests that the streak itself may act as a source of cells and not just as a route for relocation. Certainly, an elevated mitotic index is found in the primitive streak throughout early organogenesis (Tam & Beddington, 1986), which would be consistent with the notion that it generates cells to supplement the prospective populations passing through it. A somewhat similar function for the primitive streak has been proposed in the chick embryo, where experiments involving extirpation and grafting of the streak indicate that the presomitic mesoderm has a dual origin: one population coming from the presumptive somitic mesoderm and the other derived from a continual influx of cells migrating away from the regressing streak (Bellairs & Veini, 1984; Bellairs, 1985; Ooi *et al.* 1986).

In the chick embryo, using labels such as vital dyes, carbon particles and [^3H]thymidine or grafting quail tissue, it has been shown that epiblast cells lateral to

Figs 12, 13. 8th day primitive-streak-stage embryos labelled with WGA–gold conjugate by intra-amniotic injection and examined after 3 h of labelling. The WGA–Au label is found in the embryonic ectoderm (*e*) of the embryo (Fig. 12). No leakage has occurred and the label is confined to the ectodermal side of the amnion (*am*). At higher magnification (Fig. 13), the WGA–Au label is seen in the deeper aspect of the primitive streak (*ps*) and occasionally in the mesoderm at the streak (arrowheads) but not in the endoderm. Silver enhanced and counterstained with fast green. Bar, 50 μm .

Fig. 14. 9th day early-somite-stage embryos labelled with WGA–gold conjugates by intra-amniotic injection and examined after 3 h of labelling. The conjugate labels the entire neurectoderm (NE) and the primitive streak region of the 4-somite-stage embryo. Label is not found in the mesoderm of the cranial region or in the paraxial mesoderm but some mesenchymal cells beneath the streak are labelled. Only the ectoderm of the amnion (*am*) is labelled. Some cells at the base of the allantois (*al*) are also labelled with the conjugate. *fg*, foregut; *sm*, somite. Silver enhanced and fast-green stained. Bar, 100 μm .

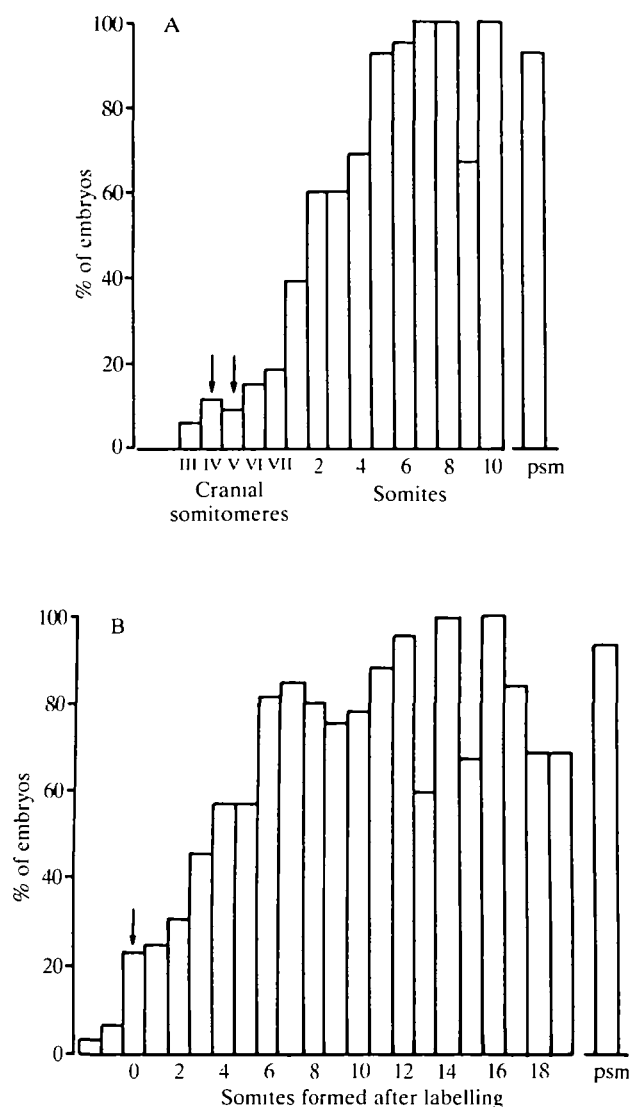


Fig. 15. The distribution of WGA–Au-labelled cells in the paraxial mesoderm of embryos labelled on (A) the 8th day and (B) the 9th day, followed by 20–22 h of culture. The arrows indicate the stage of development of embryos at the beginning of labelling. At this stage, the 8th day embryo has formed the 4th or 5th somitomers in the cranial mesenchyme. For the 9th day embryo, the arrow marks the last-formed somite (somite 3 to 7) in the paraxial mesoderm at the commencement of labelling, with reference to which somites formed subsequently are scored. A total of 33 8th day embryos and 47 9th day embryos were studied. In these embryos, somites are classified as labelled if they contain more than five cells with silver grains. The cranial somitomers (I to VII) are identified by their topographical relation to the brain parts and are classified as labelled when silver-grain-bearing cells are found in the mesenchyme at the base of the brain and in the perinotochordal mesenchyme. The histograms show the percentage of embryos having labelled metameric units at various segmental levels of the embryonic axis. psm, presomitic mesoderm.

the anterior part of the streak represent the presumptive somitic mesoderm (Gallera, 1975; Nicolet, 1971; Vakaet, 1984). These cells converge towards the streak and become situated along its anterior margins as the streak starts to regress. At the same time the prospective neurectoderm cells move caudally to flank the prospective paraxial mesoderm (see

Nicolet, 1971). If similar morphogenetic movements are occurring in the mouse this would account for the colonization patterns seen in grafts to region *E* (Fig. 1; Table 3), where contribution to paraxial mesoderm and to neurectoderm are observed. The colonization of presomitic mesoderm in grafts to the anterior streak (region *B*) also mimics the situation in

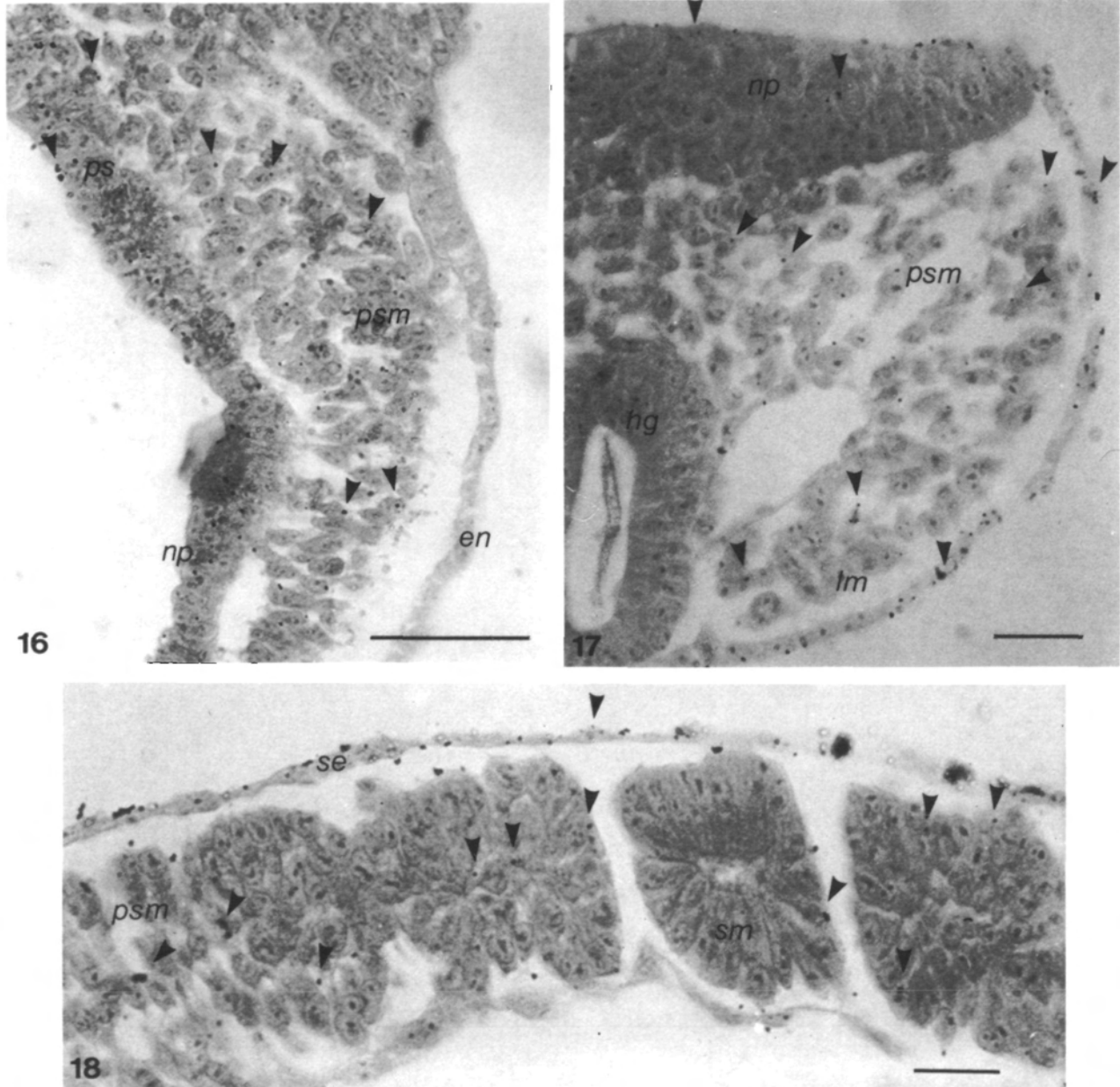


Fig. 16. The labelled neural plate (*np*), primitive streak (*ps*) and presomitic mesoderm (*psm*) of an embryo labelled with WGA-Au on the 8th day and cultured for 20–22 h. *en*, endoderm. Examples of labelled cells are marked by arrowheads. Silver enhanced and fast-green stained. Bar, 50 μ m.

Fig. 17. The posterior region of an embryo labelled with WGA-Au on the 9th day showing labelling in the neural plate (*np*) and the presomitic mesoderm (*psm*). The prospective lateral mesoderm (*lm*) is also labelled. Only a few labelled cells are seen in the hindgut endoderm (*hg*). Silver enhanced and fast-green stained. Examples of labelled cells are marked by arrowheads. Bar, 50 μ m.

Fig. 18. The labelled somites (*s*) and presomitic mesoderm (*psm*) of an embryo labelled with WGA-Au on the 9th day. *se*, surface ectoderm. Examples of labelled cells are marked by arrowheads. Silver enhanced and fast-green stained. Bar, 50 μ m.

the chick. The chimaerism in cranial mesenchyme observed in two embryos receiving grafts in region *B* is consistent with the experimental evidence that cranial somitomeres are located in the vicinity of the Hensen's node in the chick at stages 3–4 (Meier & Jacobson, 1982). Cranial somitomeres give rise to cranial mesenchyme (Meier & Tam, 1982), which may be considered as an integral part of the paraxial mesoderm of the embryo. It has been proposed that the cranial somitomeric mesoderm emerges from the primitive streak immediately before that of the somites. The contribution of cells to the cranial mesenchyme by grafts to region *B* but not by those to region *E* lends some support to this notion. In other words, cells located in the anterior streak, which invaginate before those lying lateral to the streak, are those giving rise to cranial mesenchyme in this series. These results, therefore, are compatible with the idea that paraxial mesoderm emerges from the streak in strict temporal order corresponding to its final cranio-caudal location.

It is unlikely that the regionalization found in the primitive streak reflects prior commitment of the embryonic ectoderm to form different kinds of mesodermal tissues. Heterotopic grafts of both cranial and caudal regions of the streak into the anterior part of the embryo result in colonization of definitive ectodermal derivatives (Beddington, 1982) and introduction of prospective definitive ectoderm into the caudal end of the streak produces chimaerism in embryonic and extraembryonic mesoderm (Beddington, 1982; Copp *et al.* 1986). In addition, most regions of the embryonic ectoderm appear pluripotent in ectopic grafts (Beddington, 1983*b*; Chan & Tam, 1986; Švajger, Levak-Švajger & Škreb, 1986). Therefore it seems probable that the emergence of distinctive mesodermal populations from different regions of the streak may relate more to morphogenetic constraints on cell movements through and away from the streak, distributing hitherto uncommitted cells to different parts of the embryo where subsequent tissues interactions determined their eventual differentiation, than to any intrinsic differences in the developmental potential of the cells themselves. Alternatively, the streak itself could be acting as an organizing centre in the sense that as cells pass through it they become committed to a particular developmental pathway (Tam, 1981; Tam & Beddington, 1986).

Cell recruitment into the paraxial mesoderm

The pattern of cell recruitment into paraxial mesoderm was analysed both in *in vitro* chimaeras and by observing the contribution of initially ectodermal cells, collectively labelled with WGA–Au, to the file of somites and presomitic mesoderm. Injection of

WGA–Au resulted in extensive and specific labelling of the embryonic ectoderm (8th day), neurectoderm and surface ectoderm (9th day), amniotic ectoderm and the superficial cells of the primitive streak. There was no evidence, even after short-term culture, for nonspecific transfer of gold particles to other tissue layers. This corresponds to the pattern of labelling reported previously for conjugated and free WGA in embryos of a similar stage (Gesink, Poelmann, Smits-van Prooije & Vermeij-Keers, 1983; Smits-van Prooije *et al.* 1986; Tan & Morriss-Kay, 1986). However, it is probable, due to unequal partition of gold particles, or aggregates, during cytokinesis, that only a sample of the progeny of this labelled population can be detected after prolonged culture. The likelihood of spurious negative cells precludes the use of this method to determine whether or not presomitic mesoderm comprises two populations: a resident population, which is supplemented by an influx of cells from the streak (Bellairs, 1985). However, the sequence of recruitment of labelled cells into somites, in both chimaeras and WGA–Au-labelled embryos, should reflect the behaviour of cells within the presomitic mesoderm, with respect to the existence or otherwise of a stable metameric prepatter. In other words, if the somitomeric pattern apparent in the presomitic mesoderm (Tam *et al.* 1982; Tam, 1986) is a morphological manifestation of a stable segregation of mesodermal cells into prospective somites, one would not expect to see labelled cells appearing in somites derived from those somitomeres present at the time of labelling.

The pattern of [³H]thymidine-labelled donor cell colonization and the appearance of WGA–Au-labelled cells in somites is basically similar. In both 8th and 9th day embryos the most posterior somites, presomitic mesoderm and primitive streak are labelled or contain donor cells. This supports the notion that the paraxial mesoderm is continually supplemented with cells emerging from the streak. Primitive-streak-stage embryos, at the time of injection, would be expected to have four to five somitomeres (Tam & Meier, 1982). The most anterior location of colonization donor cells is in the myelencephalic mesenchyme (Fig. 10) which is thought to be derived from the seventh somitomere (Meier & Tam, 1982). WGA–Au labelling resulted in a few chimaeras containing labelled cells anterior to the myelencephalon but the majority were labelled caudal to the first somite (Fig. 15). Therefore, in general, there does not appear to be much mixing within the earliest population of paraxial mesoderm cells.

The results from 9th day embryos are somewhat confusing, although the general impression gained is of cell mixing within the presomitic mesoderm superimposed on a net caudocranial movement of cells

through this tissue. Precise localization of grafts is more difficult at this stage and, therefore, some atypical patterns of colonization are inevitable. For example, in six chimaeras, donor cells were identified in a total of seven somites that had already formed at the time of grafting. Colonization of pre-existing somites was almost invariably restricted to the sclerotome and may represent intermingling between dispersing sclerotomal cells. Alternatively, a few cells may have been introduced directly into the anterior region during grafting. In 2 (out of 15) embryos fixed immediately after grafting about five donor cells were seen in the cranial mesenchyme, suggesting that the injection pipette may have been pushed too far through the primitive streak and consequently penetrated the anterior region. It is also possible that cells inadvertently deposited in the amniotic cavity may find their way into the embryo in a manner analogous to that of injected neural crest cells (Jaenisch, 1985).

Despite these few anomalous results, in essence both the *in vitro* chimaeras and the WGA-Au-labelled 9th day embryos present a similar picture. At the time of manipulation the presomitic mesoderm would be expected to contain six distinct somitomers (Tam *et al.* 1982). Nonetheless, progeny of grafted tissues and cells containing gold particles were found in somites that formed immediately after grafting or labelling, although the incidence of colonization or labelling increased in somites formed later (Figs 11, 15). The most plausible explanation for the colonization of somites formed immediately after manipulation is that there may be extensive cell mixing within the presomitic mesoderm, such that cells entering from the primitive streak are rapidly distributed along the length of the presomitic mesoderm. However, this mixing probably coincides with a net caudocranial movement of the total population because grafts of presomitic mesoderm produced only three or four chimaeric somites in the middle of an otherwise nonchimaeric sequence of somites and no donor cells were found in the presomitic mesoderm or primitive streak. Both the ultrastructure of somitomers (Tam *et al.* 1982) and the absence of complex junctions between mesodermal cells (Flint & Ede, 1978) would be compatible with cell mixing within the presomitic mesoderm, although such mixing argues against somitomers being self-contained precursors of somites.

We thank Professor Richard Gardner, Dr Michael Snow, Dr David Cockroft and Dr Andy Copp for their helpful discussion and advice, and Mrs Jo Williamson for preparing the manuscript. P.P.L.T. is supported by a Croucher Foundation Fellowship and R.S.P.B. by a Fellowship from the Lister Institute of Preventive Medicine.

References

- BATTEN, B. E. & HAAR, J. L. (1979). Fine structural differentiation of germ layers in the mouse at the time of mesoderm formation. *Anat. Rec.* **194**, 125–142.
- BEDDINGTON, R. S. P. (1981). An autoradiographic analysis of the potency of embryonic ectoderm in the 8th day postimplantation mouse embryo. *J. Embryol. exp. Morph.* **64**, 87–104.
- BEDDINGTON, R. S. P. (1982). An autoradiographic analysis of tissue potency in different regions of the embryonic ectoderm during gastrulation in the mouse. *J. Embryol. exp. Morph.* **69**, 265–285.
- BEDDINGTON, R. S. P. (1983a). The origin of the foetal tissues during gastrulation in the rodent. In *Development in Mammals*, vol. 5 (ed. M. H. Johnson), pp. 1–32. Amsterdam: Elsevier.
- BEDDINGTON, R. S. P. (1983b). Histogenetic and neoplastic potential of different regions of the mouse embryonic egg cylinder. *J. Embryol. exp. Morph.* **75**, 189–204.
- BELLAIRS, R. (1985). A new theory about somite formation in the chick. In *Developmental Mechanisms: Normal and Abnormal*, pp. 25–44. New York: Alan R. Liss Inc.
- BELLAIRS, R. (1986). The primitive streak. *Anat. Embryol.* **174**, 1–14.
- BELLAIRS, R. & VEINI, M. (1984). Experimental analysis of control mechanisms in somite segmentation in avian embryos. II. Reduction of material in the gastrula stages of the chick. *J. Embryol. exp. Morph.* **79**, 183–200.
- BURGOYNE, P. S., TAM, P. P. L. & EVANS, E. P. (1983). Retarded development of XO conceptuses during pregnancy in the mouse. *J. Reprod. Fert.* **68**, 387–393.
- CHAN, W. Y. & TAM, P. P. L. (1986). The histogenetic potential of neural plate cells of early-somite-stage mouse embryos. *J. Embryol. exp. Morph.* **96**, 183–193.
- COPP, A. J., ROBERTS, H. M. & POLANI, P. E. (1986). Chimaerism of primordial germ cells in the early postimplantation mouse embryo following microsurgical grafting of posterior primitive streak cells *in vitro*. *J. Embryol. exp. Morph.* **95**, 95–115.
- CRILEY, B. (1969). Analysis of the embryonic sources and mechanisms of development of posterior levels of chick neural tube. *J. Morph.* **128**, 465–502.
- FLINT, O. P. & EDE, D. A. (1978). Cell interactions in the developing somite: *in vivo* comparisons between *amputated (am/am)* and normal mouse embryos. *J. Cell Sci.* **31**, 275–291.
- FLINT, O. P., EDE, D. A., WILBY, O. K. & PROCTOR, J. (1978). Control of somite number in normal and *amputated* mouse embryos: an experimental and a theoretical analysis. *J. Embryol. exp. Morph.* **45**, 189–202.
- GALLERA, J. (1975). At what stage of development does the somitic mesoblast invaginate into the primitive streak of the chick embryo? *Experientia* **31**, 584–585.
- GESINK, A. F., POELMANN, R. E., SMITS-VAN PROOIJ, A. E. & VERMEIJ-KEERS, CHR. (1983). The cell surface coat during closure of the neural tube, as revealed by

- concanavalin A and wheat germ agglutinin. *J. Anat.* **137**, 418–419.
- HORISBERGER, M. & ROSSET, J. (1977). Colloidal gold, a useful marker for transmission and scanning microscopy. *J. Histochem. Cytochem.* **25**, 295–305.
- JACOBSON, A. G. & TAM, P. P. L. (1982). Cephalic neurulation in the mouse embryo analyzed by SEM and morphometry. *Anat. Rec.* **203**, 375–396.
- JAENISCH, R. (1985). Mammalian neural crest cells participate in normal embryonic development on microinjection into post-implantation mouse embryos. *Nature, Lond.* **318**, 181–183.
- JOLLY, J. & FERESTER-TADIE, M. (1936). Recherches sur l'oeuf du rat et de la souris. *Archs Anat. microsc. Morph. exp.* **32**, 323–390.
- JURAND, A. (1974). Some aspects of the development of notochord in mouse embryos. *J. Embryol. exp. Morph.* **32**, 1–33.
- MEIER, S. & JACOBSON, A. G. (1982). Experimental studies of the origin and expression of metamerie pattern in the chick embryo. *J. exp. Zool.* **219**, 217–232.
- MEIER, S. & TAM, P. P. L. (1982). Metamerie pattern development in the embryonic axis of the mouse. I. Differentiation of the cranial segments. *Differentiation* **21**, 95–108.
- NEW, D. A. T., COPPOLA, P. T. & COCKROFT, D. (1976). Comparison of growth *in vitro* and *in vivo* of postimplantation rat embryos. *J. Embryol. exp. Morph.* **36**, 133–144.
- NICOLET, G. (1970). Analyse autoradiographique de la localisation des différentes ébauches présomptives dans la ligne primitive de l'embryon de Poulet. *J. Embryol. exp. Morph.* **23**, 79–108.
- NICOLET, G. (1971). Avian gastrulation. *Adv. Morph.* **9**, 231–262.
- OOL, E. C. V., SANDERS, E. J. & BELLAIRS, R. (1986). The contribution of the primitive streak to the somites in the avian embryo. *J. Embryol. exp. Morph.* **92**, 193–206.
- OŹDŹENSKI, W. (1967). Observations on the origin of primordial germ cells in the mouse. *Zool. Pol.* **17**, 367–379.
- PACKARD, D. S. JR & JACOBSON, A. G. (1976). The influence of axial structures on chick somite formation. *Devl Biol.* **53**, 36–48.
- PASTEELS, J. (1937). Etudes sur la gastrulation des vertébrés meroblastiques. III. Oiseaux. IV. Conclusions generales. *Archs Biol.* **48**, 381–488.
- POELMANN, R. E. (1981a). The formation of the embryonic mesoderm in the early post-implantation mouse embryos. *Anat. Embryol.* **162**, 29–40.
- POELMANN, R. E. (1981b). The head process and the formation of the definitive endoderm in the mouse embryo. *Anat. Embryol.* **162**, 41–49.
- ROSENQUIST, G. (1966). A radioautographic study of labelled grafts in the chick blastoderm. Development from primitive streak stage to stage 12. *Contr. Embryol. Carneg. Inst.* **38**, 71–110.
- RUGH, R. (1968). *The Mouse*. Minneapolis: Burgess.
- SCHOENWOLF, G. C. (1977). Tail (end) bud contributions to the posterior region of the chick embryo. *J. exp. Zool.* **201**, 227–246.
- SCHOENWOLF, G. C. (1984). Histological and ultrastructural studies of the secondary neurulation in mouse embryos. *Am. J. Anat.* **169**, 361–376.
- 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.
- SMITS-VAN PROOIJ, A. E., POELMANN, R. E., DUBBELDAM, J. A., MENTINK, M. M. T. & VERMEIJ-KEERS, CHR. (1986). WGA-Au as a novel marker for mesectoderm formation in mouse embryos cultured *in vitro*. *Stain Tech.* **61**, 97–106.
- SNELL, G. D. & STEVENS, L. C. (1966). Early embryology. In *Biology of the Laboratory Mouse* (ed. E. L. Green), pp. 205–245. New York: Dover Pub. Inc.
- SNOW, M. H. L. (1981). Autonomous development of parts isolated from primitive-streak-stage mouse embryos. Is development clonal? *J. Embryol. exp. Morph.* **65 Supplement**, 269–287.
- SPRATT, N. T. JR (1955). Analysis of the organizer center of the early chick embryo. Localization of prospective notochord and somitic cells. *J. exp. Zool.* **128**, 121–164.
- STEELE, C. E. & NEW, D. A. T. (1974). Serum variants causing the formation of double hearts and other abnormalities in explanted rat embryos. *J. Embryol. exp. Morph.* **31**, 707–719.
- ŠVAJGER, A., KOSTOVIĆ-KNEŽEVIĆ, L., BRADAMANTE, Ž. & WIRCHER, M. (1985). Tail gut formation in the rat embryo. *Wilhelm Roux Arch. devl Biol.* **194**, 429–432.
- ŠVAJGER, A., LEVAK-ŠVAJGER, B. & ŠKREB, N. (1986). Review article. Rat embryonic ectoderm as renal isograft. *J. Embryol. exp. Morph.* **94**, 1–27.
- 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 in the caudal end of the embryonic axis of the mouse. *J. Embryol. exp. Morph.* **82**, 253–266.
- TAM, P. P. L. (1986). A study on the pattern of prospective somites in the presomitic mesoderm of mouse embryos. *J. Embryol. exp. Morph.* **92**, 269–285.
- TAM, P. P. L. & BEDDINGTON, R. S. P. (1986). The metamerie organization of the presomitic mesoderm and somite specification in the mouse embryo. In *Somites in Developing Embryos* (ed. R. Bellairs, D. A. Ede & J. Lash). New York: Plenum Pub. Co. (in press).
- TAM, P. P. L. & MEIER, S. (1982). The establishment of a somitomerie pattern in the mesoderm of the gastrulating mouse embryo. *Am. J. Anat.* **164**, 209–225.

- TAM, P. P. L., MEIER, S. & JACOBSON, A. G. (1982). Differentiation of the metameric pattern in the embryonic axis of the mouse. II. Somitomic organization of the presomitic mesoderm. *Differentiation* **21**, 109–122.
- TAM, P. P. L. & SNOW, M. H. L. (1980). The *in vitro* culture of primitive-streak-stage mouse embryos. *J. Embryol. exp. Morph.* **59**, 131–143.
- TAN, S. S. & MORRIS-KAY, G. (1986). Analysis of cranial neural crest cell migration and early fates in postimplantation rat chimaeras. *J. Embryol. exp. Morph.* **98**, 21–58.
- VAKAET, L. (1984). Early development of birds. In *Chimaeras in Developmental Biology* (ed. N. LeDouarin & A. McLaren), pp. 71–88. London: Academic Press.
- WHITTINGHAM, D. G. & WALES, R. G. (1969). Storage of two-cell mouse embryos *in vitro*. *Austr. J. biol. Sci.* **22**, 1065–1068.

(Accepted 8 October 1986)