J. Embryol. exp. Morph. Vol. 49, pp. 295–306, 1979
Printed in Great Britain © Company of Biologists Limited 1979

The influence of local environment on the organization of mesenchyme cells

By MARILYN FISHER[1] AND MICHAEL SOLURSH[2]
From the Department of Zoology, University of Iowa

SUMMARY

Limb, somite, and neural crest mesenchyme from quail embryos were implanted orthotopically and heterotopically into chick hosts to ascertain the relative importance of the local environment on mesenchyme migration. It was found that mesenchyme behavior is strongly influenced by the environment. Normally non-migratory, limb mesenchyme is capable of spreading like sclerotome when placed in the somite region. A somite placed in the limb acquires an appearance typical of limb mesenchyme. Neural crest placed in the limb migrates only along the co-implanted neural tube or axons growing out from it. The orthotopic transplantations showed that quail mesenchyme behaves normally in chick embryos. Furthermore, it was observed in the orthotopic transplants that there was no intermingling of quail and chick cells even at the edge of the graft. This result indicates that cells within mesenchyme are normally not locomotory; rather, the mesenchyme 'migrates' by spreading and expansion of the tissue as a unit in response to local influences.

INTRODUCTION

Early in the development of the amniote embryo a number of complex cell rearrangements occur which establish new associations between previously separated groups of cells. These rearrangements are vital to the continuing formation of a complex, coordinately functioning individual. The morphogenetic activities of mesenchyme are of particular interest since the entire mesoderm arises as mesenchyme, the primary mesenchyme, and is mesenchymal during much of the organogenic phase of embryonic development.

The association of mesenchyme with appropriate embryonic rudiments often involves the translocation of cells over some distance. The neural crest is the most extreme example of this. These cells arise from ectoderm at the dorsal surface of the neural tube. They subsequently become distributed throughout the body where they will give rise to the numerous crest derivatives, including: spinal ganglia, pigment cells, enteric ganglia and plexi, Schwann cells, and chromaffin cells of the adrenal medulla (reviewed by Weston, 1970). A less extreme example of mesenchyme translocation can be found in the sclerotome

[1] *Author's address:* The Jackson Laboratory, Bar Harbor, Maine 04609, U.S.A.
[2] *Author's address for reprints:* Department of Zoology, University of Iowa, Iowa City, Iowa 52242, U.S.A.

of a differentiating somite. These cells must translocate over a short distance to surround the neural tube and notochord. An example of a non-migratory mesenchyme is the limb. This mesenchyme undergoes extensive proliferation, but there is no evidence of migration of cells within the mesenchyme (Searls, 1967).

However, direct observation of mesenchymal cell behavior is, in most cases, not possible due to the internal position of the mesenchyme within growing embryos. Thus, it is difficult to obtain accurate information concerning the translocation of the mesenchymal mass and, particularly, the behavior of individual cells. It might be inferred from the shape of mesenchymal cells and the presence of filopodial processes in contact with surrounding cells and extracellular matrix material that individual cells within a migratory mesenchyme are actively locomotory.

While all mesenchymes are morphologically similar, they exhibit diverse morphogenetic behaviors – from the extensive migration of the neural crest to the absence of migration exhibited by limb mesenchyme. It is of interest to determine the basis for these differences in morphogenetic activity.

The purpose of this study is to determine the relative importance of environment and cell origin to mesenchyme morphogenesis. It is reasoned that if environmental influences are relatively more important, then mesenchyme will behave in accordance with its environment. For example, if limb mesenchyme is normally nonmigratory, it is because the limb environment is not appropriate for cell migration; but limb mesenchyme could be made to undergo extensive morphogenesis if placed in an appropriate environment, e.g. the somite region. Conversely, if morphogenetic behavior is an inherent property of each mesenchyme, then altering the environment should have little effect on the observed behavior of the mesenchyme.

These possibilities were tested in a series of orthotopic and heterotopic transplantations making use of the naturally occurring quail cell marker introduced by Le Douarin (1973). The results provide evidence of the influence of the environment on mesenchyme morphogenesis as well as some insight into the mechanism of mesenchyme translocation.

MATERIALS AND METHODS

Tissue preparation

Tissue for transplantation was obtained from quail embryos (*Coturnix coturnix japonica*) incubated at 38 °C until reaching stage 11–12 (Hamburger & Hamilton, 1951) for neural crest and somite or to stage 22–23 for limb mesenchyme. Tissues were removed from quail embryos and prepared for transplantation as follows:

To obtain somites or neural crest the axial region containing the last three somites was excised from the embryo in Tyrode saline. The lateral plate mesoderm

along with ectoderm and endoderm in this region were dissected away, and the remaining axial tissue was placed in 0·1 % Trypsin (Difco 1:250) in calcium and magnesium-free (CMF) Tyrode for 5–10 min at 37 °C. Following trypsinization the tissue was placed in a mixture containing equal parts of horse serum and CMF Saline G where the separation of somites and cleaning of the neural tube were completed by dissection. This neural tube, from which neural crest had not yet begun to migrate (Weston, 1963), was then cut into pieces approximately equal in length to one somite diameter and implanted intact. Limb mesenchyme was obtained by removing a limb-bud and excising a transverse slice from the middle of the bud. The ectoderm was cut off of the slice using tungsten needles and the remaining mesenchyme was cut into pieces of approximately the same size as the young somites. Epithelial somites were isolated intact by mechanical dissection from the trypsinized tissue.

Tissues to be implanted were stained lightly with Nile blue sulfate-impregnated agar chips (Hamburger, 1960) and kept on ice while the host embryos were prepared.

Host preparation

The host chick embryos (Hubbard Golden Comet, obtained locally) were incubated at 38 °C to stage 11–12 for orthotopic neural crest and somite or heterotopic limb transplantations, and to stage 22–23 for orthotopic limb or heterotopic neural crest and somite transplantations. The eggs were candled to determine the position of the embryo, and then a small opening was made through the shell directly above the embryo. The vitelline membrane and, where appropriate, the extra-embryonic membranes were torn over the desired region. The surface of the host was in some cases also stained lightly with Nile blue sulfate. For orthotopic somite or neural crest transplants a somite or a small region of neural tube, respectively, was removed from the host and replaced with the appropriate quail tissue. In some cases, somites were implanted in the unsegmented mesoderm immediately posterior to the last fully condensed somite. After the quail tissue was in place the egg was resealed with Parafilm and returned to the incubator for 24–48 h.

Fixation

The chick hosts were removed from the eggs 24–48 h after the operation, fixed with Zenker's fluid for 4–6 h, washed overnight in running water, treated with saturated iodine in 70 % ethanol for 5–8 h to remove excess $HgCl_2$, and then dehydrated for paraffin embedding. Seven to ten micron sections were stained by the Feulgen method and counter-stained with fast green (Humason, 1972).

Table 1. *Orthotopic and heterotopic mesenchyme transplantation*

Mesenchyme	Transplanted to
Neural crest	Neural crest region
	Limb
Sclerotome	Somite region
	Limb
Limb	Limb
	Somite region

RESULTS

To study the possible influence of the immediate environment on mesenchyme morphogenesis, mesenchyme was removed from quail embryos and implanted orthotopically or heterotopically into chick embryos. The various transplantations, summarized in Table 1, were designed such that presumed migratory mesenchymes were implanted into a non-migratory region, and a non-migratory mesenchyme was implanted into a region normally occupied by migratory mesenchyme. Between five and ten successful implants were examined in each case.

Orthotopic transplantations

Somites or neural crest from quail embryos, equivalent to stage 11–12 chick embryos, were removed and implanted in the corresponding location in chick embryos of the same stage. Quail limb mesenchyme was derived from embryos of stage 22–23 and likewise implanted into chick hosts of comparable stage. The following observations were made in sections from embryos fixed 24–48 h after the operation.

Neural crest

Neural tube pieces implanted orthotopically in the trunk give rise to neural crest cells which can be seen lateral to the neural tube between the dorsal wall of the somite and the overlying ectoderm and between the neural tube and medial wall of the somite. These are the normal paths for neural crest cell migration (Weston, 1963).

Somite

Orthotopic somite implants show a degree of somite differentiation appropriate to the stage of the embryo. Although no attention was paid to the orientation of the somite when implanted, in all cases observed, these somites appeared to differentiate with the normal orientation of dermatome, myotome

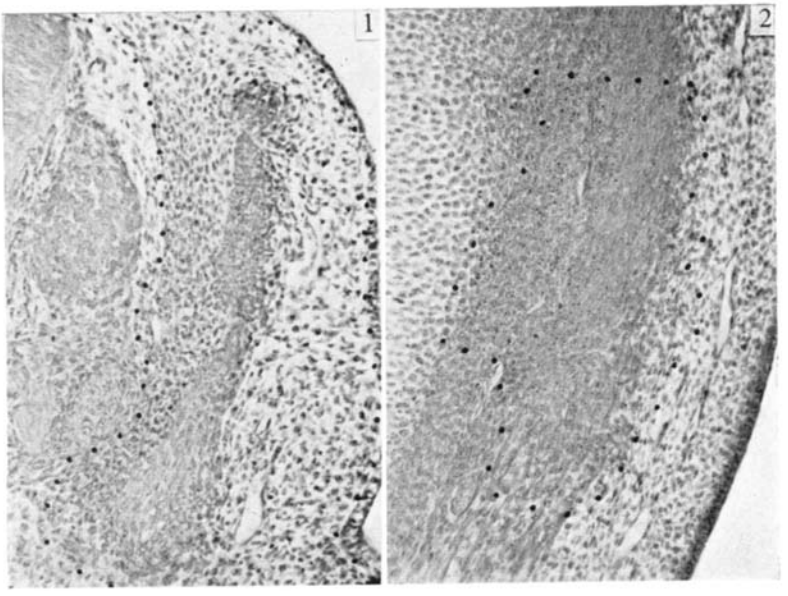

Fig. 1. Orthotopic somite implant (48 h post-implantation) which shows differentiating quail dermatome, myotome and sclerotome. The quail tissue is marked with a dotted line. Note that there are chick cells in the sclerotome region, but they are clearly delimited from the implanted quail tissue. 180×.

Fig. 2. Orthotopic limb implants (48 h post-implantation) show quail tissue smoothly incorporated, in this case, in the perichondrial core region. The quail tissue is marked by a dotted line. 180×.

and sclerotome (Fig. 1). In three of six cases observed, transverse sections through the operated region reveal one or two somites with both host and donor components. Presumably this is due to incomplete removal of host tissue prior to implantation.

Limb

Orthotopic limb implants likewise appear smoothly incorporated within the limb of the host. They show the typical arrangement of compact cells in the central core of the limb with a peripheral zone of more disperse cells underlying the limb ectoderm (Fig. 2).

General

In the cases of somite and limb implants there is no mixing of quail and chick cells even at the edges of the implant. The somite has changed from an epithelial ball to a roughly triangular prism of mesenchyme with regions consisting of epithelium and compact and dispersed mesenchyme, but it has undergone this transformation as a unit. Indeed, even in somites with both quail and chick components a distinct border is seen between chick and quail cells. Likewise,

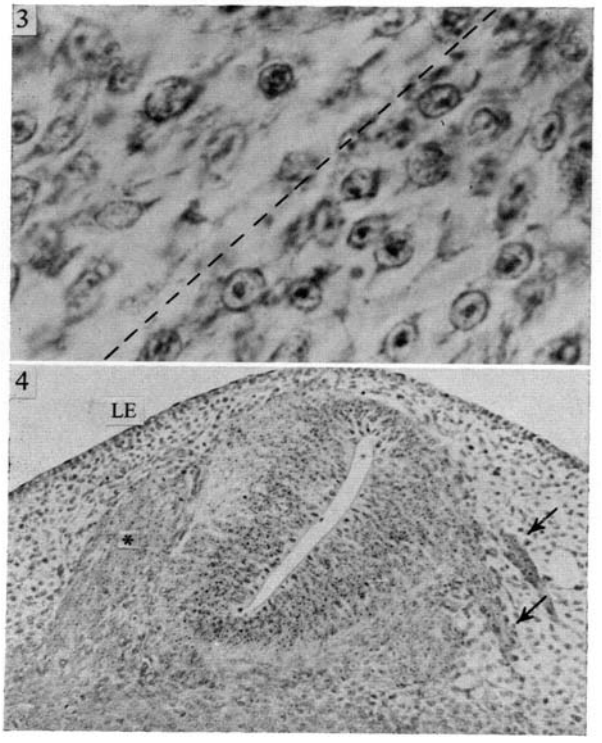

Fig. 3. This figure illustrates part of the boundary between quail and chick sclerotomal cells in an orthotopic somite implant. The quail cells are to the right of the dashed line and the chick cells on the left. 1000×.

Fig. 4. Heterotopic neural tube/neural crest implant (48 h post-implantation) in the limb of a chick host shows the formation of spinal ganglia (*) adjacent to the neural tube and outgrowth of axons (arrows) from the neural tube. No migratory neural crest cells could be seen beneath the limb ectoderm (LE). 180×.

an initially spherical or cuboidal limb implant becomes elongated along the proximodistal axis of the limb as a unit with no intermingling of quail and chick cells. The distinct border between the quail and chick cells is particularly apparent upon examination of serial sections through the implant region, and is illustrated in Fig. 3.

Heterotopic transplantations

Neural crest

Neural crest, when implanted in the limb, migrates along the co-implanted neural tube, forms spinal ganglia, and migrates along axons arising from the neural tube piece. Neural crest cells are otherwise not found dispersed through the limb (Fig. 4).

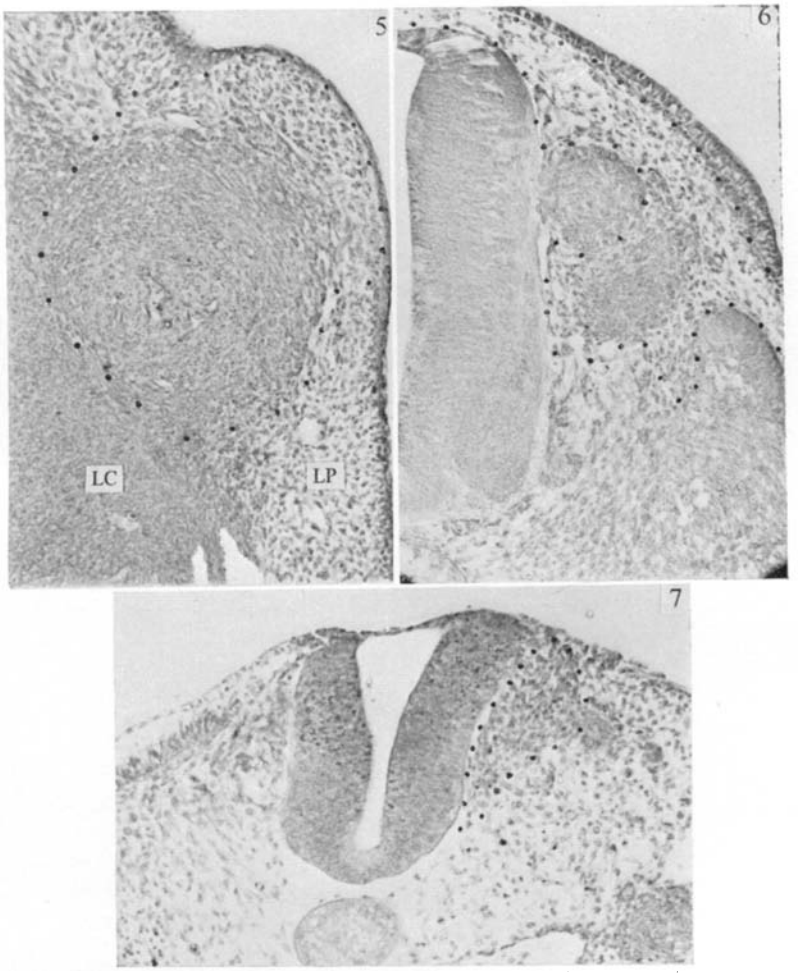

Fig. 5. Heterotopic somite implant in the limb of a chick host (48 h post-implantation), in this case shows a ball of condensed cells and a zone of more disperse cells underlying the limb ectoderm. The quail tissue is marked by a dotted line. Note especially that the condensed somite cells are similar in appearance to the adjacent limb core cells (LC), and the dispersed somite cells are similar in appearance to the adjacent limb periphery (LP) cells. 180×.

Fig. 6. Limb mesenchyme implanted in the somite region of a chick host (48 h post-implantation) consisting of a condensed mass of cells between the spinal ganglion and the remaining piece of host somite with dispersed cells extending into the sclerotome and dermatome regions. The quail tissue has been outlined to illustrate the extent and direction of spreading of the limb mesenchyme. The thickening of the ectoderm seen here adjacent to the limb implant is typical of such implants and resembles the apical ectodermal ridge of the limb. Its presence here underscores the separation between the differentiative and morphogenetic potentials of a mesenchyme. 180×.

Fig. 7. This figure shows another example of limb mesenchyme implanted near the neural tube of the host (24 h post-implantation). This section shows a vertically oriented mass of quail cells (outlined) adjacent to the neural tube. Other sections, not shown, reveal that part of the quail tissue extends laterally, immediately subjacent to the ectoderm. This implant shows limb mesenchyme spreading along tracks normally followed by neural crest. 180×.

Somite

Somites implanted in the limb lose their epithelial organization and appear as a solid sphere of rounded cells similar in appearance to surrounding limb mesenchyme. The configuration of the somite-derived mesenchyme is very much dependent on the location of the implant within the limb. The cells of implants which are located entirely within the core region are condensed in accordance with their location. In addition, such an implant is elongated along the proximodistal axis. The cells in implants which overlap the core and peripheral regions are dispersed in that part closest to the ectoderm and condensed in that part closest to the limb's core. In general, it may be said that where somite-derived cells contact limb ectoderm there is an even zone of dispersed cells which look very similar to limb peripheral cells, while the somite cells further away from the limb ectoderm are condensed, similar to limb core cells (Fig. 5).

In one case a piece of tissue containing both neural tube and somite was implanted in a limb. In this instance the somite was clearly recognizable 24 h later, and had undergone sclerotome formation while retaining a clear dermatome, myotome epithelium. When neural tube is not implanted with somite, in every case observed, after 24 h the somite is seen as a spherical mass of mesenchyme.

Limb

Implants of limb mesenchyme in the somite region are similarly influenced by their environment. Limb cells will not organize into an epithelial dermatome-myotome band, but they will disperse throughout the area normally occupied by somite-derived mesenchyme. As in the limb, there is a correlation between proximity to the surface ectoderm or neural ectoderm and the degree of dispersal of the implanted mesenchyme (Fig. 6). In one case, a piece of limb mesenchyme inserted just beneath the ectoderm adjacent to the neural tube became a L-shaped, spreading ventrally along the neural tube and laterally under the ectoderm in the paths normally followed by neural crest cells (Fig. 7).

DISCUSSION

When small pieces of quail limb, neural tube (with associated neural crest) and whole somites are orthotopically transplanted to chick hosts, mesenchyme derived from these implants behaves in the expected manner. Neural crest cells migrate along the usual paths; limb mesenchyme is smoothly incorporated into the host limb; and a somite will differentiate normally into sclerotome, dermatome and myotome regions. A perhaps unexpected observation, particularly in the case of the differentiating somite, is the lack of mixing of quail and chick cells even around the edge of the orthotopic implants. A similar

result was observed by Searls (1967) when he implanted pieces of [^{3}H] thymidine-labeled chick limb into unlabeled chick host limbs of comparable stage. As discussed by Ede & Agerbak (1968), cellular rearrangement is probably necessary during limb development to account for the observed proximodistal elongation of the limb and the orientation of the cartilage cells. However, the result obtained by Searls and repeated here using a different labeling technique suggests that locomotory activity of individual cells is not involved. It appears instead, that the cell rearrangement which occurs in the developing limb, as evidenced by the change in shape of the implanted tissue, is brought about by some mechanism which acts on cells as a group.

The similar behavior of a quail somite developing in the axial region of a chick host is somewhat more unexpected. Particularly in somites with quail and chick components, one might expect extensive intermingling of the migrating sclerotome cells. That the absence of cell mixing is a real representation of the way somite differentiation occurs normally and cannot be explained by a tendency for quail and chick cells to sort out from one another is supported by several observations: (1) experiments done in this laboratory clearly demonstrate that quail and chick limb cells mixed together *in vitro* do not sort out from one another on the basis of species (Ahrens & Solursh, in preparation); (2) Armstrong & Armstrong (1973) reported that fused aggregates of quail and chick mesonephrogenic mesenchyme show a moderate degree of intermingling – similar to what is observed between fused aggregates of [^{3}H]thymidine-labeled and unlabeled chick mesonephrogenic mesenchyme; (3) studies by Le Douarin and colleagues (e.g. Le Douarin & Teillet, 1974; Le Lievre & Le Douarin, 1975) demonstrate that quail neural crest cells are capable of undergoing normal migration and differentiation in chick hosts. Thus, there is no reason to suspect that the lack of intermingling of quail and chick cells in orthotopic implants is due to cell segregation based on the difference between these closely related species.

Therefore, even though mesenchymal cells have a morphology consistent with their being freely moving individuals and do have the capability of moving as individuals through a mesenchymal mass *in vitro* (Armstrong & Armstrong, 1973), the results presented here suggest that limb and somite mesenchymal cells do not normally migrate as individuals, but as a unit. It is not possible from these studies to determine whether this is also true of migrating neural crest cells. It would appear, however, that the migration of somite mesenchyme involves the expansion of a coherent mass of cells – similar perhaps to the spreading of an epithelial sheet, only in three dimensions rather than two. It is possible, too, that the cell rearrangement which occurs normally in the limb proceeds by the same mechanism, but to a lesser degree.

If mesenchyme translocation is brought about by the dispersion of a coherent unit of cells, what then might determine the extent to which a mesenchyme will spread *in vivo*? A partial answer to this question is suggested by the observa-

tions made of the various heterotopic transplantations. In each type of heterotopic transplant there is strong evidence of the environment's influence on mesenchyme morphogenesis. Neural crest cells placed in the limb, which is devoid of the cell-free paths through which these cells normally migrate, show greatly reduced migratory activity. They remain close to the implanted neural tube or axonal outgrowths from it. They do not move under the limb ectoderm as they would under the trunk ectoderm. Somites placed in the limb lose their epithelial organization and show regions of highly condensed and dispersed cells, appropriate to the condensed core and dispersed peripheral cells of the limb. Finally, the altered morphology of a piece of limb mesenchyme implanted next to the neural tube is another striking indication of environmental control. These cells spread out in a manner appropriate to the somite derivatives, only with the exception that limb cells do not organize into a dermatome-myotome epithelium.

The combined results of the orthotopic and heterotopic transplantations suggest that the morphogenetic potentials – but not necessarily the differentiative potentials – of limb and somite mesenchyme are quite similar, and that the difference normally observed between their behaviors is due largely to environmental influence. The nature of this influence is unknown, but several observations support the speculation that the sources of the influence are the various epithelia normally associated with these mesenchymes: (1) where somite implants lie close to limb ectoderm there is a peripheral zone of uniform width of disperse cells overlying more compact mesenchyme; (2) limb mesenchyme in the somite region shows varying degrees of cell dispersion in accordance with cell distance from either the surface ectoderm or neural ectoderm; (3) the result obtained in the case where a tissue piece containing somite and neural tube was implanted in the limb is quite different from that obtained when a somite is implanted alone. In the former instance, apparently normal somite differentiation occurs in 24 h. In the latter instance, the somite shows no trace of normal somite organization 24 h after the operation; (4) Armstrong & Armstrong (1973) reported that in two cases of mesonephrogenic mesenchymal fragment fusions no intermingling of quail and chick cells occurred. In both cases there was extensive epithelial development due to incomplete removal of the epithelium during the initial cell preparation procedures.

To understand how an epithelium might influence the degree of spreading of a mesenchyme we need to know the mechanism of mesenchyme spreading. A model is presented here which will be described in more detail and supported by additional experimental evidence in a later paper (Solursh *et al.* 1979). Briefly, the main points are as follows: if mesenchyme translocation is brought about by the dispersion of a coherent unit of cells as suggested by the results reported here, then this activity may be similar in some respects to the spreading of an epithelial sheet. In epithelial spreading most of the locomotory force is provided by cells at the periphery (Dipasquale, 1975). Similarly, peripheral

mesenchyme cells may move along suitable substrata, such as epithelial basal laminae, and thus guide the expanding mass. Unlike a spreading epithelium, however, the mesenchyme is moving in three dimensions. This is accomplished by filling the intercellular spaces with an expanding matrix, an important component of which is hyaluronic acid (reviewed in Toole, 1976; and Fisher & Solursh, 1977).

It is possible that particular epithelia contribute to the mesenchyme-associated extracellular matrix. For example, hyaluronate production by the lateral, non-neural ectoderm can be demonstrated autoradiographically by the incorporation of [^{3}H]glucosamine into hyaluronidase sensitive material (Solursh, Fisher and Singley 1979).

A second possible mechanism by which an epithelium could influence the degree of mesenchyme spreading is by providing substrata of varying suitabilities to support such spreading. Very little is known about the compositions of different epithelial basal laminae, but it seems reasonable that the composition could vary from epithelium to epithelium. In fact, corneal epithelium (Meier & Hay, 1973; Trelstad, Hayashi & Toole, 1974) and salivary gland epithelium (Cohn, Banerjee & Bernfield, 1977) have been shown to produce basal laminae which differ from each other in glycosaminoglycan content. Salivary gland epithelium produces hyaluronic acid and sulfated glycosaminoglycans, while corneal epithelium makes only sulfated glycosaminoglycans. The possibility of other differences in the composition of various basal laminae warrants study.

These investigations were supported by NSF grant PCM 77-01154 and NIH grant HD-05505 to M. S. and USPHS training grant No. HD-00152 from the National Institute of Child Health and Human Development, while M. F. was a predoctoral trainee.

REFERENCES

ARMSTRONG, P. B. & ARMSTRONG, M. T. (1973). Are cells in solid tissues immobile? Mesonephric mesenchyme studied *in vitro*. *Devl Biol.* **35**, 187–209.

COHN, R. H., BANERJEE, S. D., & BERNFIELD, M. R. (1977). Basal lamina of embryonic salivary epithelia. Nature of glycosaminoglycan and organization of extracellular materials. *J. Cell Biol.* **73**, 464–478.

DIPASQUALE, A. (1975). Locomotory activity of epithelial cells in culture. *Expl Cell Res.* **94**, 191–215.

EDE, D. A. & AGERBAK, G. S. (1968). Cell adhesion and movement in relation to the developing limb pattern in normal and *talpid*^{3} mutant chick embryos. *J. Embryol. exp. Morph.* **20**, 81–100.

FISHER, M. & SOLURSH, M. (1977). Glycosaminoglycan localization and role in maintenance of tissue spaces in the early chick embryo. *J. Embryol. exp. Morph.* **42**, 195–207.

HAMBURGER, V. (1960). *A Manual of Experimental Embryology*, p. 61. Chicago: University of Chicago Press.

HAMBURGER, V. & HAMILTON, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.

HUMASON, G. L. (1972). *Animal Tissue Techniques*, 3rd ed., pp. 317, 320, 332. San Francisco: W. H. Freeman & Co.

LE DOUARIN, N. (1973). A biological cell labelling technique and its use in experimental embryology. *Devl Biol.* **30**, 217–222.

LE DOUARIN, N. M. & TEILLET, M. A. (1974). Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neuroectodermal mesenchymal derivatives using a biological cell marking technique. *Devl Biol.* **41**, 162–184.

LE LIEVRE, C. S. & LE DOUARIN, N. M. (1975). Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. *J. Embryol. exp. Morph.* **34**, 125–154.

MEIER, S. & HAY, E. D. (1973). Synthesis of sulfated glycosaminoglycans by embryonic corneal epithelium. *Devl Biol.* **35**, 318–331.

SEARLS, R. L. (1967). The role of cell migration in the development of the embryonic chick limb bud. *J. exp. Zool.* **166**, 39–50.

SOLURSH, M., FISHER, M. & SINGLEY, C. T. (1979). The synthesis of hyaluronic acid by ectoderm during early organogenesis in the chick embryo (manuscript submitted).

SOLURSH, M., MEIER, L., FISHER, M. & SINGLEY, C. T. (1979). The formation of the sclerotome (manuscript submitted).

TOOLE, B. P. (1976). Morphogenetic role of glycosaminoglycans (acid mucopolysaccharides) in brain and other tissues. In *Neuronal Recognition* (ed. S. H. Barondes), pp. 275–329. New York: Plenum.

TRELSTAD, R. L., HAYASHI, K. & TOOLE, B. P. (1974). Epithelial collagens and glycosaminoglycans in the embryonic cornea. Macromolecular order and morphogenesis in the basement membrane. *J. Cell Biol.* **62**, 815–830.

WESTON, J. A. (1963). A radioautographic analysis of the migration and localization of trunk neural crest cells in the chick. *Devl Biol.* **6**, 279–310.

WESTON, J. A. (1970). The migration and differentiation of neural crest cells. *Advances Morph.* **8**, 41–114.

(Received 6 July 1978, revised 1 September 1978)