An Analysis of the Postgastrula Differentiation of the Hypomere

II. The influence of endoderm and tissue mass in *Taricha torosa*

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INTRODUCTION

The initial report in this series (Finnegan, 1961) emphasized the role of the endoderm in the postgastrula differentiation of the hypomeric mesoderm in *Ambystoma punctatum*. The effect of the endoderm appeared to be modified when the mass of mesoderm involved was increased and, under the *in vitro* experimental conditions employed, the endoderm did not influence the splanchnic layer of the hypomere into new types of histogenesis (induction). Thus it was concluded that the endoderm aided the histogenesis of the splanchnic mesoderm in its vicinity in a synergistic manner rather than as an inductive tissue.

Further evidence of the mechanism of this assistance by the endoderm of the postgastrula development of the mesoderm has been obtained from similar *in vitro* studies with tissues from *Taricha torosa* neurulae. This report is concerned with results which substantiate the previously derived conclusions and, further, indicate at least one manner in which the endoderm effects its synergistic role.

EXPERIMENTAL PROCEDURES

*T. torosa* eggs were shipped air express from St. Mary's, California, and maintained in this laboratory in pond water at 7° C. I should like to note with gratitude my appreciation to Brother Lawrence Cory, F.S.C., St. Mary's College, for supplying eggs to this laboratory during the past several years. Explants were prepared as described in the earlier report on *A. punctatum* (Finnegan, 1961) and were cultured in modified Holtfreter or Niu-Twitty medium at 18° C. Briefly, the explants were prepared by excising a limited amount (approximately 0.5x0.5 mm.) of hypomeric mesoderm from the ventro-lateral flank of postgastrula stages (stages 13–21; Twitty & Bodenstein stages—see Rugh, 1948) along with the overlying ectoderm. These explants quickly formed an ectodermal ball with the hypomere internal and constituted the hypomere only or (*M*) series. A second group of explants was prepared in a similar manner and a small mass of endoderm cells, taken from the lateral pharyngeal wall, was
added to the ectodermal ball; these explants constitute the hypomere plus endoderm \((M+E)\) series. The hypomere doubled \((2M)\) series was prepared by combining two hypomere only \((M)\) explants from similar-aged donors prior to the formation of ectodermal balls by these explants. A second method of increasing the amount of material from the mesoderm mantle was by increasing the length along the dorso-ventral axis. The anterior, posterior, and ventral borders of these explants were prepared as in the hypomere only \((M)\) series (Finnegan, 1961) and the incisions were then continued dorsally through the intermediate mesoderm (mesomere) and into the somitic mesoderm (epimere), typically of somites 4–6. That portion of the somites lying immediately adjacent to the neural tube and notochord was excluded in order to delete known or suspected axial influences (see Yamada, 1950; Muchmore, 1951, 1958; Holtzer & Detwiler, 1953; Ebert, 1959). The ectodermal covering for these explants was restricted to that ectoderm lying ventral to a line approximately one-half way down the somite mesoderm, any additional ectoderm required to complete the covering being taken from the ventral-anterior area of the donor animal. These explants constituted the hypomere plus epimere \((M+Ep)\) series.

The vitally stained explants were prepared by placing small pieces of cellophane previously stained with Nile blue sulphate (Rugh, 1948) on the anterior or posterior area of the explant immediately following its removal from the donor. The cellophane was held in place on the ectoderm so that the cells of the superficial epithelium were intensely stained in the small area of contact while the surrounding ectoderm cells were only lightly stained. Some penetration or diffusion of the stain to the more superficial mesoderm cells may have occurred during or following the short exposure of the explant to the cellophane. A similar procedure was used to produce superficial vital stain marks on the flanks of control embryos.

Explants were examined daily and sketches made of their development. At intervals during the culture period explants were fixed in Michaelis’ fluid, sectioned at 8–10 \(\mu\) and stained with haematoxylin and eosin for histological examination. The descriptions of histogenesis are based on the study of sections from approximately 50 per cent. of the explants in the larger groups, \((M)\) and \((M+E)\), and all of the explants in the two smaller groups, \((2M)\) and \((M+Ep)\).

**EXPERIMENTAL RESULTS**

Since, within an experimental series, the explants from all the postgastrula stages (i.e. stages 13–21) appeared to develop in a similar manner, they are discussed together, variation being noted where pertinent.

**Hypomeric mesoderm \((M)\) series (66 cases)**

All the explants in this experimental series became vesicular in the first 2 to 4 days. Of the 17 cases with vital stain marks, those on which the mark was located anteriorly indicated a posteriad stretching of the stained cells by the
third to fifth day of culture and the posteriad migration of these superficial cells continued into the second week (Text-fig. 1). When the posterior area was stained the superficial cells did not migrate anteriad but the stain became less intense during the first week as some of the cells moved to the interior of the ball and then migrated anteriad. A small circular patch of mesoderm was present internally in the posterior-ventral area of these stained explants. In the unstained cases the ectoderm superficial to this patch appeared roughened and the slight external pigment characteristic of this species was more concentrated in the area. These observations are taken to be indicative of cell migrations similar to those demonstrated in the vital stained cases. At the end of the first week (control stage 36–39) or early in the second week (control stage 40) the mesoderm patch appeared to show a pink coloration in some cases but no further evidence of haematopoiesis was visible macroscopically. In the explants from the stage 20 donor group there occasionally appeared a second small internal mass of mesoderm cells in the dorso-posterior aspect of the ball (Text-fig. 1).

These observations are indicative of a consistent migration of cells, and in control animals (stages 17–21) prepared with superficial vital stain-marks along the dorso-ventral axis at mid-trunk the displacement of cells (particularly noticeable following stage 24) indicated that the superficial flank tissue ventral to the yolk border elongated along the antero-posterior axis. Such results,
along with the confirming observations on the direction of ciliary beat (see Twitty & Bodenstein, 1941), demonstrate the maintenance of the original antero-posterior axis by the explanted tissues.

Examination of the sectioned material revealed, in the posteriorly located small mesoderm mass, haematoblasts and cells morphologically resembling erythrocytes but with unstained cytoplasm. Elsewhere the mesoderm appeared as a peritoneum underlying the epithelium and, in those explants fixed after 2 weeks of culture, the splanchnic mesoderm had differentiated as a second peritoneum lying internal to the somatic peritoneum. No granulopoiesis was evident though the stage 20 group produced small collections of acidophil cells and fibrous matrix in the splanchnic layer.

It could be seen that in none of these *T. torosa* explants did the degree of differentiation approach that obtained in the hypomere explants from stage 20 *A. punctatum* embryos (Finnegan, 1961) and thus 10 cases were prepared from *T. torosa* donors of stages 23-28. Both macroscopically and histologically these explants resembled those prepared from younger *T. torosa* developmental stages, though they did indicate the differentiation of endothelium and a larger number of acidophil cell groups in the splanchnic mesoderm, but no definite granulopoiesis could be identified. In general it appears that, under similar experimental conditions, the splanchnic mesoderm of *T. torosa* tends to disperse while that of *A. punctatum* more frequently remains as an internal mass, and the histogenesis observed reflects this difference.

Hypomeric mesoderm plus endoderm (*M+E*) series (47 cases)

For the most part the behaviour of these explants in culture was similar to that observed in the mesoderm (*M*) series reported above. In a number of cases the endoderm resided in the ventral or anterior end of the ball accompanied by a group of mesoderm cells. Otherwise the endodermal group was located in the posterior end of the ball in the vicinity of the apparently enlarged posterior-ventral internal patch of mesoderm.

Histological examination showed the large collection of splanchnic cells in the vicinity of the endoderm to be continuous with the splanchnic peritoneum found elsewhere in the explant. Within the splanchnic mesodermal mass those cells located immediately adjacent to the endoderm retained their yolk content longer than those further removed and, in the stage 20 group after 2 weeks in culture, these latter cells of the splanchnic mass appeared to be differentiating a reticular tissue (acidophil cells and matrix) while those mesoderm cells more closely juxtaposed to the endoderm demonstrated mitotic figures and basophil cytoplasm. In all cases, the explant areas distinct from the endodermal group were similar in histogenesis to the mesoderm (*M*) series (Text-fig. 3).

It seems then that the splanchnic mesoderm cells are retained in a group in the vicinity of the endoderm cells while elsewhere in the explant the dispersal encountered in the preceding (*M*) series occurs. The response of cytoplasm to
dye (basophil or acidophil) in these cell masses might be taken, along with the position of the mitotic figures, to indicate that the more superficial splanchnic cells somewhat removed from the endoderm, but responding to the mesoderm mass, were undergoing histogenesis, while cells closely associated with the endoderm remained undifferentiated (Text-fig. 3). The production of a second peritoneum by the splanchnic mesoderm in the \((M)\) series and in these \((M+E)\) explants in areas removed from the endoderm would appear to be in agreement with the observations of Jacobson (1960) on Taricha and Nieuwkoop (1947) on Triton that removal of the endoderm from early embryos resulted in an increased formation of peritoneum from splanchnic mesoderm.

_Hypomere doubled (2M) series (7 cases)_

These few explants were prepared from late stage 17 and stage 20 donor groups and since the results were consistent they are included here. In the main, the histogenesis observed resembled that of the mesoderm \((M)\) series and, though the stage 20 group produced a small endothelial area, the major splanchnic development continued to be peritoneum with a loose group of haematoblasts. Endoderm was added to two stage 20 explants and again there occurred the large collection of splanchnic mesoderm and the previously described histogenesis in the vicinity of these endoderm cells. Elsewhere these explants resembled the other \((2M)\) cases and no evidence of axial organization such as had been observed in similar \((2M)\) punctatum explants (Finnegan, 1961) was visible.

_Hypomere plus epimere (M+Ep) series (16 cases)_

Macroscopically, these explants from stage 17 and stage 20 donors resembled the \((M)\) and the \((M+E)\) series during the culture period in that they showed posteriad migration of the superficial material, an apparent involution of cells at the posterior end, and the appearance of two mesodermal groups within the vesicular ball (at the anterior and at the posterior-ventral ends of the original axis). At the end of the first week a duct-like structure could be seen coursing a short distance posteriad from the anterior mesoderm group, being particularly noticeable in several cases where vital stain had been placed anteriorly and the duct cells appeared as a short blue line along the wall of the ball (see discussion in Burns, 1955, of the origin of the pronephric duct from intermediate mesoderm.
associated with somites 5–7 in *Ambystoma*). In the second week this duct increased in length; it reached the posterior mesoderm group in only one case.

**TEXT-FIG. 4.** Hypomeric mesoderm plus epimere (*M+Ep*) series (stage 20 group—10 days). A nephric duct (*N.D.*) has differentiated and in the associated epimere (*Ep.*) some attempt at the formation of a nephric tubule (*Nt.*) seems to have occurred. ×160.

**TEXT-FIG. 5.** Epimeric mesoderm from a stage 17 *T. torosa* donor cultured in an ectodermal ball for 16 days. A well-differentiated nephric tubule (*Nt.*) was produced on the edge of a mass of epimere cells (located one section prior to illustrated area). ×160.

Histological examination showed that the anteriorly located group of mesoderm cells initially appeared as a loose collection of cells (parenchyma) which became aligned into columns of acidophil cells in the second week of culture and only in the stage 20 group produced a nephric tubule or two (Text-fig. 4). The hypomere lying ventral to the nephric duct appeared to remain as a more substantial group of basiphil cells than elsewhere in the explant and a few fibroblasts developed in the second week. Further posteriad the histogenesis resembled that of the other experimental series; no differentiating mesoderm group could be found in either the epimere or the hypomere, other than the possible haematopoietic cells, indicating that cell dispersal had occurred.

While myogenesis had not been anticipated (Muchmore, 1951, 1957) and was not observed, the failure of these (*M+Ep*) explants to produce more in the way of nephric tubule differentiation is interesting and puzzling when contrasted with results obtained in this laboratory in experiments in which the same area of epimere from stage 17 donors was cultured alone in ectodermal balls for over 2 weeks and in which well-developed tubules were produced (Text-fig. 5).

**DISCUSSION**

In an earlier publication (Finnegan, 1953) observations were reported of the endodermal effect on the behaviour of hypomere when the two tissues were placed in a confined space in a hanging drop culture. At that time it was stated (p. 379) that 'the migration of the mesodermal elements of these explants was
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retarded considerably and it appeared that the tissue affinity described by Holtfreter for these two tissue layers of the embryo was manifested in the inhibition of the migration commonly associated with mesodermal explants. Similarly, when ectoderm, mesoderm, and endoderm were combined and cultured under the same experimental conditions (p. 381), 'the typical mesodermal migration took place from the ectodermal region of the explant' but 'the majority of the mesodermal cells entering the area between the ectoderm and endoderm rounded up and, though not physically blocked from continued movement away from the explant, ceased migration'.

The experimental results reported here demonstrate that, in an ectodermal ball, Taricha torosa hypomere taken from postgastrula stages behaves in a similar manner, that is, it spreads in the absence of endoderm but remains as a mass of cells in close association with added endoderm. In this manner the endoderm would aid the subsequent differentiation of the hypomere since within the retained mass the 'localized predispositions for differentiation' (Muchmore, 1951) which are 'system-dependent' (Grobstein, 1959) may exert their influence. Muchmore (1957, 1958; see also Ebert, 1959) has postulated such a role for the endoderm and other neighbouring tissues in preventing the migration of somitic mesoderm cells and thereby aiding their subsequent differentiation.

A further role of the endoderm is also indicated by these results. Initially, the endoderm acted to retain the mesoderm in its vicinity and, after this relationship had existed for a time (by late in the second week of culture), the splanchnic cells juxtaposed to the endoderm were observed to be engaged in mitotic activity, as though they were part of a germinal layer. It must be made clear that those splanchnic mesoderm cells within the mass but removed from the immediate vicinity of the endoderm were differentiating while those cells directly in contact with the endoderm appeared to remain undifferentiated. Again, as in previous observations on T. torosa tissue (Finnegan, 1953), it was difficult to escape the impression that the endoderm group, in addition to discouraging migration, was also maintaining the mesoderm cells on its immediate periphery in a mitotically active, undifferentiated state. Possibly in this role the endoderm is the heterogeneous tissue, contact with which in some manner releases the mesoderm cells from the mitotic inhibition of adjacent mesoderm cells, as suggested by Weiss (1959, pp. 90–91), or its role may be more specific in this case. In either event it is to be noticed that the initial activity of the endoderm in retaining a mesodermal mass favours the differentiation of the mesoderm cells, while it is only later that the endodermal effect on mitosis can be observed in those mesoderm cells of the mass which are in contact with the endoderm cells. A similar conclusion was made from the analysis of mitotic counts in the A. punctatum explants (Finnegan, 1961) in which it was shown that the endoderm initially effected differentiation and later, in the third week of culture, seemed to influence mitotic activity. In comparing the results on the two species, it would appear that
the discouragement of splanchnic mesoderm dispersal occurs earlier in *A. punctatum* than in *T. torosa*.

The results obtained in the experimental series in which hypomere and epimere mesoderm (*M+Ep*) were included in the ectodermal ball indicate that the stimulus to nephric differentiation also acts (*in vitro*) to retain the neighbouring mesoderm as cell groups by preventing their dispersal. Perhaps it is that while cells are migratory any inductive tissue must first act (with specific cell adhesions) in such a manner as to obtain quiescence in the responding tissue and that a morphogenetic field initially is composed of the cells which have so responded and are so retained.

**SUMMARY**

1. A small mass of *T. torosa* postgastrula (stages 14–21) hypomere with its associated ectoderm, when cultured *in vitro*, demonstrates cell displacement posteriad along the original antero-posterior axis.

2. Histological examination of these explants indicates that the splanchnic mesoderm disperses and differentiates, as a peritoneum for the most part, with a loose group of haematoblasts residing in the cavity formed.

3. When a small group of endoderm cells was added to the hypomere-ectoderm ball, the results were similar to the above except that splanchnic mesoderm in the vicinity of the endoderm remained as a cell mass rather than dispersing as mesothelium. Late in the second week of culture mitotic figures became more prevalent in the splanchnic mesoderm in the immediate vicinity of the endoderm than elsewhere in the splanchnic cell mass.

4. When epimere (somite) mesoderm was included in the hypomere-ectoderm ball, a nephric duct developed anteriorly and mesoderm cells remained as a cell mass in the vicinity of this duct.

5. The results are discussed as indicating that the endoderm retains a mesoderm mass in its vicinity and thereby assists the differentiation of this mesoderm.

**RÉSUMÉ**

*Analyse de la différenciation de l'hypomère après la gastrulation*

II. L'influence de l'endoderme et de la quantité de tissu chez *Taricha torosa*

1. Une petite quantité d'hypomère de *Taricha torosa* après la gastrulation (stade 14–21), avec son ectoderme associé, montre, quand elle est cultivée *in vitro*, un déplacement de cellules vers l'arrière, le long de l'axe antéro-postérieur initial.

2. L'examen histologique de ces explants montre que le mésoderme splanchnique se disperse et se différencie principalement en péritoine, avec un groupe résiduel d'hématoblastes qui prennent place dans la cavité formée.

3. Lorsqu'un petit groupe de cellules endodermiques est ajouté à la sphérule formée d'hypomère et d'ectoderme, les résultats sont les mêmes que ci-dessus,
à part le fait que le mésoderme splanchnique, au voisinage de l'endoderme, constitue une masse de cellules au lieu de se répandre en un mésothélium. Plus tard, dans la deuxième semaine de culture, les figures mitotiques deviennent plus fréquentes dans le mésoderme splanchnique au voisinage immédiat de l'endoderme qu'aux autres niveaux de la masse cellulaire splanchnique.

4. Lorsque du mésoderme d'épimère (somite) est inclus dans la sphérule formée d'hypomère et d'ectoderme, un tube néphritique se développe vers l'avant, et les cellules mésodermiques restent massées au voisinage de ce tube.

5. La discussion des résultats porte sur l'indication que l'endoderme retient du matériel mésodermique dans son voisinage et de cette façon joue un rôle dans la différenciation de ce mésoderme.

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REFERENCES


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