Control of muscle differentiation by embryonic neural tissues

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INTRODUCTION

There have been several demonstrations that embryonic neural tissues can affect the differentiation of somitic muscle in amphibian embryos. In Triturus pyrrhogaster, presumptive somite mesoderm from the neurula, when explanted into an envelope of gastrula ectoderm, differentiates into muscle tissue under the influence of neural tissue developing from the ectoderm, though it will not differentiate into muscle when isolated in a jacket of neurula ectoderm (Yamada, 1939). In Ambystoma maculatum, somite mesoderm from tailbud embryos transplanted into the tail fin mesenchyme of older larvae shows poor muscle differentiation when alone or with notochord, but very good muscle differentiation in the presence of spinal cord (Holtzer, Lash & Holtzer, 1956). In the same species, presumptive somite mesoderm from the early neurula explanted into jackets of neurula ectoderm shows good muscle differentiation in the presence of embryonic spinal cord but not in its absence; and embryonic brain tissue seems to inhibit rather than encourage the differentiation of muscle in such explants (Muchmore, 1958a).

The following experiments were performed in an effort to elucidate further the effects of developing neural tissues upon the early differentiation of somitic muscle.

Part of this work has been reported to the American Society of Zoologists and has appeared in abstract form (Muchmore, 1958b).

MATERIALS AND METHODS

As in all previous work, the experiments were performed on early neurulae (stages 12–13) of the spotted salamander, Ambystoma maculatum. Freshly laid eggs were collected from ponds in the vicinity of Nashville, Tennessee and of Rochester, New York. The technique of preparing the embryos for operation was the same as described previously (Muchmore, 1951). Operations were
performed in Niu-Twitty solution and the animals or explants were then main-
tained in the same solution diluted to half or quarter strength.

In the transplantation experiments, donor and host were always at the same
stage of development. Donor areas and host sites are shown diagrammatically
in Text-fig. 1. The piece of tissue removed from the host site was purposely cut
somewhat smaller than the donor piece in order to compensate for retraction of
the edges of the wound. All operations were confined to the right side of the
animal, leaving the opposite side to serve as a control. Healing-in of the transplant
was rapid and complete in nearly all cases.

After about 10 days, the animals at about stage 39 or 40 were fixed in Bouin's
fluid, embedded in paraffin wax, cut serially at 12 μ, stained with hematoxylin
and eosin and studied microscopically. Because of the variability of the material
no attempt was made to measure quantitatively the changes in muscle mass in
the host animals. In many instances, however, to assist in visualizing the trend

![Text-fig. 1. Early neurula (stage 13) of Ambystoma from the right side. A, areas of the
ectoderm from which the transplants were taken; B, areas of the mesoderm which were
covered by the transplants.](image)

of these changes, comparisons were made of the cross-sectional areas of experi-
mental and control myotomes by projecting the image of a section on to bond
paper and tracing the outlines of the myotomes, then cutting out the tracings and
weighing the paper on a torsion balance. When the axis of the embryo is straight
(as these are at the time of fixation) and the sections have been cut reasonably
transversely (as most of these have been), there is no great difficulty in demon-
strating by this method that the experimental myotome is unchanged in size or is
increased or decreased as compared with the control side. The areas of myotomes
in sections of normal animals of stage 40 usually differ by less than 10 per cent.

RESULTS

Transplantation experiments

Series M/H

Medullary plate (M) from the trunk region was excised and transplanted into
the posterior head region (H) of another embryo of the same age (see Text-fig. 1).
This served to bring presumptive spinal cord in close relation to the most anterior (post-otic) somites, which normally give rise to relatively small masses of muscle. Embryos of stages 12, 12½ and 13 were used with the following results.

**Stage 12.** Of the ten surviving animals in this group, one is abnormal in having a large fistula on the operated side. Eight are entirely normal in the head region, indicating complete regulation of the graft into the brain and no influence upon muscle development. The remaining specimen possesses a supernumerary tail attached to the head in the region of the hind-brain and directed forward, with its axis (spinal cord and myotomes) connected to the primary axis; myotomes of the host are normal.

**Stage 12½.** In all of this group the brain is abnormal in some degree, particularly in the hind and mid regions but also in the forebrain in several cases. Six of the eleven animals in the group possess supernumerary tails, extending from the region of the mid- or hind-brain. Four of these tails are attached to the left of the mid-line, that is, on the unoperated side of the animal. Apart from the tails (which contain extra myotomes) the post-otic myotomes on the right side of the host are increased in size in four cases. In the best of these, the increase in cross-sectional area of some myotomes is about 50 per cent. as compared with the contralateral control. Enlarged myotomes are limited to the region of the transplant and myotomes elsewhere in the hosts are normal. Other mesodermal tissues appear normal.

**Stage 13.** There is considerable abnormality of the brain in all cases, involving mainly the hind- and mid-brain but also the forebrain, eyes and nose in five animals. All of the latter have small tails attached dorsally near the hind- or mid-brain. Eight of the ten animals examined in this group show a distinct increase in the amount of muscle along the hind-brain—in the best of these the increase in cross-sectional area is about 45 per cent. when compared with the control.

**Series Br/S**

A large piece from the anterior part of the medullary plate (Br) was transplanted over the trunk mesoderm (S) of another embryo of the same age. This served to substitute presumptive fore- and mid-brain for spinal cord and epidermis covering the presumptive trunk somites and pronephros of the host. Experiments in this series were performed at stages 12, 12½ and 13.

**Stage 12.** Regulation was good at this early stage. Of the five embryos in this series none shows more than a slight increase in size of the hind-brain and anterior cord. In two specimens the right myotomes are normal while the remaining three have the right myotomes very slightly reduced in the region of the graft.

**Stage 12½.** At this stage the presumptive brain material is apparently less plastic with the result that less regulation into spinal cord has occurred. The host embryos generally show a considerable increase in cross-section and length of the hind-brain, which occurs mainly on the right side but may involve the left as well. The organization of the extended hind-brain is often quite irregular, but
in no case does it include anything but hind-brain structures. In all but two of the fifteen specimens examined the myotomes of the right side are noticeably reduced in size along the extensive hind-brain. In a few cases the reduction is slight but in most it is considerable, amounting to 35–40 per cent. (in terms of cross-sectional area) in those myotomes most affected. Since there is no concomitant increase in myotome size further along the trunk, it can be concluded that the observed reduction in size of the anterior myotomes is a real reduction in mass of muscle tissue. While it is very difficult to measure the amount of nephric tissue present, it appears that the pronephroi are nearly equally developed on the two sides of the embryos.

Stage 13. Results of experiments at this stage are quite similar to the preceding. The hind-brain is extended back into the trunk for varying distances, usually more so on the right side than the left. Of the seventeen embryos kept for 10–12 days after operation, nine show a clear reduction of the right myotomes along the extensive hind-brain (up to 65 per cent. reduction in cross-section in one case), six have the right myotomes only slightly reduced and two appear normal. In no case is the right muscle mass greater than the left. Five other embryos were kept for longer periods before fixation and study—three for 22 days and two for 31 days. Of the former, two show considerable reduction of the right myotomes along an extended hind-brain, while in the other the relations of tissues in the posterior head region are much disturbed and the distribution of muscle is unclear. Both of those kept for 31 days have extensive hind-brains and much reduced myotomes on the right side. In all of these animals the pronephroi are more or less normal, some showing slightly more extensive development on the right, some on the left.

Series M/S

Medullary plate (M) from the trunk region was excised and transplanted orthotopically over the somite mesoderm (S) of a host of the same age. This served as a control for series Br/S. Embryos of stages 12½ and 13 were employed. Since there are no apparent differences in the results for the two stages, they are considered together.

Development was grossly normal except that three of the eleven specimens examined showed double spinal cords in the posterior trunk. Aside from the abnormalities associated with the divided cord the histological picture is more or less normal. In three specimens the right myotome is slightly smaller than the left, but this decrease is not more than about 10 per cent.

Series V/S

Ventral ectoderm (V) from an embryo of stage 12½ was substituted for medullary plate over the presumptive trunk somite mesoderm (S) of another embryo of the same age. This was employed as a further control, in the expectation that the ventral ectoderm would differentiate into epidermis and thus exert no neural influence on the underlying mesoderm.
Contrary to expectation, the ventral ectoderm became neuralized and participated smoothly in the formation of the host's hind-brain and spinal cord, so that the animals appeared quite normal after 10 days' culture. Histological examination revealed that in two of the eleven cases studied, the central nervous system is normal while in the remaining specimens there is a slight enlargement of the posterior hind-brain and/or the anterior spinal cord. In all cases the myotomes are more or less normal, with only occasional localized small increase or decrease in size.

**Series M/L**

Medullary plate (M) from the trunk region was excised and transplanted over the lateral mesoderm (L) of another embryo of the same age. This served to increase the amount of presumptive spinal cord on the right side and to bring the neural tissue into close relation to the presumptive pronephros on that side. If developing spinal cord does promote muscle differentiation, then it might be expected that myotome development would be enhanced at the expense of the pronephros. These experiments were performed on embryos at stage 13.

Externally the embryos appeared normal, except that on the right side the gills tended to be somewhat reduced and the forelimbs were displaced anteriorly and dorsally so that in the most extreme case the limb occurred dorsal to the gills. Sections revealed that the hind-brain and anterior part of the spinal cord were increased in size on the right side in eight of the eleven specimens. In the other three animals, the spinal cord from the donor has remained separate and lies parallel to the host axis between the pronephros and the limb. There is a definite increase in myotome size in two of the embryos, of which one has a single, the other a double spinal cord. In two other embryos, both with single spinal cords, a slight increase in the myotome is apparent; while in the other seven specimens, the right and left myotomes are equal in size. The pronephros is not noticeably affected in any of the animals, even those which show a distinct increase in amount of muscle.

**Explants**

When transplantation experiments are performed, the host animal will inevitably have some regulative effect upon the donor tissues. In order to eliminate the host influence, explants were made combining mesoderm and neural tissue in an ectodermal envelope.

There is evidence (Muchmore, 1958a) that brain tissue tends to inhibit muscle differentiation which occurs in the presence of developing spinal cord. It was not understood, however, whether the brain tissue acted by neutralizing the effect of the spinal cord or whether it acted directly upon the mesoderm. Since it is also known that presumptive somite mesoderm can self-differentiate into muscle tissue when it is isolated in large quantities in ectodermal envelopes (Muchmore, 1957), the following experiment could be performed to test the direct effect of brain tissue on muscle differentiation.
The presumptive material for the first 5–6 somites was isolated from both sides of two neurulae (stage 13) and was allowed to stand overnight in the culture medium, during which time the pieces of tissue fused into a single, rounded mass. This mass of mesoderm was then wrapped in ventral ectoderm together with a piece of presumptive forebrain from the anterior medullary plate. The explants were cultured for about 10 days. The results, compared with those of explants of the same quantity of mesoderm alone, are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Muscle</th>
<th>Nephric tubules</th>
<th>Limb bud</th>
<th>Mesenchyme</th>
<th>Neural tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesoderm alone</td>
<td>46</td>
<td>43</td>
<td>43</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>Mesoderm and brain</td>
<td>41</td>
<td>18</td>
<td>40</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

While muscle tissue occurs in a large percentage of explants containing mesoderm alone, it is found in less than half of those containing brain as well as mesoderm. At the same time the proportion of explants with nephric tubules is not changed. But also the formation of limb buds appears to have been suppressed entirely in the presence of brain tissue.

An attempt was made to judge the relative amount of muscle tissue present in each explant. If all of the mesoderm in an explant had formed muscle a value of ++++ was assigned, and a trace of muscle amongst large amounts of other mesodermal structures was marked ±; intermediate proportions of muscle tissue were judged as ++++, ++, and +. These results are shown in Table 2.

Table 2

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>++++</th>
<th>+++</th>
<th>++</th>
<th>+</th>
<th>±</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesoderm alone</td>
<td>46</td>
<td>0</td>
<td>8</td>
<td>23</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Mesoderm and brain</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

From this it can be seen that in the presence of brain tissue there is a striking reduction not only of the number of explants producing muscle but also of the amount of muscle produced.

Discussion

The results reported here confirm the conclusions reached earlier (Muchmore, 1958a) that developing spinal cord promotes, and developing brain inhibits, the differentiation of muscle in somite mesoderm of *Ambystoma maculatum*. Specifically, neural tissue from the trunk region (presumptive spinal cord) when brought into close relation to the anterior (head) somite mesoderm encourages muscle
Control of muscle differentiation

Differentiation far in excess of that normally encountered in the head region. And neural tissue from the head region (presumptive fore- and mid-brain) when brought into close relation to trunk somite mesoderm interferes with differentiation into full-sized trunk myotomes. Also, developing brain tissue prevents proper differentiation of muscle cells in explanted somite mesoderm.

The mechanisms by which the spinal cord and brain affect the mesoderm are not yet obvious. In the first place, there is no certain evidence relating to the time at which the neural tissue acts. Nevertheless, it seems probable that the effective stimulus is given early, since the differences between experimental and control myotomes can be seen clearly after only 10 days of culture, during which time there is good differentiation of the muscle cells but little increase in cell number in the somites. That is, it appears that the present experiments are concerned with a primary differentiation of the initial stock of cells in the somites and not with a regulative differentiation of cells produced later by the somite growth centers (see Holtzer & Detwiler, 1953, 1954; Holtzer, Lash & Holtzer, 1956). This is also indicated by the results of Series M/S where the slight decrease in myotome size in three specimens can be explained by a loss of somitic cells during operation and the inability of the somite growth centers to compensate for this loss in the short time available before sacrifice of the animals. That the effect of the presumptive spinal cord is exerted upon somite cells already disposed towards muscle differentiation is strongly suggested by the results of Series M/L where in several cases the myotomes are enlarged while the nephric system remains normal. It appears that the extra muscle cells in these cases have come not from presumptive pronephros but entirely from cells of the somite which normally might have contributed to cartilage or mesenchyme. It is probably important, therefore, to distinguish clearly between the early effects of differentiating spinal cord and brain upon mesodermal cells which already have a tendency toward muscle differentiation and the later effects exerted by established spinal cord upon the growth center and its derivatives.

With regard to the mode of action of the neural tissue there is also no direct evidence. It does appear, however, that a strictly physical effect can be ruled out. While at first glance it might seem likely that the brain inhibits muscle development simply by its expansion and pressure upon the somites, there is no indication, either in normal animals or in the experimental material, of undue crowding of tissues around the brain. Indeed, there is always sufficient intercellular space or loose mesenchyme into which the myotomes could expand or be pushed if necessary. Also, while the neural tube in the anterior trunk region is considerably enlarged in both Series Br/S and Series M/L, the adjacent myotomes are reduced in the former but are unaffected or enlarged in the latter. The effect on the myotomes is clearly dependent on the kind rather than the amount of neural tissue present. Further, it has been demonstrated earlier (Muchmore, 1957) that crowding of somite cells in explants tends to encourage rather than inhibit the differentiation of muscle.
No information is available concerning the fate of cells prevented by the brain from becoming muscle or the differentiation of other derivatives of the somites in the vicinity of enlarged myotomes, inasmuch as the specimens were sacrificed before cartilage and dermal differentiation had occurred. It is probable, however, that the later differentiating tissues would be regulated toward normality through the activities of the somite growth centers, as suggested by Holtzer & Detwiler (1953).

In view of the recent success of Lash et al. (1962) in isolating a specific chondrogenic factor from chick spinal cords, it is reasonable to suppose that the effects described in the present paper are also mediated by chemical factors. It seems probable that the spinal cord supplies a factor (or factors) which allows or encourages the differentiation of predisposed somite cells into myoblasts, while a brain factor (or factors) inhibits this differentiation when present in high enough concentration.

From the data available one can propose a general outline of the control of muscle differentiation in somite mesoderm of the salamander. At the end of gastrulation the presumptive somite mesoderm is already disposed toward muscle differentiation, for reasons as yet unknown. During the early phases of neurulation neighbouring tissues in the trunk region, particularly the presumptive spinal cord, exert some influence, probably through chemical factors, encouraging somitic cells to differentiate into myoblasts and form the trunk myotomes. At the same time the brain produces an effect which inhibits muscle differentiation in nearby mesodermal tissues. This effect is strongest in the fore- and mid-brain regions, then it diminishes rather quickly through the hind-brain and disappears as the hind-brain merges into the spinal cord. As a result no myotomes are developed in the anterior part of the head, but they appear suddenly behind the otic vesicles and quickly gain full size along the spinal cord. Somitic cells which are not incorporated into the myotomes at this time probably contribute later, along with the cells produced by the somite growth centers, to the formation of occipital and vertebral cartilages. This development of cartilage, and probably also the subsequent growth of the myotomes, is controlled by a factor (or factors) coming from the spinal cord and notochord.

Two other observations made in the course of this work deserve some comment.

(1) When presumptive spinal cord was transplanted into the hind-brain region of the neural plate (Series M/H), small tails often grew out from the site of the transplant. Most of these tails are directed forward and several occur on the left (unoperated) side of the host. It is probable that the tail tissues are derived from the posterior part of the neural plate (see Spofford, 1945) but it is difficult to understand how they came to be oriented as they are. The problem is undoubtedly concerned with the movements of the host tissues during closure of the neural folds. More detailed data than are presently available will be required to provide the answer.

(2) When ventral ectoderm (presumptive epidermis) is transplanted into the
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trunk region of the neural plate (Series V/S), the resulting spinal cord is nearly normal. It is evident that at the stage employed (12½-early neurula) the presumptive epidermis is still competent to form neural tissue. This substantiates Lopaschov's (1941) report of neural induction of early neurula ectoderm. Also, it may well explain the occurrence of neural tissues in many of our previous somite explants which were covered with neurula ectoderm (see Table 1 and Muchmore, 1957, p. 299). In as much as most such explants did not show neural differentiation of ventral ectoderm one can conclude that there is involved a threshold of effect which is reached only occasionally under the influence of isolated mesoderm. On the other hand, the threshold can apparently always be surpassed when the ventral ectoderm is transplanted over the intact chorda-mesoderm in the early neurula. Furthermore, it is obvious that the induced neural tissue has regional characteristics of the region into which it is incorporated in as much as adjacent myotomes are normal in size and configuration.

SUMMARY

1. Previous work has suggested that in the salamander embryonic spinal cord enhances and embryonic brain suppresses differentiation of muscle from somitic mesoderm. These results have been confirmed by means of transplantation and explantation experiments performed on early neurulae of *Ambystoma maculatum*.  
2. Transplantation of presumptive spinal cord into the hind-brain region results in increased size of post-otic myotomes. On the other hand, transplantation of presumptive forebrain into the trunk neural plate usually results in a striking decrease in size of trunk myotomes. Orthotopic transplants of neural plate have little or no effect upon the development of myotomes. Ventral ectoderm transplanted into the neural plate at stage 12½ is incorporated into the host spinal cord and adjacent myotomes are usually normal. Transplants of trunk neural plate over lateral mesoderm often results in increased size of myotomes without affecting the pronephros.  
3. Explants of large amounts of somite mesoderm in an ectodermal envelope readily differentiate into muscle. Inclusion of presumptive brain cells in such explants results in a conspicuous decrease in frequency of occurrence of muscle differentiation and in quantity of muscle actually formed.  
4. The control of muscle differentiation in the early embryo is considered in the light of the known influences exerted by the neural tissues.

RÉSUMÉ

*Le contrôle de la différenciation musculaire par les tissus neuro-embryonnaires*

1. Des recherches antérieures ont suggéré que chez un uroïde la moëlle épinière de l'embryon favorise la différenciation de muscles aux dépens du mésoderme somitique tandis que le cerveau embryonnaire la supprime. Ces résultats ont été confirmés par des expériences de transplantation et d'explantation effectuées sur de jeunes neurulas d'*Ambystoma maculatum*.
2. La transplantation de moelle épinière présomptive dans la région du cerveau postérieur a pour effet une taille accrue des myotomes post-otiques. D'autre part, la transplantation de cerveau antérieur présomptif dans la partie troncale de la plaque neurale provoque une diminution frappante de la taille des myotomes du tronc. Des transplants orthotopiques de plaque neurale ont peu ou pas d'effet sur le développement des myotomes. Si de l'ectoderme ventral est transplanté au stade 12½ dans la plaque neurale, il est incorporé dans la moelle épinière de l'hôte et les myotomes adjacents ont la taille normale. La transplantation d'une partie troncale de la plaque neurale sur le mésoderme latéral a souvent pour résultat d'accroître la taille des myotomes sans affecter le pronéphros.

3. Des explants importants de mésoderme somitique, enveloppés d'ectoderme se développent pleinement en muscle. L'inclusion de cellules de cerveau présomptif dans de tels explants amène une diminution apparente dans la fréquence avec laquelle le différenciation musculaire se manifeste et dans la quantité de muscle effectivement formé.

4. Le contrôle de la différenciation musculaire dans l'embryon jeune est discuté à la lumière des données concernant les influence exercées par des tissus d'origine neurale.

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REFERENCES


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