ON THE RELATION BETWEEN MOVEMENTS AND THE SHAPE OF SOMITES IN EARLY EMBRYOS OF THE TELEOST *BRACHYDANIO RERIO*

by

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ABSTRACT

As a working hypothesis it is supposed that in the teleost *Brachydanio rerio*, the muscle contractions, the growth, and probably some other factors, cause the first changes in the shape of the somites. Furthermore, the movements of the embryo could yield the forces by which the somites are brought to their theoretically optimal shape.

In order to test this hypothesis, we investigated the somite shape and structure of spontaneously immobile embryos. Although the results are difficult to interpret, they certainly do not contradict the hypothesis.

For further analysis we applied two kinds of lesions in order to immobilize early embryos: removal of the brain, and damage to the midbody somites. The results of these experiments indicate that both the development of the shape of the somite and the arrangement of the muscle fibres are dependent on movements of the embryo.

INTRODUCTION

In the teleost *Brachydanio rerio*, the somites segregate as block-shaped unities from the mesoderm. Soon after their formation the differentiation of the muscle fibres starts, first medially near the notochord, later also in the rest of the somites (Waterman, 1969).

The first changes in the shape of the midbody somites (nos. 5—10) become apparent only after a contractile apparatus is present in these somites. On the other hand, the tail somites (nos. 18—35) change their shape immediately after their formation, before any muscle fibres are present (Van Raamsdonk et al., 1974).

From the 16-somites stage on, the embryo performs movements. So the tail somites are formed in a period in which the embryo performs already lateral swinging movements, caused by the contractions of the differentiated anterior somites. These observations suggest that movements of the embryo may play a prominent part in determining the shape of the somites. The first changes in the shape of the midbody somites might be caused by their own activity, while the rapid change in the shape of the tail somites might be caused by moulding these somites on the shape of the differentiated anterior ones, during the lateral swinging movements (Van Raamsdonk et al., 1974).

From the mechanical properties of the myosepts, Van der Stelt (1968) concluded, that in functioning somites, which have their muscle fibres orientated parallel to the notochord, the angle between myosept and body axis should be 35°. In fact, we found this angle in all embryos which had been performing lateral swinging movements for at least 24 hours.

The hypothesis we propose now is: muscle contractions and growth, and probably some other factors, cause the first changes in the shape of the somites, but the movements of the embryo yield the forces by which the somites are brought to their (theoretically) optimal shape.

In some spawns of *Brachydanio rerio* we found embryos which, in contrast to normal ones, did not show any movements. These embryos, which are immobile owing to some developmental error, could give us information about the possible relation between movements and the development of the shape of the somites. It appeared that all spontaneously immobile embryos showed aberrancies in the head region as well as in the shape of the somites.

Because of these observations, we tried to produce immobile embryos by removing a part of the brain in early embryonic stages.

We also tested the hypothesis that the rapid changes in the shape of the tail somites are

caused by moulding. Therefore we disturbed the continuity in the row of somites in an early developmental stage.

In both groups of experimental embryos and in the spontaneously immobile embryos, we studied whether any movements were performed, and how the shape of the somites developed. In some embryos we also studied the histological structure of the myotomes and myosepts.

MATERIALS AND METHODS

Brachydanio rerio was bred according to the method described by Hisaoka and Battle (1958). Twenty to twenty-four hours after spawning, the eggs were sorted on stage and vitality. The eggs were then transferred to a 1 : 1 diluted Holtfreter solution and dechorionated with watchmakers' forceps. Embryos which were found to be immobile were set apart and were reared further in the same Holtfreter solution. We frequently observed these embryos under a stereoscope to see whether they performed any movements. Three or five days later, the spontaneously immobile embryos were fixed for histological investigations. For details of fixation, see Van Raamsdonk et al., 1974.

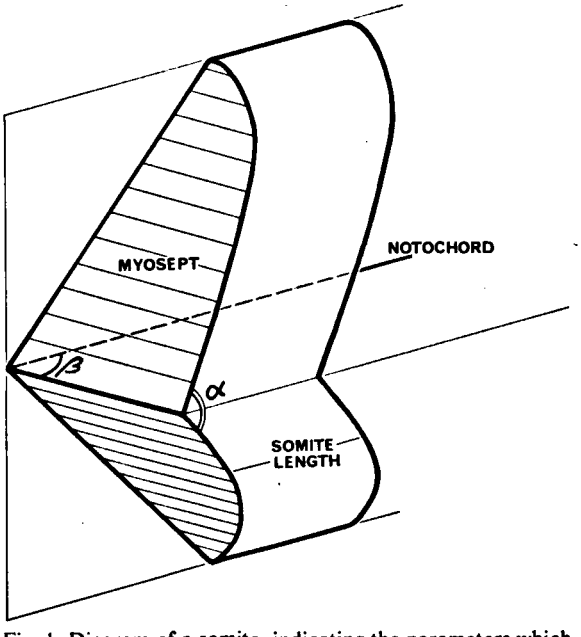

Fig. 1. Diagram of a somite, indicating the parameters which are used as characteristics of the shape of the somite: α, and the length of the somite. The angle φ between the notochord and the myosept is calculated from α and β:
$\varphi = \arcsin(1/\sqrt{\cot g\, \tfrac{1}{2}\alpha + \cot g\, \beta + 1})$

Operations

Dechorionated embryos with 15 or 25—30 somites were put on a cold (4 °C) agar plate. On each embryo some 5 % agar of about 35 °C was dropped. Then, with a glass micropipet (ø = 50—75 μm), the part of the brain anterior to the auditory pit was sucked off. In other cases we made midbody lesions. The dorsal part of two or three midbody somites (nos. 10—15) was sucked off at both sides of the embryo. In almost all cases, at least a part of the neural tube was also removed by this procedure. Immediately after the operations, we removed the surrounding agar from the embryos. The embryos were kept further in a 1 : 1 diluted Holtfreter solution.

Normal, dechorionated embryos, serving as controls, were reared in the same solution. Other control embryos were enclosed in agar for some minutes only. Immediately after the agar solidified it was removed again from the embryos.

Experimental and control embryos of 3 days old were transferred to a 1 : 10 diluted Holtfreter solution. The embryos were photographed from the lateral side. On the photograph, we measured the angle α between the dorsal and the ventral myosept, and the length of the somites. These values were taken as shape characteristics of the somites (see fig. 1).

Of course it is not in the first place α, but the angle between the myosept and the body axis φ, which is valuable for functional analysis. But changes in α always reflect changes in φ.

Unless stated otherwise, the time indications in this paper refer to hours or days after fertilization.

RESULTS

Spontaneously immobile embryos

From the 16-somites stage on, *Brachydanio rerio* embryos perform movements. Inside the chorion, the embryos are curved, but immediately after dechorionation they stretch. The immobile embryos, however, were always completely motionless. When these embryos are dechorionated, they stay as curved as they were. All these immobile embryos showed malformations in the head region. They always developed a beating heart with a considerably enlarged pericard (figs. 2, 3, and table II).

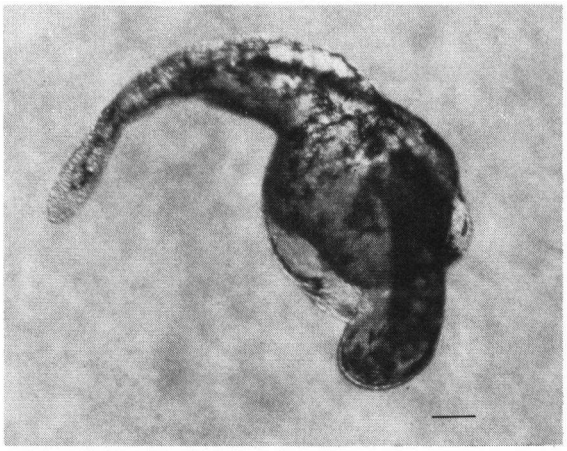

Fig. 2. Micrograph of a spontaneously immobile embryo. Note the absence of the eyes. Scale = 100 μm.

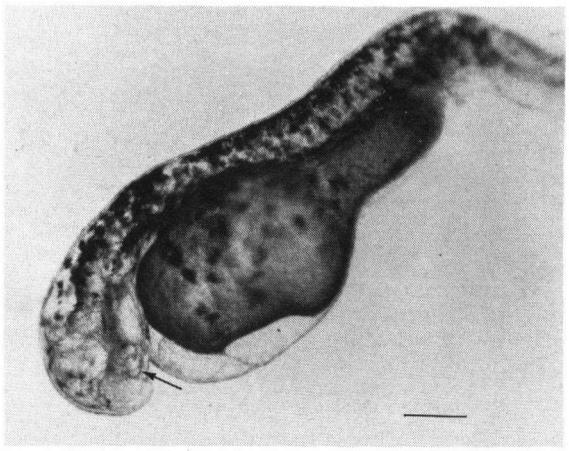

Fig. 3. Micrograph of a spontaneously immobile embryo. Note the large pericard and the poorly developed eye (arrow). Scale = 100 μm.

Table I. Development of the shape of the anal somite in control embryos.

Age	*N*	myosept angle α (°)		width of anal somite (μm)	
		mean	S.D.	mean	S.D.
30—34 hours (25—30 somites)	17	86.0	6.4	60	5.3
48 hours (35 somites)	16	82.1	4.6	70	2.8
72 hours	18	80.7	3.6	85	4.4
4 days	15	81.4	3.3	95	1.9
5 days	14	80.3	3.9	102	1.0
6 days	11	80.2	4.5	108	2.6
7 days	6	78.0	2.9	112	2.0

Although the myosept angle α shows great variation, this angle is always greater in immobile embryos than in normal embryos of about the same age. In normal embryos the somites are wider than in immobile embryos (tables I and II).

Six of these embryos have been investigated histologically. In all somites we found muscle fibres with an apparently normal banding pattern of the myofibrils and a normal sarcoplasmic reticulum and tubular system (fig. 4).

However, the arrangement of the contractile structures was strongly aberrant. The muscle fibres were oriented in almost all directions (fig. 5). The same holds for the myofibrils (fig. 6). In the myosepts we found collagen fibres as in the controls. Some myosepts were incomplete (fig. 7). In those cases some muscle fibres extended over a length of two somites. Other myosepts looked as if they were ruptured (fig. 8). In the immobile embryos, the notochord cells were hardly (if at all) vacuolated (fig. 9).

The neural tubes of the immobile embryos appeared quite similar to normal ones.

Table II. Spontaneously immobile embryos, 3—5 days after fertilization.

Embryo no.	myosept angle α (°)	width of anal somite (μm)	remarks
1 (fig. 2)	140	64	eyeless
2	145	59	one unpigmented eye
3	115	61	one unpigmented eye
4	110	59	one unpigmented eye
5 (fig. 3)	140	66	two unpigmented eyes
6	155	73	two unpigmented eyes
7	150	58	two pigmented eyes
8	165	73	two pigmented eyes
9	135	64	two pigmented eyes
control embryos	81	85	3 days after fertilization

Control embryos

Control embryos which were enclosed in agar for some minutes only, developed as the dechorionated control embryos.

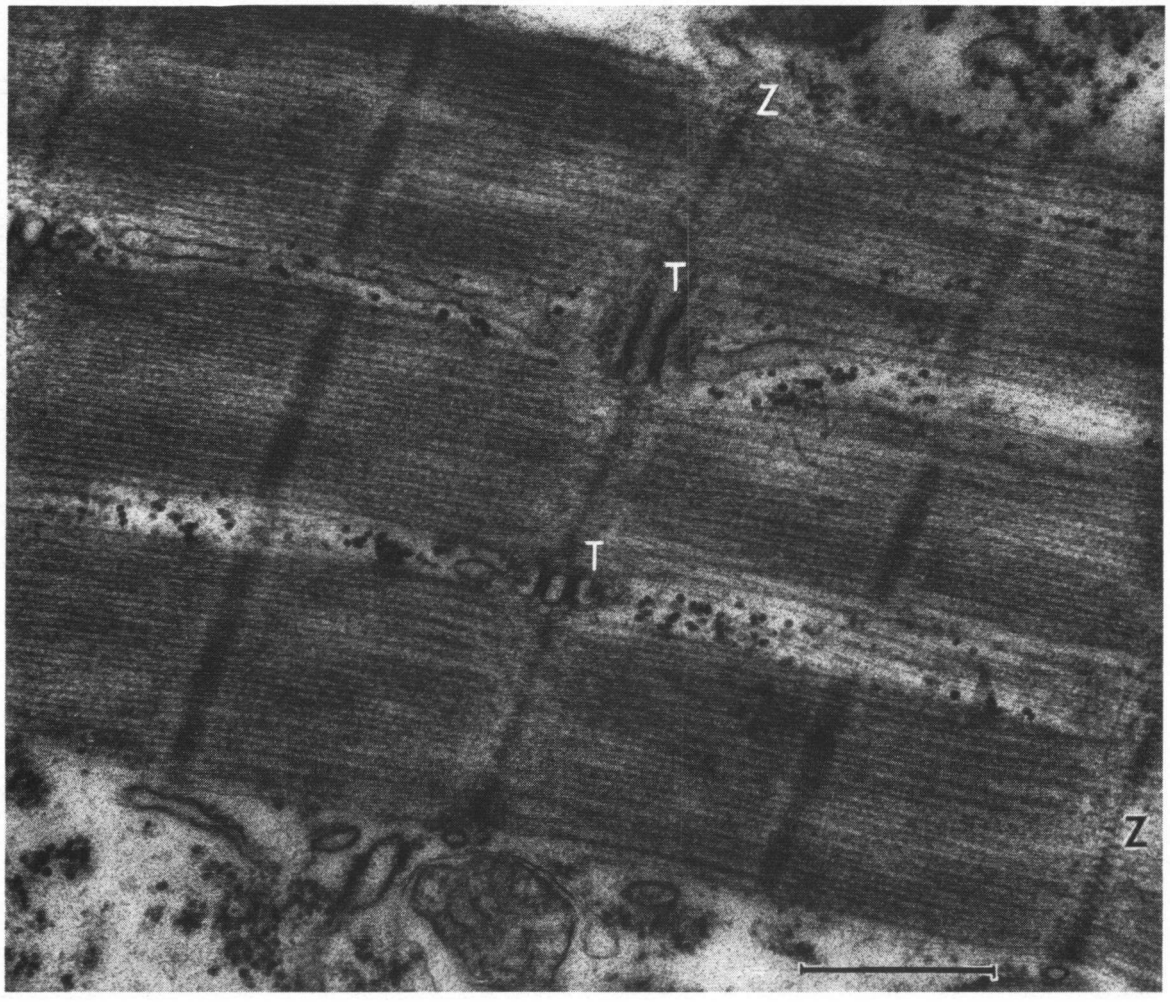

Fig. 4. Electron micrograph of sarcomeres of a spontaneously immobile embryo. Note the normal arrangement of the Z-membrane (Z), triads (T) and myofilaments. Scale = 0.5 μm.

Removal of the brain in the 15-somites stage (20 hours)

Our efforts to remove the brain from embryos in premotile stages, that is before the 16-somites stage, were not very successful. Most embryos died within several hours after the operation because of damage to the yolk sac (dead embryos become turbid, while living embryos are clear and transparent).

Until now only one embryo survived this treatment for more than 24 hours. This embryo was operated in the 15-somites stage. In this embryo, not only the brain but also the first 4 or 5

Table III. Shape of the anal somite in embryos treated in the 15-somites stage (20 hours).

Type of treatment	*N*	30 hours after treatment				remarks
		myosept angle α (°)		width of anal somite (μm)		
		mean	S.D.	mean	S.D.	
removal of brain and the first 4—5 somites	1	160		40		21 somites, shrugging tail movements
midbody lesion	7	131	23	43	1.3	25—30 somites, shrugging tail movements
control embryos	17	86	6.4	60	5.3	25—30 somites

somites turned out to be removed. This embryo did not develop a heart. After the operation, new somites were formed in the caudal part of the embryo; 30 hours after the operation we counted 21 somites, so the rate of development was slower than in normal embryos (see table III).

Sometimes the embryo's tail performed a feeble shrugging movement with little or no lateral flexure.

Table III also shows that the myosept angle of somites in the anal region is much more obtuse than in normal embryos with about 25 somites (30 hours), and moreover, that the width of the somites is less than in normal embryos.

Midbody lesions in the 15-somites stage (20 hours)

In this type of experiments we found it equally difficult to avoid yolk-sac damage.

Seven embryos survived this type of operation for more than 24 hours. At the site of the operation an unstructured outgrowth of cells was visible. Thirty hours after the operation the pigmentation in the head region was like in normal 30-somites stage (34 hours) embryos (fig. 10). By that time we saw in 4 embryos a beating heart. In the period after the operation, 10—15 new somites were formed. Thus in these cases there is slight retardation in development.

The embryos did not perform swinging movements with their tails, we only observed sometimes a feeble trembling. In all cases the myosept angle and the width of the somites were clearly different from those in normal embryos with 25—30 somites (see table III).

Removal of the brain in the 25—30 somites stage (30—34 hours)

In the 25—30 somites stage, the embryos had been performing lateral swinging movements for at least 8 hours. The anal somite contained myotubes parallel to the notochord (fig. 11). The myosept angle α in the anal somite measured 86°. These embryos had a clear blood circulation.

In this developmental stage, we tried to re-

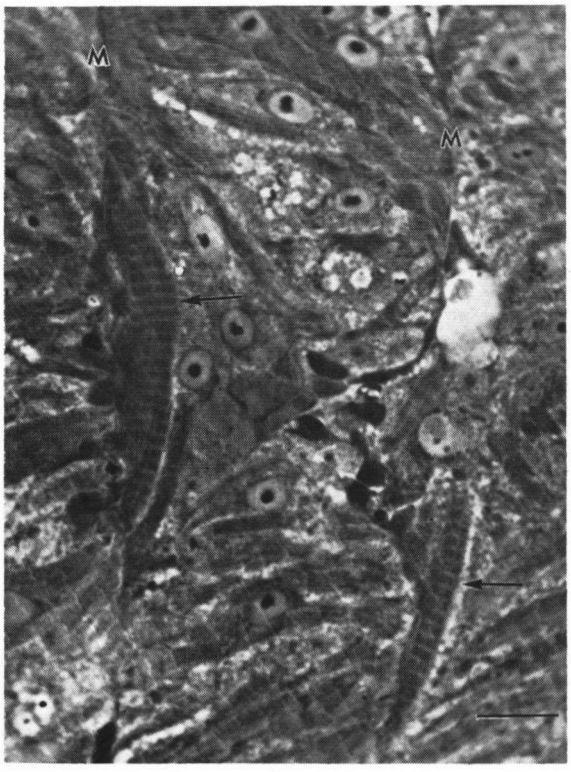

Fig. 5. Sagittal section of a somite of a spontaneously immobile embryo, showing aberrant fibre arrangement. Note the fibres along the myosepts (arrows), both sides of these fibres are connected to the same myosept. M = myosept. Scale = 10 µm.

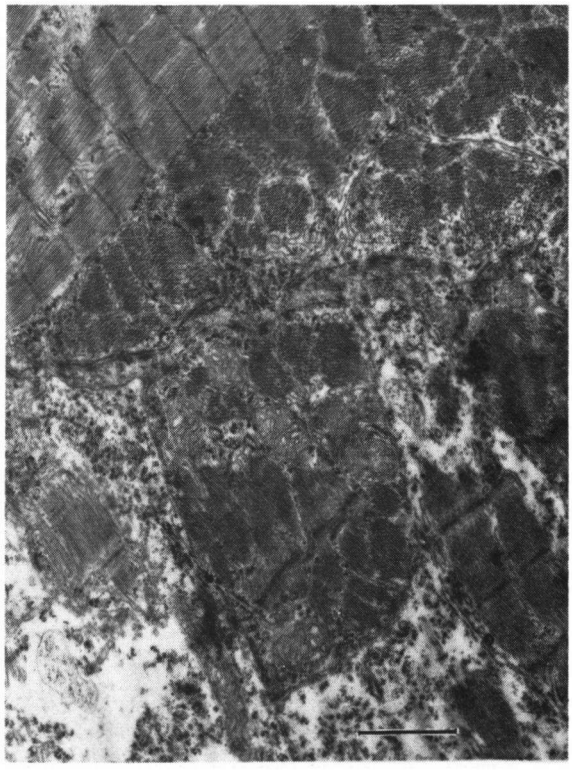

Fig. 6. Electron micrograph of myofibril arrangement within muscle cells of a spontaneously immobile embryo. Note the different orientations of the fibrils. Scale = 1 µm.

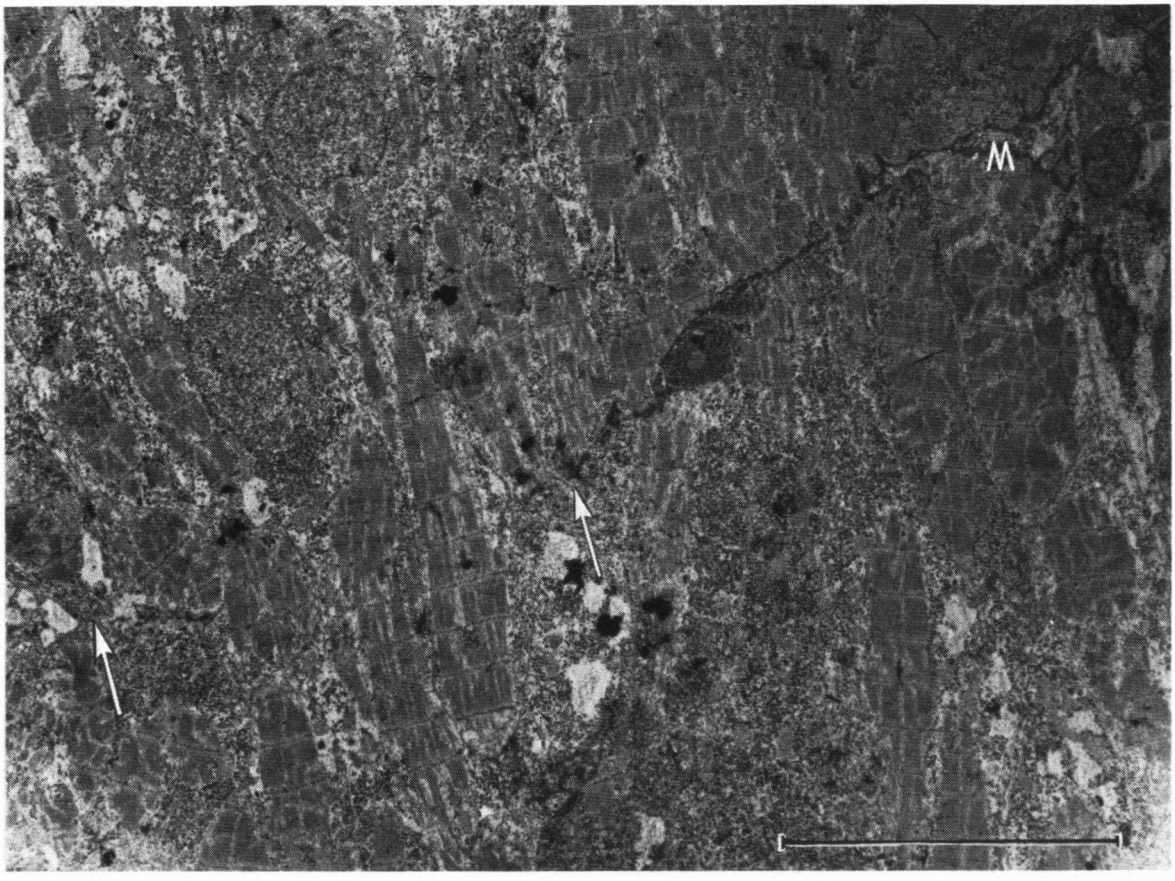

Fig. 7. Electron micrograph of an incomplete myosept from a spontaneously immobile embryo. Note the absence of the myosept between the arrows. M = myosept. Scale = 10 μm.

move the part of the brain anterior to the auditory pit. This kind of experiment is hard to standardize. In three cases (out of nine) the eyes were still present, but the part of the brain between the eyes and the auditory pit was removed anyway. In all embryos treated, the postotic somites remained completely undamaged. Only those embryos, in which the blood circulation in the tail was preserved, were used for further investigations. Nine embryos survived for more than one day, six embryos survived for even more than seven days.

Immediately after the operation, the embryos were motionless. The next day the embryos started moving again, though the movements were not as frequent as in normal embryos. Three days later, one of the treated embryos (fig. 12) moved in a way that could not be discerned from that of normal embryos. In the other embryos, the activity decreased sharply about 4 days after the operation, but they never became completely motionless. Table IV shows the data on the development of the shape of the anal somites in these embryos.

In the second period of relative inactivity, we noticed deviations from the normal muscle fibre orientation. Fig. 13 shows muscle fibres in tail somites of an embryo six days after the operation.

Midbody lesions in the 25—30 somites stage

In this case too, we only used embryos in which the blood circulation in the tail had been staying normal after the operation (figs. 14, 15). The treatment results in an outgrowth of cells, just as in the treated embryos of the 15-somites stage. In all embryos the tail somites remained undamaged. The eight embryos which were still alive after one day, all survived for more than 5 days.

Immediately after the operation, the embryos lost their motility. Table V shows the data on the shape of the anal somites in these embryos. Two

days of immobility resulted already in aberrant muscle fibre orientations. Fig. 16 shows muscle fibres in tail somites, four days after treatment.

DISCUSSION

Roux (1905) suggested that the development of organisms could be divided into two periods, an organ forming period, followed by a period of functional development. In the first period the organs are brought to a condition in which they are capable of starting their specific function, in the following period the organs are already performing their specific function. Their further perfection is helped by this activity and interfered with by its absence.

Applying this concept to the development of somites in teleosts, we put the question whether the normal exercise of function is a necessary factor in determining the shape of the somites and the organization of their myofibres.

To answer this question we have in the first place data on embryos in which the somites have never been capable to perform their normal activity, and secondly, embryos in which the somites had been following a normal course of development and were able to perform their function until they were immobilized.

Fig. 8. Electron micrograph of a discontinuous myosept of a spontaneously immobile embryo. The myosept is ruptured. Collagen fibres are only found in the neighbourhood of the endings of the myofibrils (arrows). Scale = 1 µm.

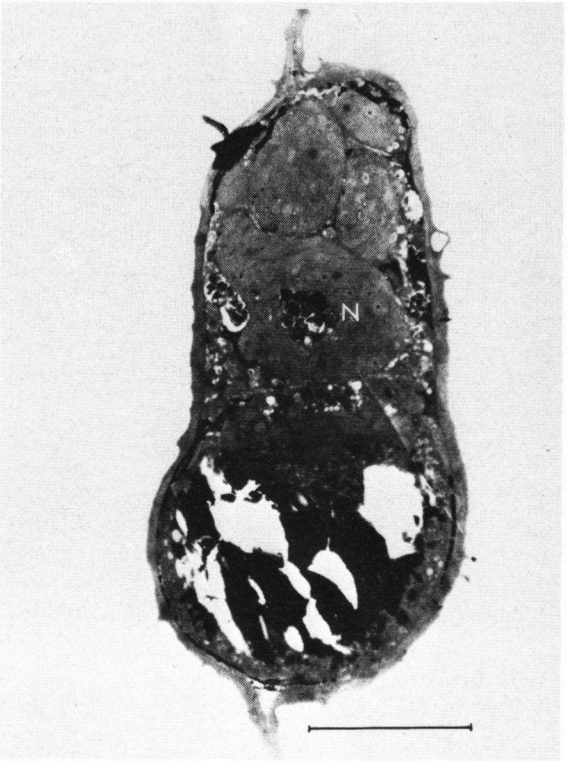

Fig. 9. Transversal section through a somite of a spontaneously immobile embryo. Note the small notochord (N). Scale = 50 µm.

Spontaneously immobile embryos

First of all, we have to speculate on the reasons why certain embryos are immobile. Aberrations of the central nervous system could be the cause. But it is difficult to draw definite conclusions upon this point.

Harris & Whiting (1954) showed that embryos of the dogfish *Scyliorhinus caniculus*, in which the central nervous system was completely removed, performed normal movements. They concluded that contractions of myotomes were initiated myogenically and transmitted myomyally in the earliest stages. In later stages the contractions were still transmitted myomyally but initiated by the nervous system. Droin & Beauchemin (1975) likewise concluded that the somite contractions originate from myotomic activity. These authors

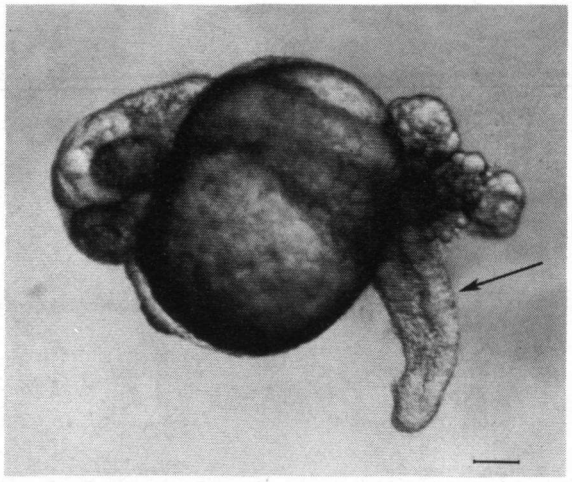

Fig. 10. Midbody lesion in a 15-somites stage embryo, 30 hours after treatment. Note the block-shaped somite (arrow). Scale = 100 μm.

rations of the central nervous system can have some relation with the motility of early embryos, because *Brachydanio rerio* embryos in which a part of the brain or the whole brain was removed, stay motionless at least for one day. But we also found that the motility can be restored later on.

Spontaneously immobile embryos are exceptionally rare. They could only be found when other embryos of the same spawn were already motile. Therefore we do not know anything about the earliest developmental stages of these immobile embryos. So we cannot decide whether the absence of movements in spontaneously immobile embryos is cause or result of the disorganization of muscle fibres.

The explanation we prefer is that the rate in which the contractile apparatus develops is not adjusted to the rate of development of other organs. E.g., the myosepts may be ruptured because their growth rate was too slow. Also the notochord was retarded in development. This could be the reason why the growth in length of the embryos was less than normal. Obviously, many muscle fibres are too long for the somites in which they are located so they cannot orient themselves in a normal way, i.e. parallel to the notochord.

In the sense of Roux's concept, one could say

Table IV. Development of the shape of the anal somites in embryos from which the brains were removed in the 25—30 somites stage (30—34 hours).

Days after treatment	*N*	myosept angle α (°)		width of anal somite (μm)		remarks
		mean	S.D.	mean	S.D.	
0	17	86.0	6.4	60	5.3	control embryos
1	9	89.9	3.1	68	3.2	immobile
2	8	91.5	6.1	76	6.1	gradual resumption of motility
3	7	86.1	3.2	90	6.1	
4	7	88.9	4.8	96	5.6	gradual loss of motility
5	6	91.6	7.4	104	6.0	
6	6	98.8	6.9	107	3.6	

made cultures of the axial system of *Xenopus laevis* embryos, in which the neural and epidermal ectoderm was removed.

On the other hand, Harrison (1904) established that frog embryos, in which the medullary tube was removed in a premotile stage (10 somites), did develop a histologically normal muscular system, but these embryos remained completely motionless.

From our experiments it appears that aber-

Table V. Shape of the anal somite in embryos treated in the 25—30 somites stage.

Type of treatment	*N*	3 days after treatment				remarks
		myosept angle α (°)		width of anal somite (μm)		
		mean	S.D.	mean	S.D.	
removal of the brain anterior to the auditory pit	7	86.1	3.2	90	6.1	sometimes swinging tail movements
midbody lesion	8	113.8	10.5	82	5.0	immobile
control embryos	15	81.4	3.3	95	1.9	4 days after fertilization

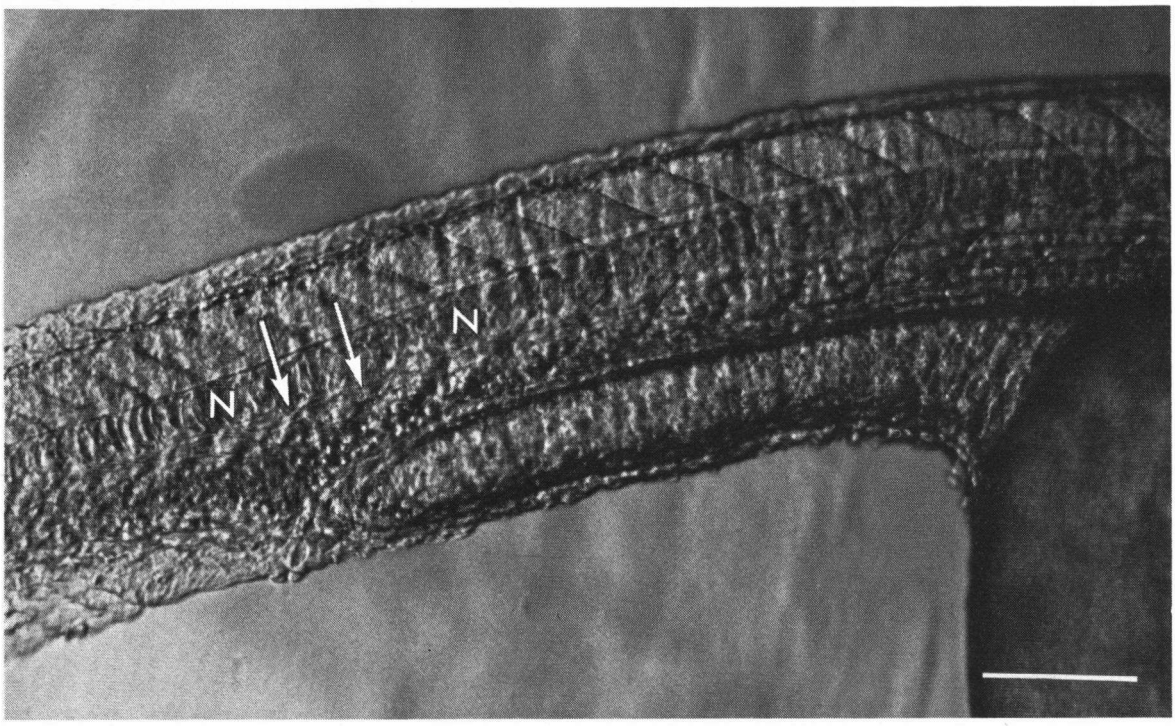

Fig. 11. Control embryo in the 25—30 somites stage. Note the somite shape and the orientation of the muscle fibres (arrows) parallel to the notochord (N). Scale = 100 μm.

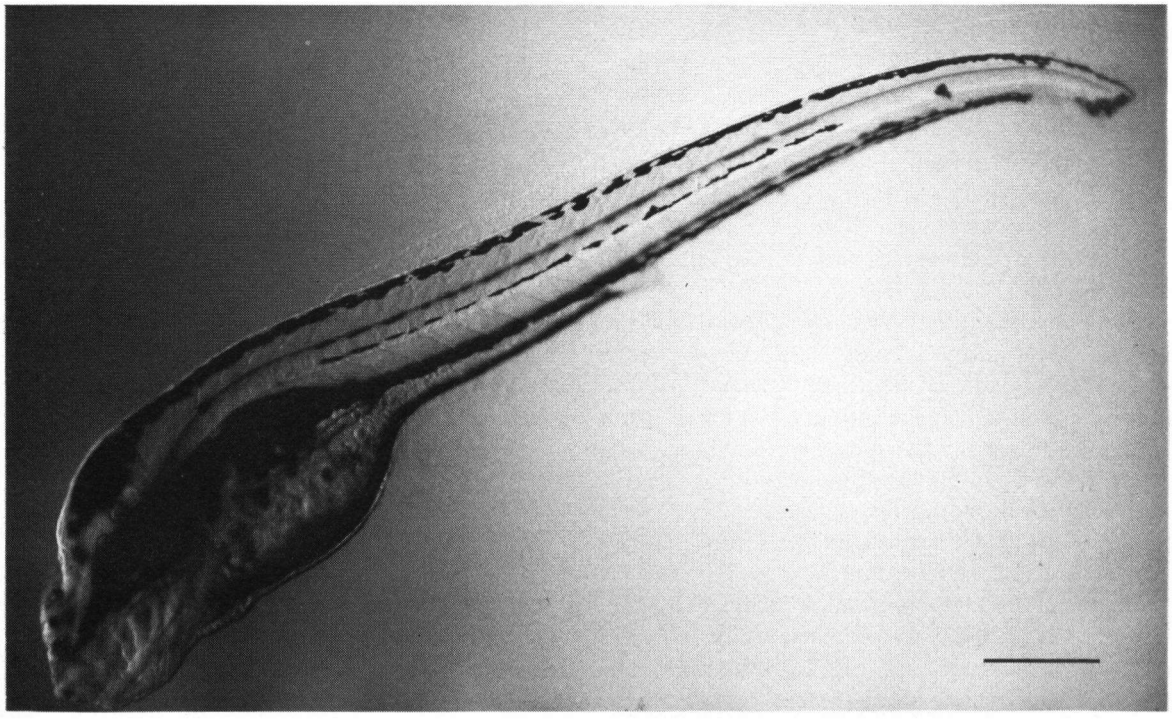

Fig. 12. Micrograph of a normally moving embryo, three days after removal of the brain. Age of the embryo is four days. Scale = 200 μm.

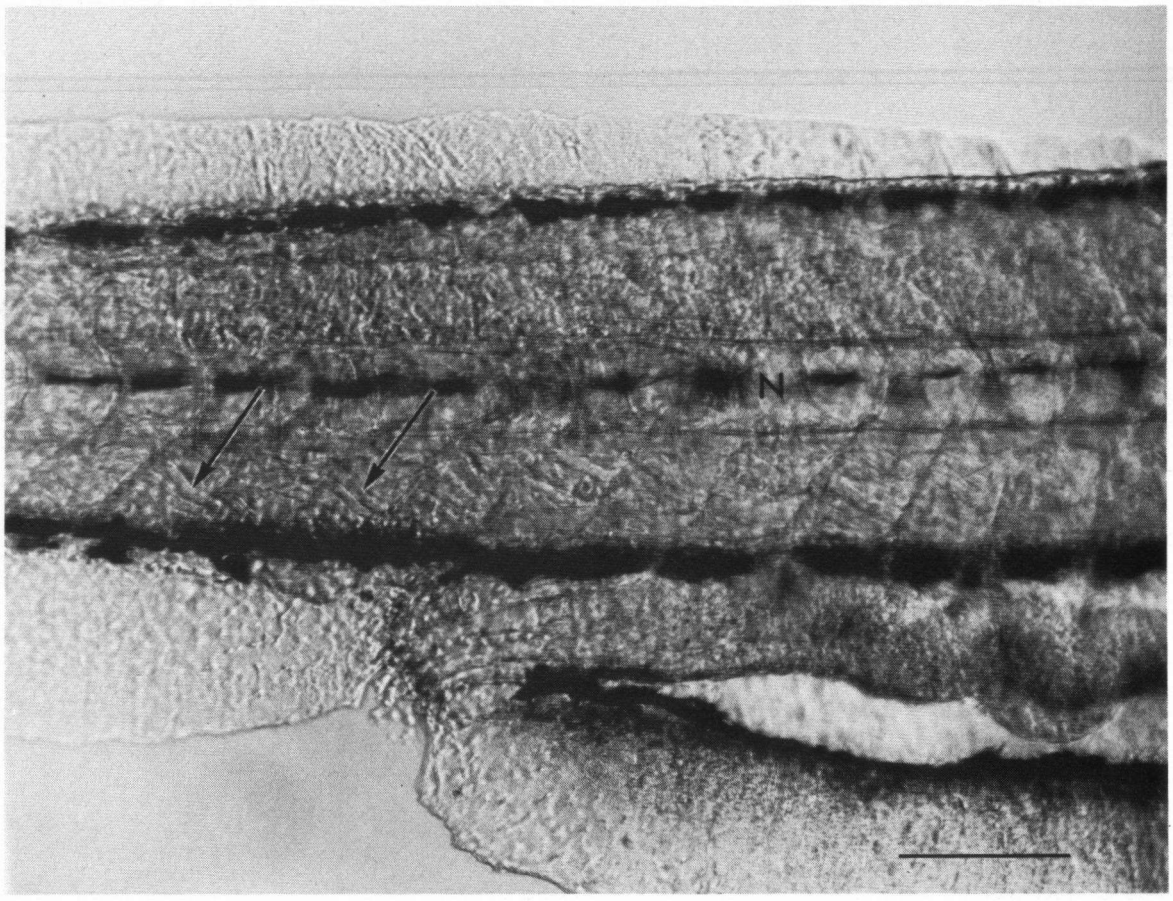

Fig. 13. Micrograph of an embryo, six days after removal of the brain. Note the aberrant orientation of the muscle fibres (arrows). Age of the embryo is seven days. N = notochord. Scale = 100 μm.

that in these embryos the somites have never been brought to a condition in which they can start to perform their normal function, because the muscle fibres in these somites are oriented in almost all directions, so they are unable to shorten.

In an earlier paper we have shown that the shape of the midbody somites does not change before muscle fibres have been formed in these somites, and that the somites achieve their theoretically optimal shape after they have been performing their specific function for at least 24 hours (Van Raamsdonk et al., 1974). These observations are very well concordant with Roux's concept.

In the spontaneously immobile embryos, the somites have never performed their specific function, so it is tempting to state that the absence of this activity is the reason why these somites did not achieve a shape as in normal embryos of the same age. However, we have to bear in mind that we do not have any knowledge of the nature of the disorder, nor of the point in the development in which the aberrance becomes operative and the period between this point and the moment of the first visible effect of it.

So, it is not very well possible to conclude from these data that the optimal somite shape is the result of the performance of the specific function indeed, but our data certainly do not contradict this hypothesis.

Experiments on embryos in the 15-somites stage (20 hours)

Normal embryos do not perform their first visible movements before the 16-somites stage, so the treated embryos had never performed lateral swinging movements.

In all cases the central nervous system was affected. Moreover, in those cases in which we

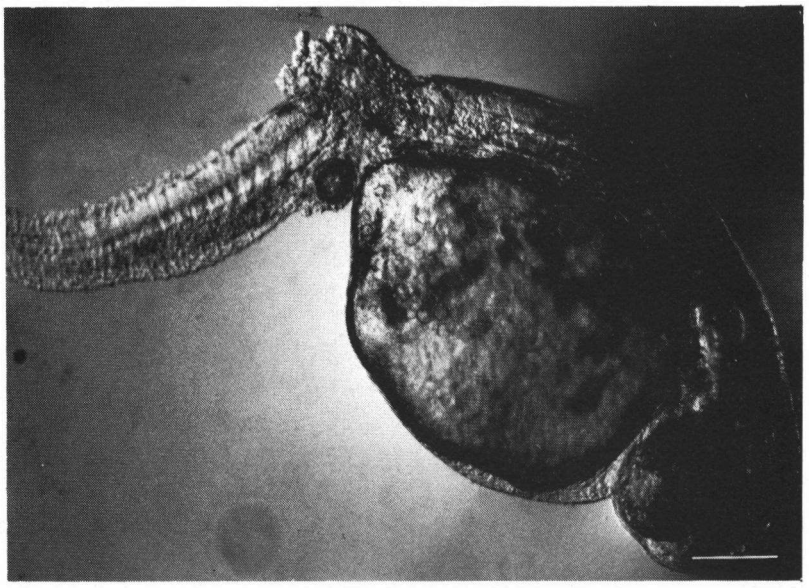

Fig. 14. Midbody lesion in the 25—30 somites stage. Micrograph of an embryo one day after treatment. Scale = 100 µm.

induced midbody lesions, the continuity in the row of somites was disturbed, because of the unstructured outgrowth of cells in the midbody region. The shrugging movements which were sometimes observed, indicate that muscle fibres had been formed in the somites of the treated embryos. However, we cannot consider these movements as the performance of the specific function of the somites. So it seems that damage of the central nervous system or of the most anterior somites affects, at least for some time, the mobility.

Where we induced midbody lesions, there could be also another reason for the relative immobility: the newly formed tail somites were probably functionally disconnected from the differentiated cranial somites, so the propagation of contraction impulses could be disturbed.

From the data on the somite shapes in these embryos, we may conclude that the inability to perform their normal function results in retardation of the development of the somite shape.

These results also support the hypothesis that the rapid deformation of the anal and postanal

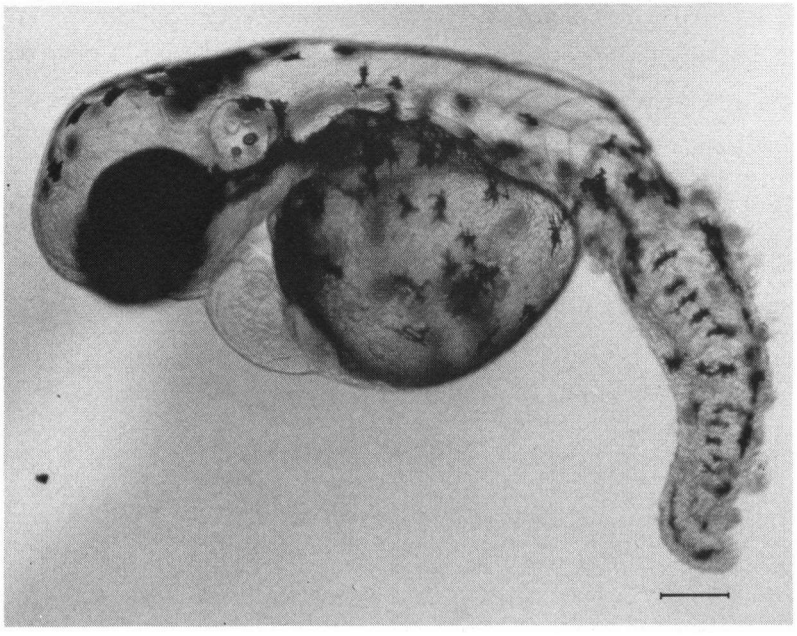

Fig. 15. Midbody lesion in the 25—30 somites stage. Micrograph of an embryo four days after treatment. Scale = 100 µm.

somites in normal embryos is due to moulding of these somites (Van Raamsdonk et al., 1974; 1975).

Experiments on embryos in the 25—30 somites stage

Before we removed the part of the brain anterior to the auditory pit, these embryos had been performing lateral swinging movements for at least 8 hours.

Immediately after treatment, the embryos were motionless. The reason for this is not clear to us. The damage due to treatment is hard to estimate, since after one day there is first a period in which

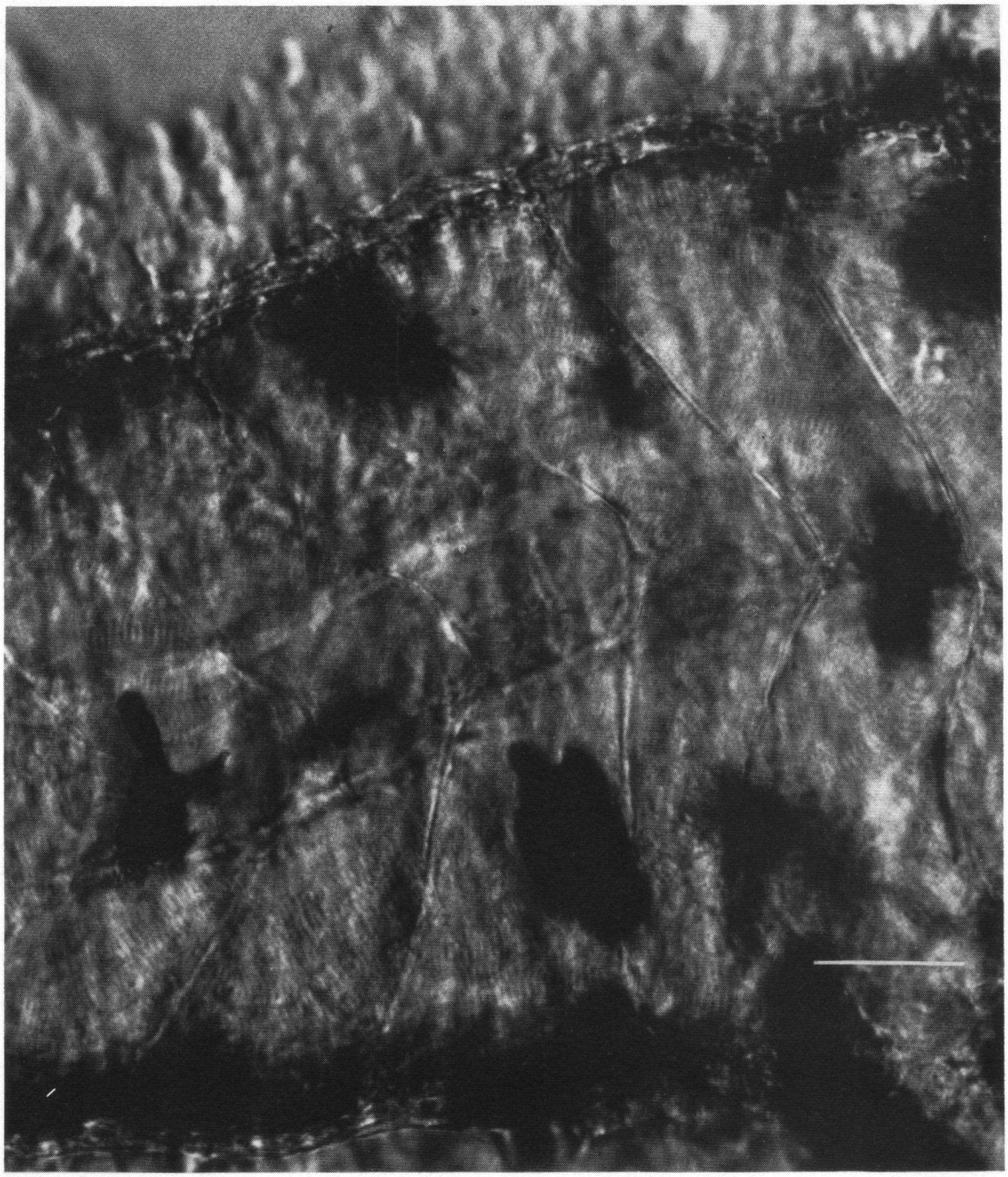

Fig. 16. Micrograph of the tail somites of an embryo with a midbody lesion, four days after treatment. The embryo is five days old. Note the aberrant fibre orientation. Scale = 50 μm.

the motility is resumed, but this is followed by a period in which the motility is gradually lost again.

We suppose that in this last period the somite contractions in normal embryos are brought under nervous control. In electron micrographs of the anal somites of 3 days old embryos, we found nervous tissue. Until now we did not find this in anal somites of younger embryos (Van Raamsdonk, unpublished). This presumption is in accordance with the findings of Harris & Whiting (1954). They supposed also that only in a later stage the myotome contractions become dependent on nervous activity.

Whatever the damage was, always, when the embryos were immobile, the myosept angle became more obtuse, and when the embryos started moving again, this angle became more acute. We conclude from these data that the morphogenesis of the somites is strongly dependent on the movements of the embryo. Moreover, in young embryos the performance of the specific function is probably not only necessary to acquire the optimal shape, but also to maintain it.

All embryos in which we induced midbody lesions lost their motility. Here too, the reason remains unclear. It could result from damage to the central nervous system or from the discontinuity in the row of somites or from both. Anyhow, also from these experiments it appears that the inability to perform the specific function is detrimental to the development of the somite shape.

Both types of experiments gave us information about the differentiation and organization of the muscle fibres. Obviously, the differentiation of muscle fibres is not affected, but the organization of muscle fibres becomes clearly aberrant. It is tempting to conclude that in early developmental stages the differentiation of muscle fibres is independent on their function, but the organization of muscle fibres is dependent on their function indeed. However, we have to keep in mind that in all immobile embryos the length growth is somewhat retarded. It is very difficult to discriminate between the effect of this and the effect of the immobility itself.

As far as we know, no other studies have been performed on the relation between function and the development of the somite shape in fishes.

In amphibian embryos, this relation has been studied by Harrison (1904) and Willemse (1973). From their studies it appears that somite differentiation in anaesthetized frog tadpoles was completely normal. On the other hand, Droin & Beauchemin (1975) found the somites in immobile *Xenopus* tadpoles to be wider and more pointed than in controls. In the muscle fibres they found a normal banding pattern in the myofibrils, but these myofibrils were rather haphazardly arranged.

So it appears that for different species the morphological development of the somites depends to a lesser or greater extent on the performance of function.

As a general conclusion it may be stated that the inability to perform the specific function affects the development of the shape of the somites and the orientation of the muscle fibres. This is also apparent from our recent experiments in which young *Brachydanio rerio* were immobilized in agar for 4 days. The myosept angle becomes more obtuse, but when the agar is removed later on, the embryos start anew performing movements and (consequently) the myosept angle gets more acute and reaches control level again (Van Raamsdonk et al., in preparation).

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