However, these inhibitors did not completely prevent growth when the medium contained HS (fig.). The different responses of blastocysts in these 2 sera probably cannot be ascribed to the presence of polyamines in horse serum for we were unable to detect putrescine, spermidine or spermine in any of the sera used in this study (unpublished). Moreover, other cell types have also been shown to respond differently to the effects of inhibitors of polyamine synthesis when grown in medium containing HS instead of FBS[19]. Nevertheless, the results reported here confirm and expand our previous conclusions that polyamine synthesis is required for activation and growth of diapausing blastocysts in vitro. Further studies are required to establish how polyamine synthesis is linked to control of growth in these embryo.

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The generation of new 'white' muscle fibers by budding in the lateral musculature of elvers *Anguilla anguilla* (L.) during normal development

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Summary. The elver is a developmental stage of the European eel Anguilla anguilla (L.). During its growth, relatively large numbers of new muscle fibers are formed in the lateral musculature. We investigated the origin of these fibers. They proved to originate from already existing fibers by budding.
Key words. Muscle fibers; budding; *Anguilla anguilla;* muscle development; eel, European.

The elvers used in the present investigations represent a stage of development of the European eel *Anguilla anguilla* (L.). The larvae migrate from the Sargasso Sea to the West-European coasts. During this journey of 2¾–3 years they develop through larval (Leptocephalus) stages into glass eels which migrate from the sea to fresh water[1]. At that time their length varies from 60–75 mm. During the initial period of freshwater life (elver stage), pigment is gradually developed in the cutaneous layers. The growth occurring in this stage includes growth of the lateral musculature which is characterized by an increase in diameter of muscle fibers as well as a considerable increase in number of fibers[2]. In the lateral muscles of the eel 2 fiber complexes may be distinguished, the superficially situated complex of slow ('red') fibers separated by a fascia from the deep, fast ('white') fiber complex[3–6]. In the white fiber complex new small fibers appear almost immediately after the start of growth. In 8 cm animals 25% of the white fibers consists of small new fibers, in 9 cm animals 50%[2].

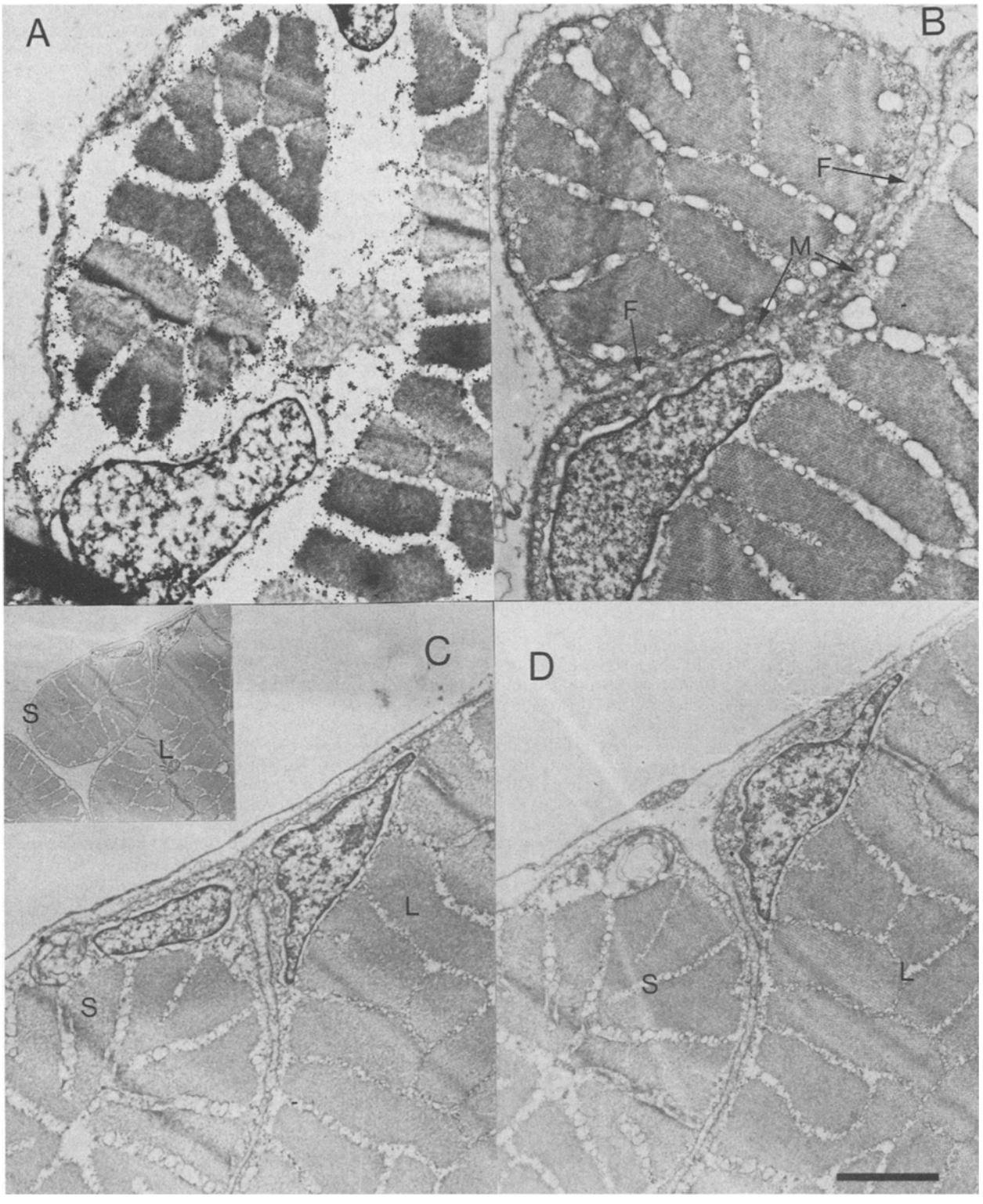
Light microscopical investigations show that the first new fibers appear in the deeper region on the white fiber complex and gradually small new fibers appear also in still more superficially-situated regions.

Electron microscopical studies of transverse and longitudinal sections of the white muscle fiber complex in elvers, ranging from 6–15 cm, revealed the total absence of myotubuli or similar stages of muscle fibre development which usually precede the appearance of new small muscle fibers. Myosatellite cells were present, accompanying less than 3% of the muscle fibers and never found in serial arrangement. However, in the transverse sections we searched carefully for satellite cells, an abundance of quite remarkable structural features was found, especially in 80–85 mm specimens. We may classify them as follows:

1. Groups of 6–10 myofibrils are situtated exremely superficially in large muscle fibers. They are separated from the other fibrils in the fibers by a zone of sarcoplasma. In this sarcoplasmatic zone nuclei and mitochondria may be found (fig. A).

2. A similar group of myofibrils is found, while additionally in the sarcoplasmatic zone membrane fragments (M) are present. In some cases invaginations (F) of the sarcolemma partially divide the sarcoplasmatic zone (fig. B).

3. Sarcolemmal membranes are present to such an extent that their presence results in the separation of a small fiber (S) from a large muscle fiber (L). In both fibers nuclei are situated in a peripheral zone of sarcoplasma, and both fibers have contact areas that are restricted in number and extent (fig. C and D, showing the same pair of fibers).



Transverse sections of white muscle fibers. Explanation in the text. Calibration bar 0.2 μm.

The sequence (1) through (3) may be present in one animal, i.e. in the same transverse section, (1) found superficially in the white muscle fiber complex, (2) intermediately and (3) deeply, together with the above-mentioned light-microscopical observations suggesting that this sequence represents successive stages of development of new white muscle fibers in elvers. The term budding gives the best description of this process of muscle fiber generation. As a part of normal growth, the phenomena described above ar unique in the field of muscle fiber development. They are only distantly related to the muscle fiber splitting, occurring rarely in regenerating or strongly overloaded muscle[7–9]. In the cases of muscle fiber splitting de-

scribed in these papers, the resulting 2 or 3 branches are about equal in diameter while the 'buds' observed in our material receive less than 5% of the mass of the mother fiber, resulting in a very unequal distribution of fiber material.
Preliminary results of investigations on the generation of new fibers in the red fiber complex indicate the occurrence of a similar budding process.

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Effect of the calmodulin inhibitor R24571 (calmidazolium) on rat embryos cultured in vitro

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Summary. The possible effects of inhibition of the calcium-binding protein, calmodulin, on mammalian morphogenesis have been investigated by culturing rat embryos in vitro from 9½ to 11½ days of development in the presence of R24571 (calmidazolium), a specific inhibitor of calmoldulin. Embryos cultured in 10^{-2} mM R24571 for 48 h show inhibited development and exhibit a range of morphogenetic abnormalities including assymetry and neural tube defects. Embryos exposed to R24571 for the first 24 h of a 48 h culture are more severely affected than embryos exposed to R24571 for the last 24 h.
Key words. Morphogenesis; mammalian embryo; calcium; calmodulin.

Morphogenesis, tissue shaping during embryogenesis, is accompanied by changes in the shapes of the individual cells of the tissue undergoing a change in form. In some examples of morphogenesis such as neurulation and gastrulation microfilaments appear at the time when cells are changing shape[2]. Since some morphogenetic cell movements are inhibited by cytochalasin-B[3], which prevents microfilament contraction, it is reasonable to suggest that morphogenesis is effected, at least in part, by co-ordinated changes in cell shape brought about by microfilament contraction. Indeed computer simulations have shown that co-ordinated changes in cell shape offer a sufficient explanation for the tissue movements seen in neurulation and other morphogenetic movements[4].
In non-embryonic systems the contraction of microfilaments is initiated by changes in the level of intracellular free calcium, and so calcium is implicated as being important to the control of morphogenesis. A number of observations support this idea. For example papaverine, which is thought to inhibit calcium fluxes, causes abnormal morphogenesis in amphibian and chick embryos, and its effect can be ameliorated or reversed by administration of the divalent cation ionophore A23187[5,6], which increases the permeability of biological membranes to calcium[7]. In a number on non-embryonic cell types the control of cellular functions by calcium is mediated via the calcium-binding protein calmodulin[8]. Thus the possibility is raised that calmodulin might play a part in morphogenesis. One of the ways in which the possible role of calmodulin has been investigated in cellular systems has been by the use of calmodulin inhibitors. Early experiments employed trifluoroperazine (TFP) but recently a more specific inhibitor of calmodulin, R24571 (calmidazolium), has become available[9]. Here we report the results of experiments to test the effects of R24571 on the morphogenesis of rodent embryos grown in vitro.
Materials and methods. Embryo culture. Rat embryos were obtained from random-bred Wistar rats at 9.5 days of gestation, timed from midnight preceding the morning on which vaginal plugs were observed. Embryos at the headfold stage were explanted in Hank's balanced saline containing 4 mM sodium bicarbonate (Flow Laboratories, Irvine, U.K.). Before culture and after 24 h of culture a small rent was made in the yolk sac and amnion to facilitate drug penetration. R24571 (Boehringer) was prepared as a stock solution at 14.5 mM in spectroscopic grade ethanol and used at a final concentration 10^{-2} mM. The membranes of control embryos were also opened and control cultures contained an equivalent concentration of ethanol. The culture medium was rat serum obtained from blood centrifuged immediately after withdrawal. The serum was inactivated immediately prior to use by heating at 56 °C for 30 min. The embryos were cultured for 48 h in rotating glass bottles[10]. Initially the bottles were equilibrated with 5% O_2; 5% CO_2; 90% N_2 gas mixture. After 24 h the cultures were re-equilibrated with a 20% O_2; 5% CO_2; 75% CO_2 gas mixture and after 43 h they were equilibrated with 40% O_2; 5% CO_2; 55% N_2. In the first experimental series embryos were exposed to R24571 for the whole of the 48 h culture period. In subsequent experiments embryos were exposed to

Table 1. Effect on the development of rat embryos cultured in vitro of the calmodulin inhibitor R24571

		Number of embryos	Apparent embryonic age after 48 h in vitro ± SEM	Yolk sac diameter (mm) ± SEM	Crown-rump length (mm) ± SEM	Protein content (μg) ± SEM
	Controls (+ ethanol)	49	11.47 ± 0.03	3.3 ± 0.08	3.0 ± 0.06	164 ± 9
10^{-2} mM R24571	24–48 h	25	11.18 ± 0.06	3.1 ± 0.08	2.8 ± 0.11	–
	0–24 h	26	10.90 ± 0.03	3.1 ± 0.08	2.0 ± 0.06	–
	0–48 h	54	10.90 ± 0.03	2.8 ± 0.08	2.1 ± 0.08	57 ± 6