SEPIA ARMS AND TENTACLES: MODEL SYSTEMS FOR STUDYING THE REGENERATION OF BRACHIAL APPENDAGES

B. ROHRBACH, H. SCHMIDTBERG*

Institut für Allgemeine und Spezielle Zoologie, Bereich EntwicklungsbioLOGIE, Justus-Liebig Universität Giessen, Stephanstrasse 24, 35390 Giessen, Germany

*Corresponding author: Henrike.Schmidtberg@allzool.bio.uni-giessen.de

ABSTRACT. – Macroscopic investigations on the capability of Sepia officinalis to regenerate lost tentacles reveal a process of renewal that can be divided into six stages of differentiation, corresponding to the studies on arm regeneration in the cuttlefish by Féral (1977, 1978): the protrusion of the central main nerve cord, the scarring of the wounded tissues, the formation of a hemispherical bud which is developing into a cone-like shape, the sucker-formation, and finally the recovery of the functional ability. Following Féral (1979) we applied a three-phase model based on histological-cytological aspects to evaluate degeneration, dedifferentiation, and re-differentiation processes of the wounded tissues in the regenerates. After amputation an intensive migration of amoebocytes into the wounded tissues begins, which is followed by the disintegration of cells at the wound surface. Inside the wound an autolysis of amoebocytes into a plasmoidal mass occurs and forms the primary wound occlusion which is replaced by a monolayered epithelium. The total differentiation of all tissues occurs in the latest phase starting in the central main nerve cord and proceeding to the peripheral regions of the tentacle. The sucker primordia become innervated only towards the end of the third month. Their regeneration is compared with sucker differentiation during embryonic development.

INTRODUCTION

Considering the enormous variety of tasks the arms and tentacles of cephalopods have to achieve (Naef 1928, Sanders & Young 1940, Messenger 1968, 1977, Kier 1982, 1996, Boletzky 1994), it seems likely that an animal that looses one or several arms while fighting with a prey, a conspecific or another predator, suffers a decrease of fitness. But this appears to be only a temporary handicap, because in all investigated cephalopod species a regeneration of the lost arms is possible.

The ability for regeneration was observed for the first time in Octopus vulgaris (Steenstrup 1856) and was initially denied for the Decabrachia (Brock 1886). Further investigations of Sepia officinalis, however, showed that the Decabrachia can regenerate the arms as well (Lange 1920, Aldrich & Aldrich 1968). Féral (1977, 1979, 1988) gives excellent observations on the regeneration of arms, tentacles and suckers in cephalopods. This author analysed in detail the wound healing processes and the morphology, histology, and cytology of arm regenerates in Sepia. Furthermore, the regeneration of amputated arms was proven in studies on Ommastrephes bartrami (Murata et al. 1981) and field investigations on Architeuthis dux also showed that regeneration of tentacles occurs (Aldrich & Aldrich 1968).

Sepiidae as nektobenthic animals stay close to the substrate or are buried in the sandy ground waiting for the prey to be caught by a surprise attack. Older Sepia can also seize prey only with its arms by jumping on it, a so called pouncing attack (Messenger 1968, Duval et al. 1984). This pattern of behaviour can be used when a part of the tentacle or the whole tentacle is lost. The growth rate of the animals is not affected by this changed behaviour (Murata et al. 1981). Thus, apparently the tentacles are not absolutely necessary for catching prey (Messenger 1968). Therefore, the question arises if sepids totally regenerate their lost tentacles as in pelagic cephalopods, or do the regenerates remain imperfect because Sepia is able to catch prawn without tentacles? Another question is whether the regeneration process is age dependent. And finally, is the regeneration process much slower or faster than in arms?

The muscular configuration of tentacles of adult squids (Kier 1982, 1985, 1988, Budelmann et al. 1997) and the differentiation of the tentacle muscle fibres in squids (Kier 1996) and cuttlefish (Grimaldi et al. 2004) have already been extensively studied. The present investigations addition-
ally provide a morphogenetic analysis of tentacle regeneration processes in juvenile *S. officinalis* L. to compare those results with previous studies on arm regeneration and on developmental processes. The removal of one tentacle allows one to show the extreme adhesive efficiency of the suckers in the remaining tentacle club (Messenger 1968). Therefore, we discuss the functional ability of the regenerating tentacles according to the differentiation status and functional morphology of their regenerating suckers.

**MATERIAL AND METHODS**

Fertilized eggs of *Sepia officinalis* (Linné, 1758) were obtained from Arcachon (Atlantic Ocean, France) and Banyuls-sur-Mer (Mediterranean Sea, France) and reared in closed aquarium systems (filtered and recycled seawater, at 18°C) at the Institute of Zoology in Giessen. The animals were chosen according to their age without considering their dorsal mantle length (dml). Prejuveniles (hatching to 30 days) and juvenile (1 to 6 months) animals were used. The studies on the sucker development were carried out on embryonic stages, newly hatched, and juvenile *S. officinalis* from Banyuls-sur-Mer.

Once the animals were anaesthetized in a 2% ethanol-seawater solution, the club with a part of the shaft of one tentacle in each animal was dissected. The following dissections were defined (Fig. 1A):

- **amputation site 1**: in that tentacle area where shaft length is equal to club length (prejuveniles ca. 2 mm shaft length)
- **amputation site 2**: in the tentacle area where the shaft length is equal to one half of the club length
- **amputation site 3**: at the base (3) and the centre (3°) of the club. At this position suckers exist in prejuvenile animals. This group serves as a positive control for the ability to regenerate.
- **amputation site 4**: position of the tentacle shaft from where chromatophores are developed distally.
- **amputation site 5**: shaft “base” (as far as the tentacle could be pulled out of the tentacle pocket)

For the observation of a progressive renewal of tissues a second amputation was performed on several animals at various time intervals after the first cut: 2 mm tissue samples were cut from the shaft tip where the regeneration of the tentacle takes place (Fig. 1B).

The time intervals chosen for sample preparation were 2 h, 5.5 h, 20 h, 26 h, 43 h, 62 h, 84 h, 100 h, 120 h, 140 h, 10 days, 15 days, 16 days, 21 days, 25 days, 30 days, 60 days, 90 days after dissection.

Tissue samples were fixed immediately after amputation, with preparation for scanning (SEM), transmission electron (TEM) and light microscopical (LM) embedding. For this purpose different fixation solutions were used. TEM investigations: prefixation in 2.2% or 3.8% glutardialdehyde in cacodylate buffer (pH 7.4, 1000 mosmol) and postfixation in 1% or 2% OsO₄ dissolved in cacodylate buffer. The material was embedded in Araldite (Durcupan®) or in Spurr’s medium (Spurr 1969). Semithin sections (1 μm) were stained with toluidine blue (Böck 1984) and ultrathin sections were contrasted according to Reynolds (1963). LM investigations: fixation in Bouin’s solution (Romeis 1968) or in 4% paraformaldehyde in seawater, and embedding in paraffin. Sections (7 μm) were stained with Masson’s trichrome (modified by Goldner), Azan according to Heidenhain, and Bodian (Romeis 1968). For immune histochemistry samples were fixed in a 4% formaldehyde seawater solution and were later embedded in paraffin. The sections were stained according to van Leeuwen (1986). The antibodies against serotonin (Serva) were used at a dilution of 1:5000 (overnight, 4°C). Control sections were incubated without primary antibodies. After incubation with the bridging antibody (dilution 1:20, 30 min at room temperature), sections were incubated with the PAP-complex (1:80, 2h) and made visible using substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB).

For the fluorescence histochemical investigations unfixed cryostat sections were stained according to Barber (1982). The specific acetylcholinesterase (EC no. 3.1.1.7) was localized according to the direct thiocholine method of Karnowsky & Roots (1964).

Material for SEM investigations was dehydrated in ethanol or acetone, critical point-dried, and gold-coated.

**RESULTS AND DISCUSSION**

*Morphology of untreated Sepia tentacles*

The tentacle organization in Decabrachia has been described in numerous studies (Kier 1982, 1988, Budelmann *et al.* 1997). In particular the characteristic arrangement and the interaction of the different muscular systems, which differ from the configuration observed in the arms, play an important role for the rapid elongation of the tentacular shaft in squids (Kier 1982, 1985, 1991, 1996, Smith & Kier 1989, Kier & Schachrat 1992). The tentacles of squids and cuttlefish show similar organization. Therefore we present only a short overview of the gross morphology of an intact *Sepia* tentacle (Fig. 1C).

The tentacles are surrounded by a monolayered prismatic epithelium, separated from the adjacent dermis by a lamina basalis (Fig. 2C). The dermis consists of three layers: a subepidermal connective tissue layer, a chromatophore containing layer, and a thinner internal connective tissue layer. In both connective tissue layers collagenous fibres, blood vessels and capillaries, nervous and muscular fibres, and loosely distributed fibrocytes are located (Fig. 2D). In contrast to the arms (Féral 1977) the muscular tissue in the dermis is more strongly developed. At least in the investigated animals an iridophore layer could not be located, neither in the tentacle shaft nor in the club. The
Fig. 1. – A. Schematic representation of tentacle club and shaft with various amputation sites, lower arrow: proximal sucker primordia on tentacle shaft. B. Stump of a regenerating tentacle, arrow: second amputation site. C. Cutaway diagram of a tentacle shaft of *S. officinalis*. AN axial main nerve cord, AR tentacle artery, B connective tissue, CM circular musculature, E epidermis, HM helical muscular system, LM longitudinal musculature, LMe longitudinal extrinsic musculature, Np peripheral nerve cord, TM transversal musculature, V vein.
Fig. 2. – A, Aboral side of juvenile *Sepia* tentacle, B, Upper tentacle six months after amputation, stage VI, untreated tentacle below. C, Cross section of tentacle club with the different muscular systems. D, Extrinsic musculature. E, Intrinsic muscle system. F, Tractus cerebrotentacularis consisting of two fibers of different thickness on the oral and aboral side. AN axial main nerve cord, B connective tissue (adventitia), Ch chromatophore, CM circular musculature, D dermis, E epidermis, GC cortex, HM helical muscular system, LM longitudinal musculature, LMe longitudinal extrinsic musculature, Np peripheral nerve cord, Tc tractus cerebrotentacularis, TM transversal musculature, TR trabeculae.
In the vicinity of the infundibulum and the rim of the muscular wall, and a thick nerve bundle in the peduncle can be traced to the main nerve cord of the tentacle.

**General observations on tentacle regeneration**

Animals of different ages do not in general differ in their regeneration abilities. However, a second amputation of regenerating tentacles causes a delay of 2 to 3 days in tissue regeneration. Additionally, temperature has an influence: lower temperatures decelerate the healing process, as already demonstrated in earlier studies (Féral 1977, 1978). Both the regeneration and the development process of a new tentacle club are independent of the existence of suckers on the remaining tentacle shaft. Tissue regeneration seems to proceed according to a given pattern: the tentacle club is starting to be renewed, and this is followed by the extension of the tentacle shaft. This pattern is realized regardless of which site is amputated. The closer the cut is located to the body the sooner the elongation of the shaft begins after the formation of the club primordium. A cut leads to a regeneration of a club at any rate and the developing cones look the same.

At all amputation sites the tentacle club was completely repaired after three months, and already before the whole tentacle length was achieved the functional efficiency of the tentacle club was re-established; this became obvious in the usability of the largest suckers and the general functioning of the regenerated tentacles. The time of the complete tentacle renewal (i.e. club and shaft have the same size and form as the second untreated tentacle) depends on the site of amputation and results exclusively from the different growth rates of the tentacle shaft. The regeneration process of complete tentacles cut at amputation site 3 was completed about 3 months after amputation; tentacles cut at amputation site 2 after 5 to 6 months; tentacles cut at amputation site 1 between 6 and 8 months later. Tentacles without chromatophores on its shaft cut at amputation site 4 and 5 showed regeneration processes as well, but the total regeneration time could not be defined because the animals died (between 9 to 23 months) before regeneration was complete.

**Macroscopic findings on regeneration**

Based on the studies of Féral (1977, 1978) on the arms of *S. officinalis*, we propose a similar classification of the tentacle regeneration process in six stages, because of the highly visible morphological similarity (Table 1).

In stage I (0 to 7 days after amputation) the main nerve cord emerges from the surrounding transversal muscular tissue while the rim of the wound contracts (Fig. 3A). 4 h to 8 h later the...
wound constriction is dorsolaterally extended. An amputation in the immediate vicinity of suckers leads to a rejection of these suckers 2 h to 7 h later. Suckers that are not located in the wound area remain intact. In stage II (Fig. 3B), 5 to 7 days after amputation, the external and internal epidermis merge over the wound while the axial nerve cord is withdrawn. This process, which lasts from 5 to 16 days after amputation, is completing the phase of wound healing and starting the phase of regeneration. In stage III (Fig. 3C, 3D), 8 to 19 days after amputation, a thin cone is growing aborally. In stage IV (Fig. 3E), 15 to 25 days after amputation, a concave bulge appears on the oral side of the tentacle where the primordia of tentacle suckers emerge. At stage V (Fig. 3F, 3G), 25 to 37 days after amputation, the cone is considerably elongated, and chromatophores appear on the aboral tentacle side, dispersing from the base to the tip of the regenerate. The surfaces of the more basal suckers start to invaginate their epidermis where their infundibulum later appears. In the terminal stage VI (> 30 days) the regenerate extends after an intensive growth phase (Fig. 2B). At that time the tentacle has regained its functional ability, as demonstrated by the completed differentiation of the largest suckers.

A comparison of the observations by Féral (1977, 1978) on arm regeneration clearly shows a delay of regeneration processes to occur only in the formation of the tentacle suckers (Table I), even though the water temperature was higher than in the earlier study and thus some acceleration of growth was expected. It remains questionable if this phenomenon is caused by different rearing conditions (laboratory raised animals, addition of artificial seawater). Moreover, the dwarfism of animals observed in our aquarium system (Versen & Boletzky 1992) as a result of the rearing conditions (relatively small aquaria holding numerous animals) is unlikely to cause a decelerated sucker development. Several authors point out that diminutive animals show normal behaviour and reach sexual maturity (Boletzky & Hanlon 1983, Versen & Boletzky 1992). A partial answer to this question may be the observation that juvenile Sepia reared in the aquarium often do not use their tentacles after unsuccessful attempts at catching prey and therefore move forward by using their arms instead (Boletzky 1972, 1974a,b, 1993, Versen & Boletzky 1992, Chen et al. 1996). This means that the total regeneration of the tentacle suckers during this particular time of postembryonic development may not be absolutely necessary for the young animals, because another equally profitable behaviour exists, which is supported by fully differentiated and powerful suckers on the arms.

Table I. – Comparison of time scales for morphological stages of regeneration processes in arms and tentacles of S. officinalis.

<table>
<thead>
<tr>
<th>stages</th>
<th>Sepia arms (Féral 1977)</th>
<th>Sepia tentacles (Féral 1979)</th>
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<tbody>
<tr>
<td></td>
<td>rearing temperature 16 °C</td>
<td>rearing temperature 18 °C</td>
</tr>
<tr>
<td>Stage I</td>
<td>1 to 7 days</td>
<td>0 to 7 days</td>
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<tr>
<td>wound healing, cicatrisation of wound</td>
<td>wound healing, cicatrisation of wound</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>5 to 14 days</td>
<td>5 to 7 days</td>
</tr>
<tr>
<td>hemispherical shape of arm tip, bud formation</td>
<td>hemispherical shape of tentacle tip</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>10 to 21 days</td>
<td>8 to 19 days</td>
</tr>
<tr>
<td>arm growth, cone formation</td>
<td>cone formation</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>17 to 25 days</td>
<td>15 to 25 days</td>
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<tr>
<td>sucker formation</td>
<td></td>
<td>tentacle growth</td>
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<tr>
<td>Stage V</td>
<td>25 to 35 days</td>
<td>25 to 37 days</td>
</tr>
<tr>
<td>first chromatophores</td>
<td>first chromatophores, sucker primordia</td>
<td></td>
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<tr>
<td>Stage VI</td>
<td>&gt; 30 days</td>
<td>35 days to 3 months</td>
</tr>
<tr>
<td>thickening of regenerate, recovery of functional ability</td>
<td>differentiation of suckers, thickening of regenerate, recovery of functional ability</td>
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Structural reorganization after amputation

The regeneration of the tentacle may also be divided into three separate phases according to the histological and ultrastructural characteristics of amputated arms as described by Féral (1979):

Phase I starts with the amputation of the tentacle and lasts about five days. It consists of three main processes: closure of the wound associated with migration of blood cells; massive synthesis of collagen; degeneration, and dedifferentiation of the surrounding tissues. According to Féral (1979) the degeneration process is characterized by lysis of cells in the wound tissue. The dedifferentiation process is determined by cells that lose their morphological and physiological characteristics and acquire an embryonic nature. In phase II, between the 5th and 20th day after amputation, a blastema
Fig. 3. – Stages of regeneration, macroscopic findings: A, Stage I, immediately after cut. B, Stage II: hemispherical shape of tentacle tip (x). C and D, Stage III, cone formation at aboral side. E, Stage IV, regenerate turns to the aboral side. F, Stage V, cone growth. G, differentiation of sucker primordia (SA) in a regenerating club. Ü amputation site in the middle of club.
develops. Numerous mitotic cells are indicative of an intensive growth phase. In phase III (20 days to 3 months) the cells start to differentiate, and tissues and organs develop. Finally, the tentacle regains its mobility and functional abilities. Let us now consider the different phases in greater detail.

Phase I: from the time of amputation to the fifth day, the central nerve cord protrudes from the amputated stump. This protrusion is caused by contractions of the surrounding longitudinal muscular tissue (Fig. 4A). At the edge of the wound bulges arise from the surrounding epithelium and cells disintegrate in the wound region (Fig. 4B). An intensive migration of amoebocytes takes place between and among all tissues of the tentacle (Fig. 4C). In parallel with an increased protocollagen synthesis inside the wound the autolysis and agglutination of amoebocytes into a "plasmoidal mass" begins. It forms a primary wound occlusion (Fig. 4D). At the transition from phase I to phase II, between 5 to 7 days, the wound tissue is covered by an epidermal layer consisting of flattened epithelial cells. Initially no lamina basalis is developed, and in contrast to other tissues no degeneration or dedifferentiation process is detectable in the epithelium (Fig. 4E).

Degeneration of tissue, revealed by a necrosis in the periphery of the wound, can be noticed immediately after the amputation, starting with the neuron cells of the cortex of the main nerve cord, and continuing with the musculature and up to the dermis. In comparison with other tissues, the degeneration of muscle cells, especially of the longitudinal muscle system, is weak. The extrinsic muscle system detaches the connection to the muscular system. Afterwards the transversal muscles regenerate and extending along the extrinsic muscular system. Afterwards the transversal muscles are reorganized. The sucker "anlagen" become innervated only towards the end of the third month. Before reaching its full length, the club’s functional ability has already been restored. At this point the club is still slender and reaches its final diameter only when the tentacle has completed its extension and has stopped growing (Fig. 2B).

The histological and cytological differentiation of tissues in regenerating tentacles proceeds almost exactly, morphologically and timewise, as the renewing process in arms as described by Féral (1979).

Numerous invertebrates are able to regenerate central and peripheral neural connections (Moffet 1995, 1996, Bale et al. 2001). Our investigations on the regeneration of both nervous tissue and the main nerve cord proceed in agreement with those studies.

One of the first responses to neural injury in molluscs is the activation of blood cells such as macrophages, NK-like cells, and granulocytes which migrate to the body wall and then to wound surfaces: such processes have been characterized by morphological, histochemical, and immunohistochemical methods in numerous studies (e.g. Ruddell 1971, Sminia 1974, Glinski & Jarosz 1997, de Eguileor et al. 1999). In cephalopods earlier studies revealed that wounds are closed by local vasoconstriction and haemocyte aggregation, forming a blastema while the injured muscle and nerve cells are phagocytized (Stuart 1968, Browning 1979, Malham et al. 1997, Beuerlein et al. 2002a, b).

Our investigations yielded no chronological differences in the regeneration processes involving the three different muscular systems. In the de-differentiating process the myofibrils disintegrate, subsequently the sarcomeres of the muscular cell disintegrate. For re-differentiation a reverse process is presumed to exist, corresponding to the situation of developing myoblasts as described in the central heart of S. officinalis (Versen 1991): first of all the z-patches differentiate, followed by the formation of myofilaments. But more data are needed to accurately characterize the differentiation of myoblasts in Sepia.
Fig. 4. – Structural reorganization. A, Phase I, protruding nerve cord immediately after cut. B, Phase I, beginning disintegration of cells in the wound region (arrowheads). C, Phase I, migrating amoebocytes inside the wounded tissue. D, Phase I, plasmoidal mass of amoebocytes (X). E, End of phase I, wound tissue covered by an epidermal layer. F, Phase II, cone growth. G, Phase III, nerve fibers (arrowhead) proceeding from the main to peripheral nerve cord. 

Abbreviations: see Fig. 2.
The role of neurotransmitters

Generally, the reaction of the regenerating neurones is influenced by neurotransmitters (Chiasson et al. 1994), and this appears to be true also in tentacle regenerates of *Sepia*.

Acetylcholine-esterase (AchE) as an indicator for the presence of acetylcholine was detected in neuromuscular end-plates (Fig. 5A) of the transversal (Fig. 5B) and circular muscular system as well as in the synapses of the tractus cerebrotentacularis (Fig. 5A) in untreated tentacles and regenerates of late phase III. This corresponds to the results obtained by Bone et al. (1982) who described the presence of acetylcholine in the inner longitudinal muscles of the arms and tentacles. The localization of the acetylcholine esterase in different areas of the central nervous system (Chichery & Chichery 1974) and in the central circulatory organs (Kling 1986, Schipp et al. 1986) suggests that acetylcholine is a common neurotransmitter not only in *S. officinalis* but in cephalopods in general as suggested by Messenger (1996) and has an excitatory effect on the muscles of the arms, tentacles, head retractors and siphon (Bone et al. 1982).

Studies on non-amputated parts of the tentacle, performed by means of glyoxilic acid induced fluorescence, demonstrate the presence of catecholamines in the neuropil of the main nerve cord (Fig. 5C) and in the tractus cerebrotentacularis (Fig. 5E). Investigations on 25-day old regenerates indicate the presence of catecholamines on maximum emission (480-490 nm) primarily in the neuropil of the main nerve cord (Fig. 5D). These

Fig. 5. – Neurotransmitters. A, AchE in the axial nerve cord, inset: AchE in neuromuscular end-plates B, AchE in the muscular system. C, Catecholamines in intact tentacles in the neuropil. D, Catecholamines in 25-day old regenerates primarily in the neuropil. E, Catecholamines in the tractus cerebrotentacularis of untreated tentacle. AR tentacle artery, B connective tissue, CM circular musculature, LM longitudinal musculature, NP neuropil, Tc tractus cerebrotentacularis, TM transversal musculature, TR trabeculae.
results correspond well with other findings demonstrating the presence of catecholamines in the nervous system of Sepia (Juorio 1971, Tansey 1980, Kling 1986). But the above results also indicate that the transmitter status with monoamines in the newly formed tissues is completed only at the end of the regeneration process.

No serotonin fluorescence (5-HT, 520–530 nm) could be traced in the stumps of any treated Sepia tentacle, in contrast to the situation observed in untreated tentacles where serotonin is detectable. According to our results the tentacles of Sepia possess a dual cholinergic/catecholaminergic innervation. Additionally, a peptidergic mechanism regulated by FMRF-amides, as described for cephalopods in earlier studies (Westermann et al. 1997, 2002, Schipp et al. 1991, Marschinke 1997), is supposed to also function as a neurotransmitter. But this and the possible role of further peptides in intact and regenerating tentacles has to be proved in further studies.

**Sucker differentiation**

The expression of suckers in untreated embryonic arms and tentacles starts with a single row of ridgelike buds on the oral side of the tentacle tip at stage XI of Naef (1928) (see also Nolte & Fioroni 1983, Haas 1989). Histologically the sucker primordia can be detected by the bulging ectodermal epithelium. Below the epithelium a mesodermal tissue is developing. In stage XII the proximal suckers arrange themselves in two rows and furthermore in four rows on the newly developing tentacle club (Fig. 6A, 6B). In histological sections the suckers become spheroidal due to the narrowing sucker base (Fig. 6C). In stage XIII the proximal suckers are arranged in six to seven rows (Fig. 6C). All of them show the same form and size. In developmental stages XIV to XVI the epithelium of the apical sucker surface is differentiating (Fig. 6D), then invaginating the prospective region of the infundibulum and finally the acetalbum. The connective tissue and the muscular systems emerge, as well as the tentacle peduncle. In stage XVII to XVIII (Fig. 6E, 6F, 7C) the typical asymmetry of decabrachian suckers appears, and a high prismatic sucker epithelium is lining the sucker chamber. At stage XIX (Fig. 7D) adult-like suckers with a well-differentiated infundibulum with pegs are present. First muscle and nerve fibres differentiate but the intrinsic and extrinsic muscular and nervous systems are not totally finished before the hatching stage (Fig. 6G, 6H, 7E). All in all the differentiation of functional suckers depends also on the ambient temperature. At 20°C the embryonic development of the cuttlefish lasts about 40 to 50 days. The last embryonic phases (stage XV to XX) take 50% longer than the whole embryonic development and the last growing phase (stage XVIII to XX) actually requires 80% of the whole embryonic development (Fioroni 1964). Thus, generally the sucker development during embryogenesis up to hatching lasts about 30 days.

As mentioned above in tentacle regenerates the development of suckers begins for the first time in phase III, approximately four weeks after amputation, and becomes visible by the ridge-like buds of the epidermis. Thus, time of appearance and the structural organisation of sucker primordia in untreated and regenerating tentacles are similar. But in comparison with the situation in embryonic tentacles where the proximal suckers initially are the largest (Fig. 6B, 6C), knoblike tiny buds also appear in that region, where the tentacle shaft terminates and the prospective proximal part of the regenerating club develops (Fig. 1A, lower arrow). In tentacles of adult animals, no matter whether amputated or untreated tentacles are considered, these suckers are already incorporated into the tentacle club.

The differentiation of the various tissues in the suckers of regenerates proceeds in the same way as in embryonic development (Fig. 7F), it is delayed, however. In particular the innervation of the suckers, the connection between nerve cord of the sucker peduncle, and the main nerve cord in the tentacle is re-established only in the third month (see above: phase III), which means that the usability is delayed in regenerates.

**Prey-capture behaviour**

The transverse muscles of the intrinsic muscular system are responsible for the extremely rapid extension of squid tentacles, as described by Kier (1982). In comparison to the obliquely striated muscle cells of transverse muscles of the arms, they are cross-striated muscle cells that form a specialized ultrastructure with relatively short sarcomeres (Kier 1985, 1991, 1996). Kier (1996) states for the tentacles of Sepioteuthis lessoriana that the transverse muscles are obliquely striated for the first three weeks after hatching. Afterwards they change to cross striation like in adult animals. This muscle cell differentiation is correlated with behaviour. Immediately after hatching, animals perform the pouncing attack using the arms, and only after the transformation of the musculature the tentacles are used. In our investigations on the cuttlefish the transverse muscle cells of the tentacles are likely cross striated and possess short sarcomeres already at hatching. At this time hatchlings are already able to use their tentacles but they have to exercise this behaviour, as suggested by many failing attempts. Generally the animals show an individual behaviour independent of the length
Fig. 6. – Sucker differentiation during embryonic development (stages according to Naef 1928). A, Stage XII, tentacle tip with lamellae-like sucker primordia in one longitudinal row. B, Stage XII, proximal tentacle region, bud-like sucker primordia in two rows. C, Stage XIII, proximal tentacle region with suckers beginning to organize in eight rows. D, Arm crown of Sepia at stage XIV-XV. E, Stage XVIII, distal suckers with first invagination of apical surface (arrowhead). F, Stage XVIII, largest tentacle sucker with opening of prospective sucker chamber, surrounded by polygonal infundibulum cells. G, Tentacle club at hatching stage XX. H, Differentiated tentacle sucker at stage XX, polygonal processes with pegs cover the infundibulum; the inner horny ring is still smooth and shows no teeth or cones, in contrast to suckers of adult animals. I infundibulum, Pe piston epithelium, pH polygonal infundibulum cells, Sö sucker opening to acetabular chamber.
REGENERATION OF TENTACLES IN SEPIA OFFICINALIS

Fig. 7. – Sucker differentiation during embryonic development (stages according to Naef 1928) and during tentacle regeneration. A, Stage XV, tentacle sucker primordia with ectodermal epithelium and mesodermal tissue. B, Transverse section of a tentacle sucker in stage XVI, invagination of apical sucker epithelium, first differentiation of muscle tissue, and tapering of sucker stalk. C, Transverse section of tentacle sucker in stage XVIII, high prismatic cells are lining the prospective sucker chamber. D, Tentacle sucker in stage XIX with well-differentiated infundibulum and acetabulum. E, Tentacle sucker at hatching stage XX, highly differentiated, asymmetrical sucker with fully functional acetabulum and infundibulum, like in suckers of adult animals. F, Suckers of different developmental stages on a regenerating tentacle (stage VI); the morphological and histological organization is comparable with the sucker development during the different embryonic stages. Ac acetabulum, Bg blood vessel, D mucous cells, Ek ectodermal epithelium, Es invagination of apical sucker epithelium, G prospective sucker ganglion, If infundibulum, iH inner horny ring, Mu muscle tissue, P piston epithelium, Ra radial muscle tissue, R sucker rim with numerous chemoreceptor cells, So sucker opening to actebular chamber.
of the tentacle stump or the stage of regeneration: some of them prefer the “pouncing” attack, others use the ejectible tentacles or the regenerates. And even a change of food from dead Crangon crangon to living, fast moving Mysis spec. does not cause a change in behaviour: some animals retain the slower “pouncing” attack with the arms even if failing attempts accumulate.

CONCLUSION

The structural organisation of the tentacle of Sepia officinalis is comparable to conditions described in the literature for other decabrachian cephalopods. Processes during regeneration (after amputation) such as degeneration, dedifferentiation and re-differentiation correspond to the observations on arm regeneration in the cuttlefish reported by Féral (1977, 1979). But in contrast to his results the full regeneration of the tentacle, which is defined by reaching full length and motility of the tentacle shaft and innervation of suckers, takes at least three months.

With the arms Sepia has the ability to achieve all vital functions; prey capture, warning, camouflage, defence etc. The tentacles, however, seem to be an auxiliary equipment that enable the animals to catch their prey from some distance. Thus, tentacles are of great importance for the animals to survive in their natural environment. In laboratory investigations the natural prey-capture behaviour cannot be reproduced entirely but it is well known that in their natural habitat animals absolutely rely on tentacles to catch fast fish, shrimp or other cephalopods. The extreme velocity of the tentacle movement and the possibility to catch prey from some distance provides a great advantage in the interspecific competition. For this reason Sepia cannot abandon the possibility of tentacle regeneration which is an essential advantage of survival for these highly developed decabrachians.

DEDICATION. – We dedicate this work to Professor Pio Fioroni (1933-2003) who conveyed his enthusiasm for cephalopods to us.
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Received October 5, 2005  
Accepted December 15, 2005