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THE EPIMORPHOSIS OF A WALKING LEG IN THE EARLY STAGES OF POSTEMBRYOGENY IN *TEGENARIA ATRICA* C.L. KOCH (*ARACHNIDAE, AGELENIDAE*)

EPIMORFOZA ODNÓŻA CHODOWEGO WE WCZESNYCH ETAPACH POSTEMBRIOGENEZY *TEGENARIA ATRICA* C.L. KOCH (*ARACHNIDAE, AGELENIDAE*)

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ABSTRACT: The epimorphosis of a waking leg in the early stages of postembryogeny in *Tegenaria atrica* C.L. Koch has been studied and compared with the normal development of a leg.

Experiments were made in larvae, nymphae I and nymphae III whose first left walking legs were amputated in the middle of their length (in the middle of a tibia). The amputation was carried out by the method of sashes made of a steelon thread. The proper part of the leg was cast off at the nearest moulting time.

It was found out that the course of regeneration corresponded to the stages of ontogeny. The regeneration occurred after the first moult following the amputation and most often began with forming a club–like callus, which morphologically corresponded to an appendage of an embryonic type, at the place of the cut. The clublet lengthened and developed into a leg of a larval type, and afterwards into one of a nymphal type. The complete, seven–segmented appendage regrew at this time.

In larvae and nymphae I the regeneration of the cut off parts of legs was completed after four or five consecutive moults while in nymphae III – already after four moults. The number of moults necessary for the regeneration of the cut off leg in larvae and nymphae I depended on the structure of the scar surface which appeared immediately after the tied part of the appendage had been cast off.

All developmental stages of *Tegenaria atrica* C.L. Koch under investigation retain the capacity to regenerate the lost part of the leg, however, the highest rate of this process was found in the oldest of the investigated stages, i.e. in nymphae III.

INTRODUCTION

Regeneration is a biological process which enables the regrowth of damaged or lost parts of the body (organs, tissues, cells) and the restitution of biochemio-physiological activities occurring in them. It may concern various levels of organisation of an organism and occur permanently or periodically, making possible the exchange of used up cells (physiological regeneration), the regrowth of a lost organism (epimorphosis) or the restitution of a whole organism from its fragments (morphallaxis) (Dobrowolski, Klimaszewski, Szelęgiewicz, 1971).

The power to regenerate is found in almost all groups of animals, but the type and quality of these powers depend on, among others, the level of ontogenesis (the level of differentiation of cells) and the level of phylogenesis of an unit of classification to which an animal belongs. The lower level of morphological, biochemical and physiological differentation of cells, that is the less complex structure of an organism and the lower level of phylogenesis, the greater powers to regenerate, especially those of morphallaxis and morphogenesis (Pautch, 1939; Rajski 1983). An essential factor conditioning the capacity to regenerate and the launching of this process as well as influencing its course is the age of an animal. A younger animal posseses greater powers of regeneration (Grodziński, Jura, Szarski, 1970; Rajski, op.cit.). Also the degree of the capacity to regenerate considerably varies within particular units of classification (Beklemiszew, 1958; Grabda, 1984).

Problems of regeneration in spiders are relatively poorly known and were treated incidentally in the past. Phenomena of physiological regeneration were most often described in connection with laboratory breeding of various species (Savory, 1936). Standard observations of the regeneration of organs were carried out in *Dolomedes* in regard to the capacity for autotomy of this spider (Bonnet, 1930). However, more extensive reports on the regeneration of various morphological structures, mainly organs of appendage origin in various species of arthropods, and thus of spiders, are relatively scarse (Bordage, 1889, 1901; Gabritchevsky, 1927, 1930; Randall, 1981; Vachon, 1967; Weiss, 1907). Only do few works touch these problems more widely – with regard to a definite unit of classification (Friedrich, 1966; Locket, 1936; Oppenheim, 1908; Vollrath, 1987; Wood, 1926), sometimes in connection with the problems of phylogenesis (Vollrath, 1990) or in relation with early stages of postembryogeny (Mikulska, Jacuński, Weychert, 1975). Some researchers pointed out biochemical and physiological aspects of these processes (Moskwa, 1966; Nagornyj, Nitkin, Bułankin, 1963).

Authors of this publication have decided to deal with the regeneration of a walking leg of *Tegenaria atrica* C.L. Koch, thereby initiating a series of publications on the quality and rate of repair processes related to legs and other structures of appendage origin.

MATERIAL AND METHODS

In order to obtain selected developmental stages of *Tegenaria atrica* C.L. Koch, a ground breeding of *Tegenaria atrica* was established near Borzyszkowo (the Bydgoszcz province). Spiders were held in favourable conditions in drainage tubes 80 mm in diameter. In July and August spiders were caught and kept in laboratory breeding in optimal conditions for this species until they reached sexual maturity (Mikulska, Jacuński, 1968). All individuals were placed singly in glass containers with 500 cm^3 capasity. To facilitate building a dwelling trap web, X–shaped wooden statlets were put into each container. Spiders were alternately fed on larvae of domestic crickets *Acheta domesticus* and of meal worms *Tenebrio molitor* L. in excess, and every other day water for drinking was supplied by putting damp pieces of cotton wool into each container.

Each of the forty sexually matured females was held with a few males in turn for several days to secure maximum insemination. Egg sacs, appearing from the beginning of October, were taken away and cut open, and afterwards, the eggs were placed in to glass pans and kept at 23°C until they hatched out and proper nymphal stages developed.

For experiments, 50 larvae, 40 nymphae I, and 30 nymphae III were used. The actual phase of the experiment was launched when first larvae appeared.

In larvae and nymphae, which appeared later, the distal part of the left walking leg of the first pair was amputated. The amputation was made with aid of a stereoscopic magnifying glass on a special table, using the sash made of steelon thread 40 um in diameter, which was tied in the middle of a tibia (Fig. 1).

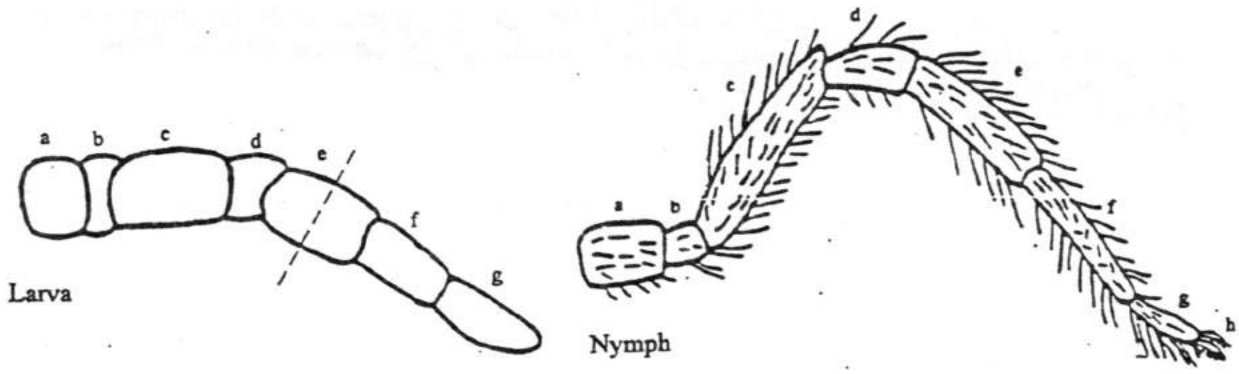

Fig. 1. Schematic diagram of the structure of a larval and nymphal leg in *Tegenaria atrica* C.L. Koch with the marked place of the tying of a sash; a – coxa, b – trochanter, c – femur, d – patella, e – tibia, f – metatarsus, g – tarsus, h – claws

The sashes were tied to larvae immediately after the hatch while to nymphae after moulting (Fig. 2).

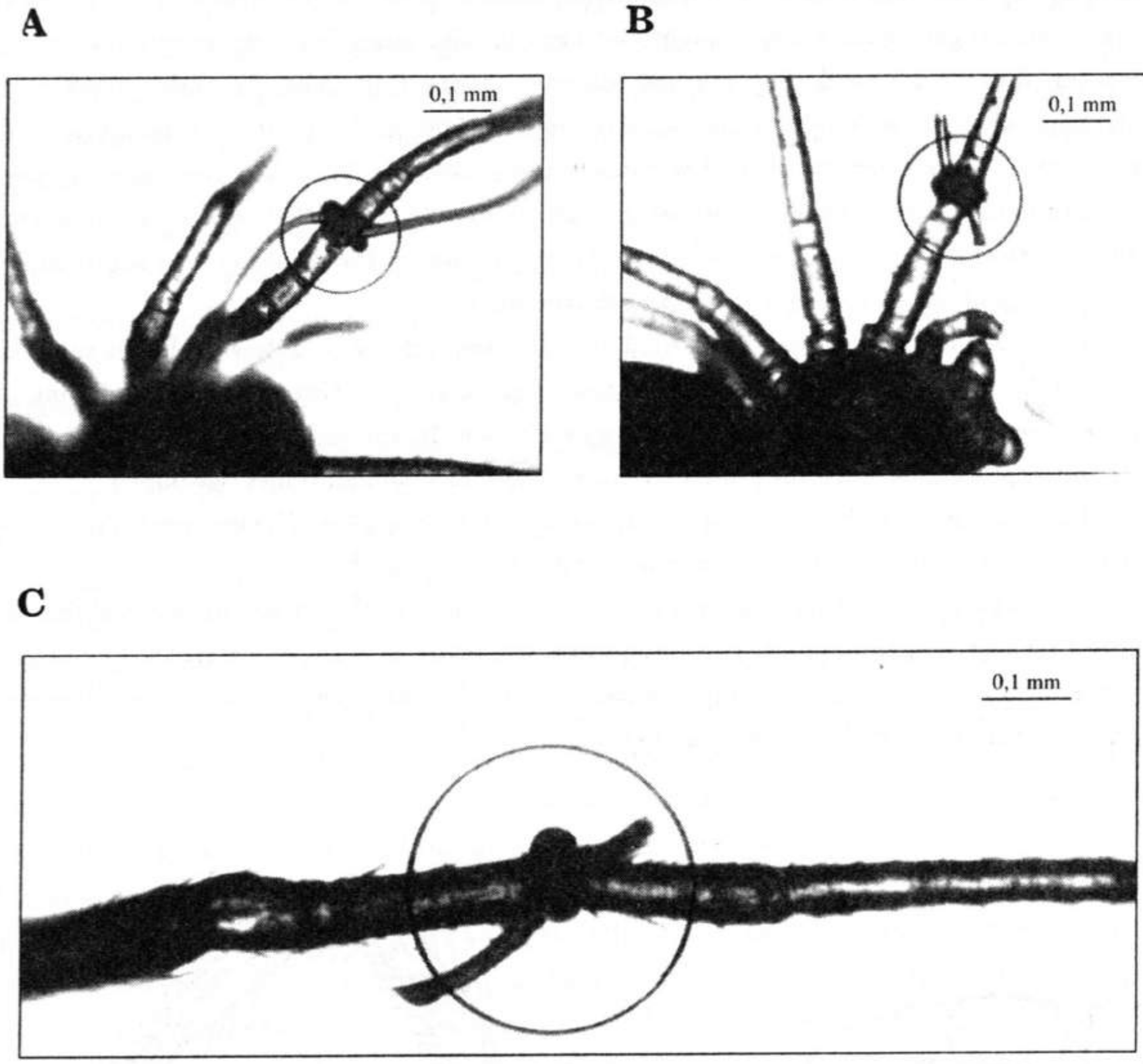

Fig. 2. Larva (A). nymph I (B), and nymph III's leg (C) with the sash tied in the middle of the tibia of a left walking leg in *Tegenaria atrica* C.L. Koch. (photographed by L. Jacuński)

The results of measurement and observation of regenerating legs and of those developing normally (legs for comparing) were compared in tables and illustrated graphically.

The tied part of the leg was cast off at the nearest moulting time. The described way of amputation was relatively little injurious as it did not result in opening myxocoel, and thus flowing out of hemolymph. The loop of the sash closed a hemocoel at the definite place breaking the continuity of this cavity, however, hemolymph pressure was not drastically reduced, which is often the reason of the death of spiders in the case of an injury to walking legs.

During the experiment nymphae were kept singly in Petri's pans and fed on larvae of *Tribolium confusum Dur.* in excess. Water was supplied everyday by putting damp pieces of cotton wool into the pans.

The course of the regeneration was usually checked every ten hours, or if necessary, more often. The measurement was carried out by means of scaled spectacles. During the observation attention was paid to the:

– the appearance of the regenerate in comparison with the remaining part of the leg,
– the size of the regenerating leg in comparison with the normal leg placed on the right side of the body (the latter served for comparing),
– the increase of the length of the regenerate; it enabled to determine the rate of the growth of a regrowing leg in the investigated developmental stages,
– the number of segments, their structure and equipment with bristle–like hairs (setae) and distal claws,
– the number of moults necessary for complete regeneration.

RESULTS

1. The regeneration of the leg amputated at the larva stage

Despite employing the little injurious method of amputation (without loss of hemolymph), 70 per cent of the individuals subjected to the operation died in various stages of the experiment, of these 28 per cent died already before the first nymphal moulting. Twenty individuals lived until the nymph I stage (c. 40 per cent). One may certainly state that the first moulting time was extremely critical for the larvae with the tied sashes. Another critical moment was the moult of nymphae II when 5 individuals died. Because of these reasons further observations and measurement were carried out on 15 individuals.

After moulting at the nymph I stage the regenerate was not found at the place of amputation. The place of the cut (the place where the amputated part of the leg was cast off) resembled a skinned over wound (Fig. 3).

The actual regeneration process began after the next moult, i.e. after the second one, which occurs at the nymph II stage. At that time the regenerating part of the leg had the structure of a larval leg. It was characterized by light colour, which testified to the poor formation of a cuticle. On the other hand, it was not armed with the setae and nymphal claws. The leg with the regenerate was shorter than the normal leg by 0.5 mm (15.62 %). The nymph II stage lasted 24 days at this variant of the experiment.

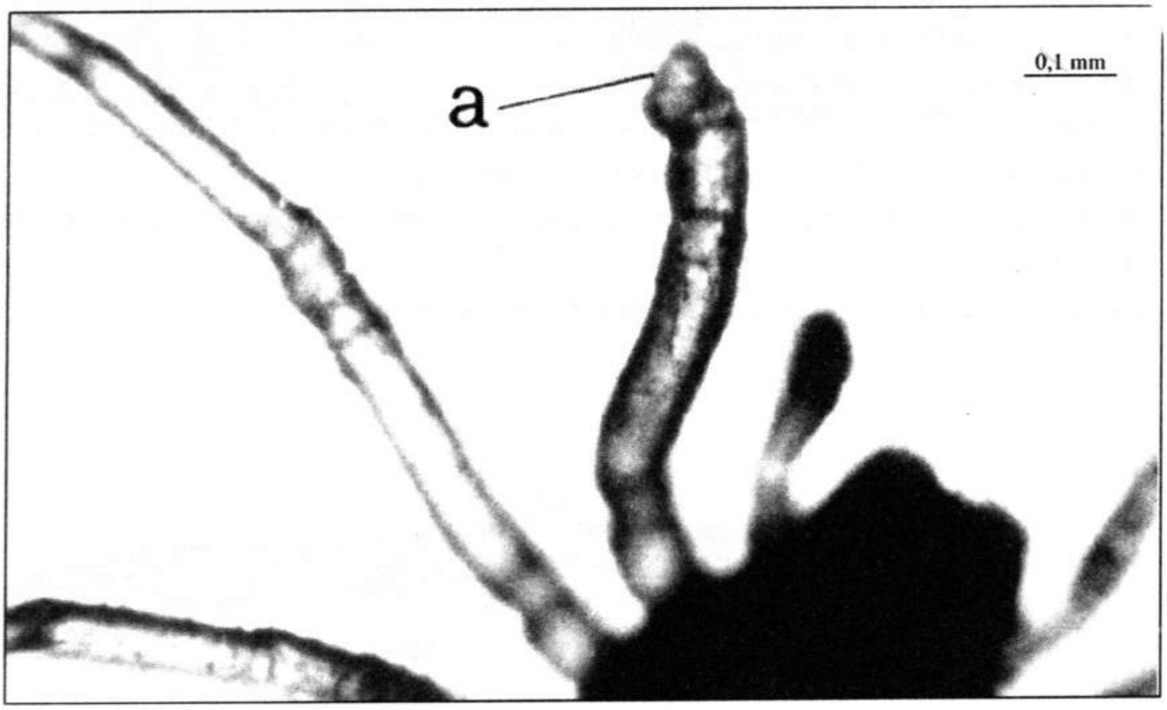

Fig. 3. Nymph I of *Tegenaria atrica* C.L. Koch after it has cast off the fragment of the leg tied at the larva stage; a – ending of the amputated leg with a skinned over wound (photographed by L. Jacuński)

After the third moult (the nymph III stage) the regenerate took shape of a nymphal leg with a thicker cuticle and characteristic setae and claws. The particular segments of the appendage lengthened proportionally to each other. The leg with the regenerate was shorter than the normal leg by 0.44 mm (11.46 %). At this developmental stage, the regenerating leg was characterized by greater growth than the normal leg, that is the former grew faster. The growth in comparison with the previous stage expressed as a percentage was nearly 26 per cent while that of the normal leg – 20 per cent (Tab. 1).

Tab. 1. Mean length of normal legs (right legs) and regenerating legs (left legs) after the amputation carried out at the larval stage

Developmental stage	Mean length of a leg (mm) and the percentage of the increase in length in comparison with the previous stage					
	normal leg		regenerating leg in comparison with the stage of			
			nymph V		nymph IV	
	(mm)	(%)	(mm)	(%)	(mm)	(%)
larva	1.24		0.66 amputation			
nymph I	2.13	71.7	lack of the regenerate: casting off the sash with the part of the leg			
			1.30	96.97	1.30	96.97
nymph II	3.20	50.23	2.70	107.69	2.70	107.69
nymph III	3.84	20.00	3.40	25.83	3.40	25.83
nymph IV	4.50	17.19	3.52	3.53	4.50	32.35
nymph V	5.76	28.00	5.76	63.64		

The duration of the nymph III stage ranged from 25 to 34 days. Observations revealed that individuals, in which the nymph III stage lasted shorter, and thus the moulting into nymph IV occurred earlier, were characterized by greater acceleration of the regeneration process. In these cases the completion of the restitution of the lost part of the leg took place at the next developmental stage, i.e. in nymphae IV. The extension of a intermoulting period in the remaining spiders investigated at this variant of the experiment, caused slower pace of the regeneration process. The completion of the restitution of the lost part of the leg in these cases occurred only at the nymph V stage. This all resulted in appearing extensive differences in length of the regenerating legs in nymphae IV (Tab. 1; Fig. 4).

At the nymph IV stage, lasting 17 days on average, the regeneration process was completed in 46.7 per cent of individuals. In this case the investigated leg morphologically resembled a normal appendage (that served for comparing), and its growth in comparison with the previous stage was on average 1.1 mm (32.35 %), that is 24.4 per cent of its length. In the remaining individuals the regeneration proceeded as before. This was indicated by the diminished size of the regrowing leg, which was shorter than the normal leg by 0.98 mm (21.78 %). The increase of the length of the leg with the regenerate amounted to only 3.52 mm (3.53 %) in comparison with the previous stage. At the same time the normal leg lengthened, on average, by 0.66 mm (17.19 %).

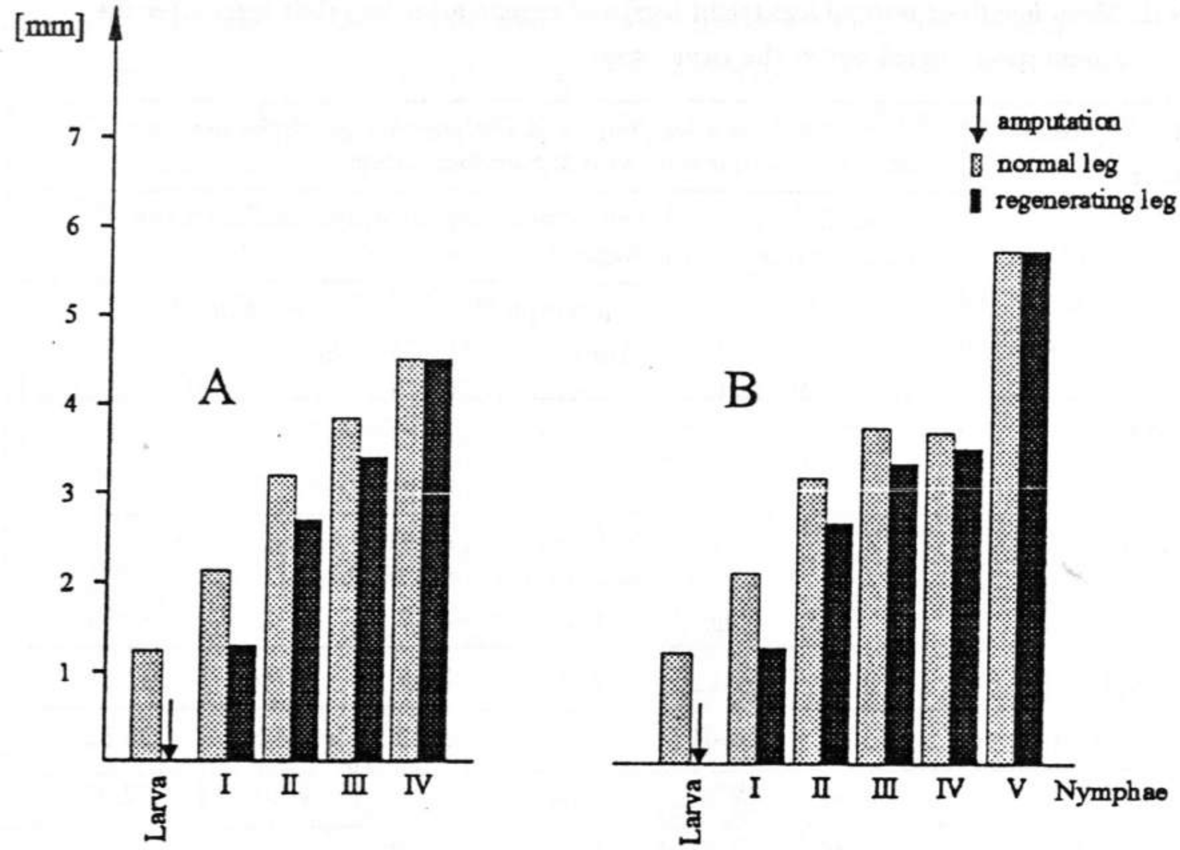

Fig. 4. Mean length of the normal legs and of the regenerating ones after the amputation carried out in larvae; A – a complete cycle of regeneration lasts till the nymph IV stage (46 days), B – a complete cycle of regeneration lasted till the nymph V stage (76 days)

The completion of the regeneration in this group of individuals occurred at the nymph V stage. The last phase of the regeneration was characterized by the rapid growth of the leg in comparison with that in nymphae IV. At this period the leg grew 2.24 mm on average, which constitutes circa 60 per cent of its initial length. The increase in amounted to length of the regenerating leg in nymph V 38.89 per cent, and of the normal leg – 21.88 per cent (Tab. 2).

2. The regeneration of the leg amputated at the nymph I stage

In this variant of the experiment the amputation sashes were tied to 40 nymphae I. Of this number, 25 individuals (62.5 %) died; 19 (47.5 %) immediately after the amputation and 6 (15 %) – at the nearest moult. Therefore, the regeneration process was investigated only in 15 individuals.

Table 2. The increase in length of the leg after consecutiye moults from the beginning of the regeneration from the first stage which occurred after the amputation expressed as a percentage

Stage at which the amputated part of the leg is cast off	Larva				Nymph I				Nymph III	
	complete regeneration				complete regeneration				normal	regen-erating
	after 4 moults		after 5 moults		after 4 moults		after 5 moults			
	normal	regen-erating	normal	regen-erating	normal	regen-erating	normal	regen-erating		
after 1 st moult	41.78	49.23	41.78	49.23	11.25	14.39	11.25	10.79	4.56	6.73
after 2 nd moult	33.44	51.85	33.44	51.85	46.07	40.09	46.06	40.09	6.25	59.38
after 3 rd moult	16.67	20.59	16.67	20.59	13.09	48.24	13.09	48.24	13.64	20.00
after 4 th moult	14.67	24.44	14.67	3.41	23.81	33.33	23.81	22.22	22.81	29.83
after 5 th mouls			21.88	38.89			7.31	20.55		

The part of the leg cut off with the sash was cast off during the moulting from nymph I into nymph II.

The nymph II stage lasted 21 days on average. At this time the beginning of the regeneration process took place only in five individuals. It consisted in forming regenerates in the form of a stump or a clublet, covered by a thin, transparent cuticular coat. Their morphological structure had the larval character, and sometimes even embryonic (Fig. 5, 6).

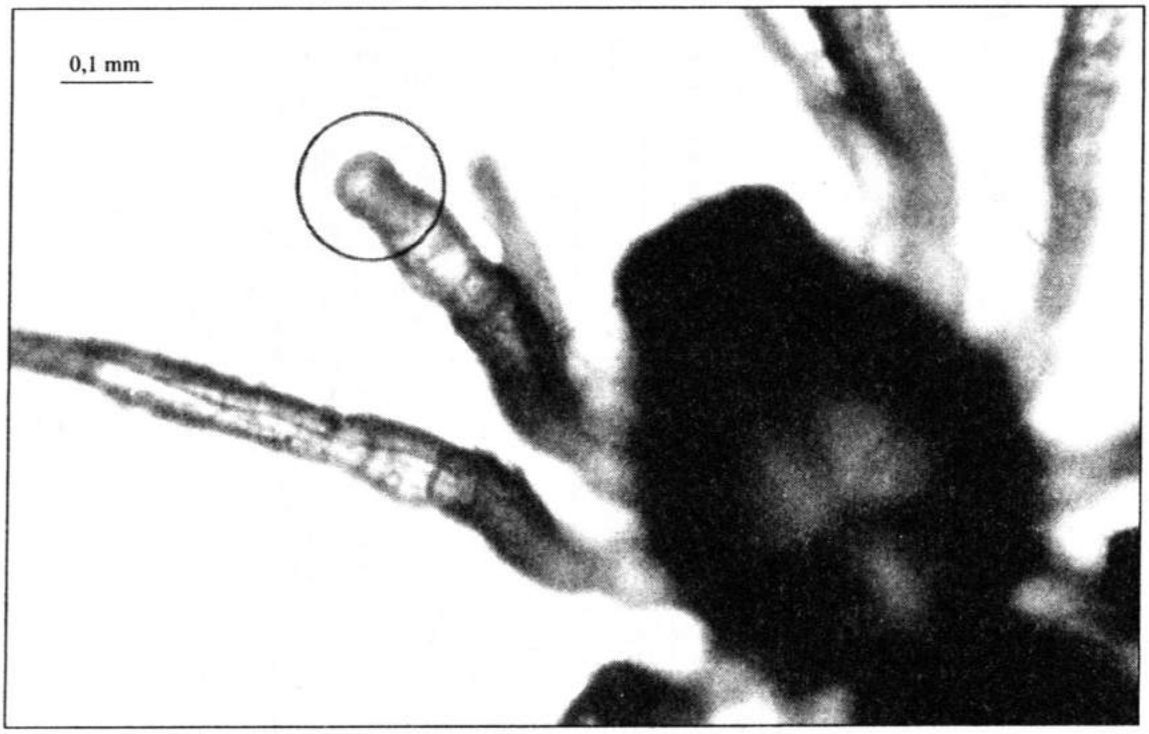

Fig. 5. The regenerate developed in the form of a clublet in nymphae II (the amputation was made at the nymph I stage) (photographed by L. Jacuński)

The part of the regenerates in structure resembled the so–called regeneration–bud, existing in some vertebrates.

The nymph III stage lasted 26 days on average. The regeneration process was found in all cases, however, it was considerably more effective in the individuals in which the regeneration buds formed earlier. In these cases (5 individuals) the regrown segments of the leg were of light colour, transparent, and in structure resembled a similar segment of larvae's walking appendages. The tarsus, a distal segment of the regenerate, was characterized by the lack of nymphal claws, which normally appear in all nymphal forms. Besides, the regrown parts of legs had visible germs of bristle–like hairs in the form of low, transparent cones.

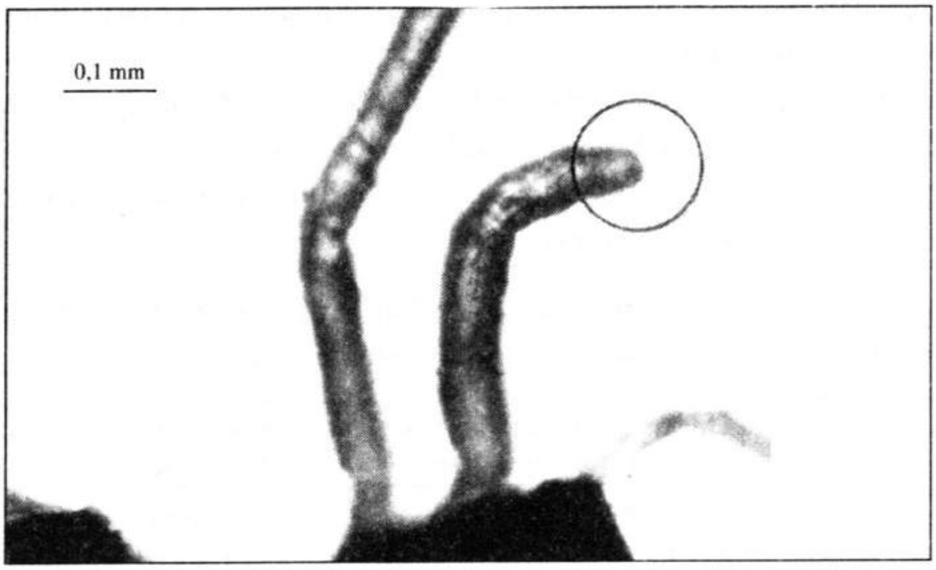

Fig. 6. The regenerate developed in the form of a short outgrowth in nymphae II (the amputation was made at the nymph I stage)

In the ten remaining individuals, the regenerates, too, had the larval character with a number of segments normal for this species, however, they did not form the germs of bristlelike hairs and claws (Fig. 1).

The regenerating leg was in comparison with the normal leg, on average, 2.13 mm shorter (c. 47.9 % of length). The increase in length of the legs in comparison with the previous stage was, on average, 79.85 per cent – 66.91 per cent for the regenerating legs and 85.42 per cent for the normal ones (Tab. 3).

The duration of the nymph IV stage varied. In the individuals developing regeneration–buds earlier, this stage lasted 16 days on average while in the remaining ones – 28 days. It was directly influenced by the intensity of the regeneration process. Individuals of the first group completed the restitution of the leg after the fourth moult – at the nymph V stage, and the remaining ones – only after the fifth moult – at the sixth nymphal stage (Tab. 3).

In all individuals at the nymph IV stage, the regenerated legs were covered by the lighter layer of the cuticle. Besides, they had proper setae and distal claws. The legs with the regenerate were, at that time, shorter than the normal legs by 0.64 mm, that is by circa 2.16 per cent while the increase in length of the amputated leg was 2.16 mm (48.2 %) and of the normal leg – 0.67 mm (13.09 %) (Tab. 2, 3; Fig. 7).

The nymph V stage lasted, on average, 17 days at this variant of the experiment. The individuals developing the regeneration buds earlier completed the regeneration process at this stage. The increase in length of the regenerates was 2.24 mm (50.00 %) in comparison with the regenerate of the previous stage. In the remaining individuals, this growth amounted to 1.28 mm (28.57 %) on average and the regenerating leg was shorter than the normal leg by 0.96 mm (14.29 %). The growth of the normal leg was 1.6 mm (23.81 % of its length at that time) (Tab. 2).

The regeneration process was thoroughly completed at the nymph VI stage. What is characteristic is that in the individual completing the regeneration process, the increase in length of the regenerate was 1.49 mm (20.55 % of its length) and in comparison with the growth of the normal leg (0.53 mm i.e. 7.31 %) was three times as large (Tab. 2).

Tab. 3. Mean length of the normal legs (right legs) and the regenerating legs (left legs) after the amputation carried out at the nymph I stage

Stage of development	Mean length of a leg (mm) and the percentageof the increase in length in comparison with the length in the previous stage					
	normal leg		regenerating leg			
	(mm)	(%)	(mm)	(%)	(mm)	(%)
nymph I	2.13				Amputation 1.19	
nymph II	2.40	12.68	scab 1.29		1.39	regeneration–bud.
nymph III	4.45	85.42	2.32	79.85	2.32	66.91
nymph IV	5.12	13.09	4.48	93.10	4.48	23.10
nymph V	6.72	31.25	5.76	28.57	6.72	50.00
nymph VI	7.25	7.89	7.25	25.87	7.25	7.89
			regeneration was completed at the nymph VI stage		regeneration was completed at the nymph V stage	

3. The regeneration of the leg amputated at the nymph III stage

At this variant of the experiment, the sashes were tied to 30 nymphae. 24 individuals survived the amputation (80 %). The remaining ones badly suffered shock caused by the tying of the sash, and tried to dispose of this inconvenience by the natural way, i.e. by autotomy. It resulted in injuring the tied appendage as well as other ones, which led to the loss of hemolymph, and consequently to death. During the following moults the eight next individuals died. Therefore, further investigations were carried out on 14 individuals (survival rate – 46.67 %).

The nymph IV stage lasted 16 days. At that time the restitution of a seven–segmented leg having the structure of a larval appengade was taking place (Fig. 8). The regenerates were shorter than the normal legs by 3.62 mm (63.51 %) (Tab. 4).

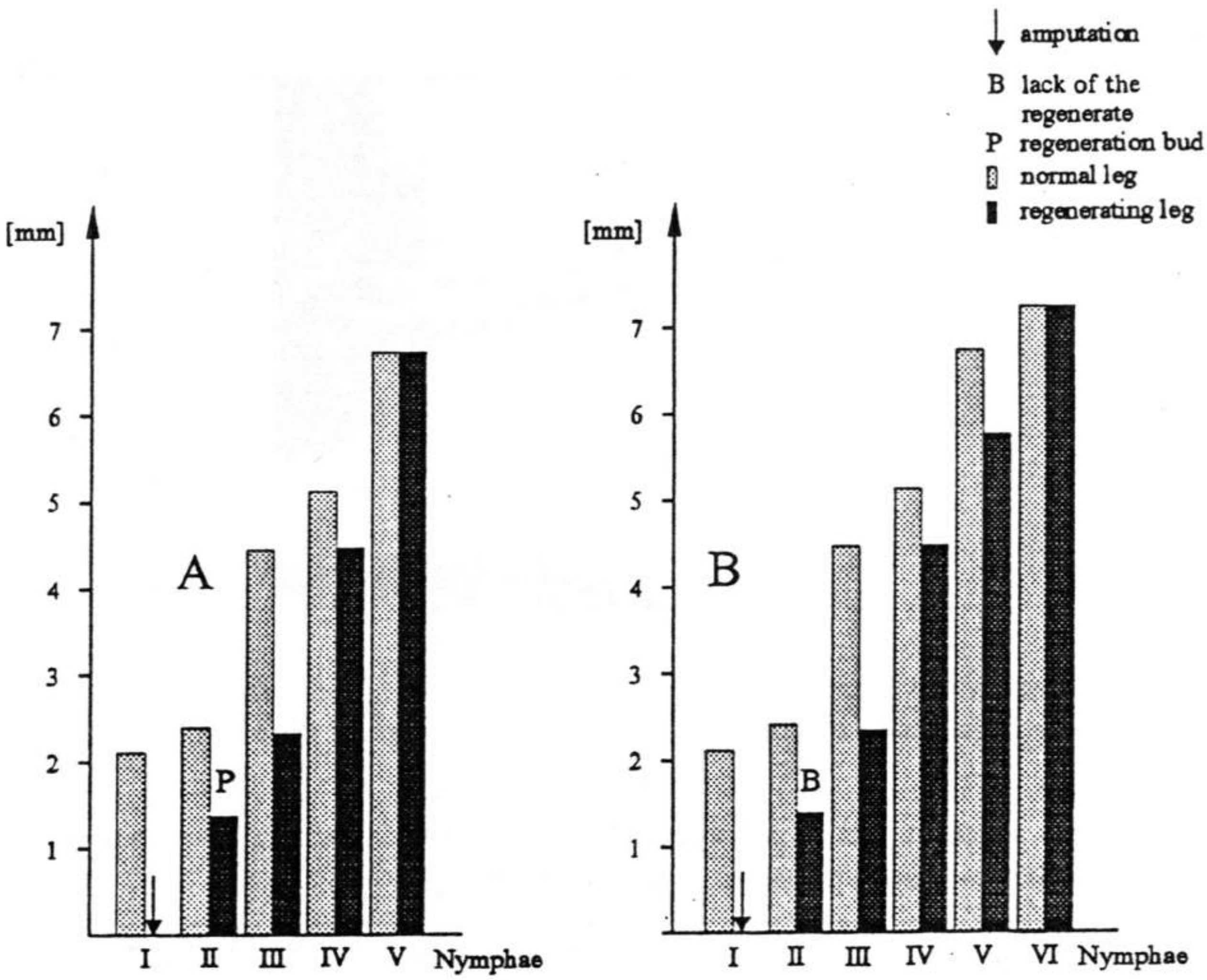

Fig. 7. Mean length of regenerating legs in particular developmental stages after the amputation carried out at the nymph I stage; A – in the individuals completing the regeneration at the nymph V stage, B – in the individuals which completed the regeneration at the nymph VI stage

The nymph V stage lasted 21 days in this form of the experiment. The regenerating legs were complete at this stage. Only in single individuals was the less number of setae and distal claws found. Although particular segments grew proportionally, the whole regenerating appendage was shorter than the normal leg by 0.96 mm (15.79 %). The increase in length of the leg in comparison with the previous stage was 0.38 mm (6.67 %) for the normal leg, and 3.04 mm (146.15 %) (59.38 % of its length) for the regenerating leg.

The nymph VI stage lasted 17 days on average, and the third moult turned out to be the critical moment of postembryogeny of the investigated species of the spider. As it has been mentioned, eight individuals did not survived this moult. The regenerating legs were 0.64 mm shorter than the normal ones (9.09 %). The increase in length in comparison

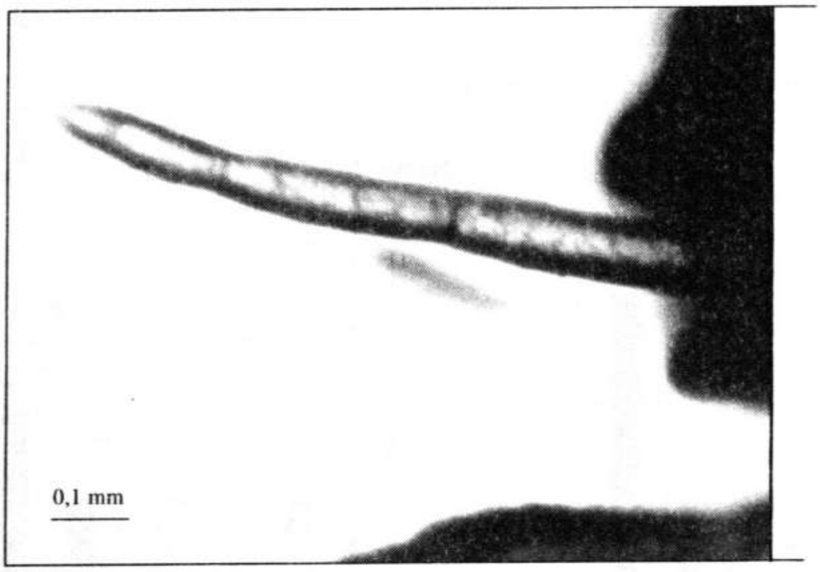

Fig. 8. The regenerated leg of the nymph IV in the individual in which the amputation of the leg in the middle of a tibia was carried out a the nymph III stage

Tab. 4. Mean length of the normal legs (right legs) and the regenerating ones (left legs) after the amputation carried out at the nymph III stage

Stage of development	Mean length of legs (mm)		Increase in length of legs as a percentage comparison with the previous stage	
	Legs			
	normal	regenerating	normal	regenerating
nymph III	5.44	amputation	–	amputation
nymph IV	5.70	2.08	4.78	1.94 7.22
nymph V	6.08	5.12	6.67	146.15
nymph VI	7.04	6.40	15.79	25.00
nymph VII	9.12	9.12	29.55	42.50

with the previous stage was 0.96 mm (15.79 %) (13.64 % of its length) for the normal legs, and 1.28 mm (25 %) (20 % of its length) for the regenerates (Tab. 2, 4, Fig. 9).

At the nymph VII stage the regeneration process was completed in all individuals. Both legs, regenerated and normal, were of equal length (9.12 mm), but the increase in length in comparison with the nymph VI's normal legs was 2.08 mm (29.55 %) while in comparison with its regenerating legs – 2.72 mm (42.50 %) (Fig. 9).

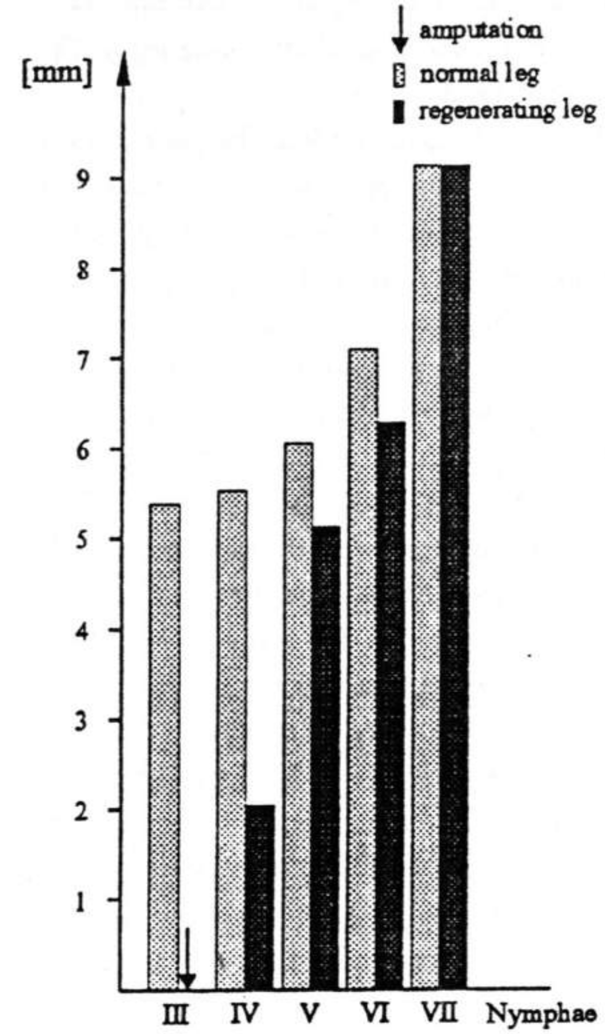

Fig. 9. Mean length of the normal legs and the regenerating ones after the amputation carried out a the nymph III stage

DISCUSSION

The hitherto existing relatively scanty literature concerning repair processes in spiders points out the existence of the power of regeneration in this group of arthropoda, however, it describes neither the course of this process nor its dynamic in various phases of postembryogeny. More detailed studies on this problem may in the future be a very interesting key to the explanation of the very important from the point of view of phylogenesis powers ensuring the survival of arthropoda in conditions of the shock related to loss of larger parts of appendages, and perhaps of other parts of the body. These powers along with autotomy are the one of more important evolutional a chievements connected with moulting processes, step growth as well as other events as the result of which greater loss of appendages takes place (Bonnet, 1930; Mikulska, Jacuński, 1970).

The authors of this work, the first of a series planned on repair mechanisms, have decided to check the dynamic and the course of the regeneration of a walking leg in *Tegenaria atrica* C.L. Koch in the early stages of embryonic development.

The walking leg was amputated in the middle of its length, that is at the place distant from the area in which autotomy most often occurs in most spiders. Before the actual amputation took place, the leg was tied with the sash squeezing the bore of the leg, which resulted in breaking the continuity of a cuticule and muscles, as well as in isolating the considerable part of a hemocoel and stopping hemolymph circulation at this place. This phase of the experiment may be called a false amputation. It was not so drastic as a purley mechanical cut, however, it caused certain changes in spiders' behaviour (alternate occurrence of states of intense activity and of halts of locomotion processes) and higher death rate.

The actual amputation, that is when the tied part of the leg was torn off, took place at the nearest moulting time. The part of the appendage isolated with the sash was cast off with exuviae. Only since that time were the following stages of the regeneration observed. At the beginning, according to a stage at which the sashes were tied, the appearance of the regenerating surface varied. In the larvae which cast off the tied part of the leg at the first nymphal stage, the regenerate was not found. We called the place where the leg was cast off the surface of the scarring over of a wound. In the individuals to which the sashes were tied at the nymph I stage, the fragment of the leg was cut off at the nymph II stage and the cut surface looked different in particular individuals. In some of them, scars formed as was the case with the amputation carried out at the larval stage (Fig. 3). On the other hand, in the remaining individuals, the regenerate developed in the form of a thin--walled stump, or sometimes of a clublet (Fig. 5, 6). The formation of these structures indicated that regeneration would proceed efficiently and quicker at the later stage of postembryogeny. In these cases the regeneration was completed one moult earlier (Tab. 1, 2, 3). In this situation one should regard the thin–walled stumps as the areas of greatly advanced fields of regeneration. The reasons of the apperance of such structures at the distal part of the leg cut off as well as those of the acceleration of repair activity are not known. The individuals used for the experiments were at the same age, and the sashes were tied to them at the same time as well. Therefore, one should suppose that repair efficiency was, between others, a result of individual differences in the rate of metabolism between particular individuals. These differences had – as one should think – to influence the rate of morphogenesis in cells situated over the place where the sash was tied. The final answer explaining the appearance of regions of more intense regeneration will be given by cytologocial and histological investigiations which will be carried out as the next of the already mentioned series.

The second phase of the regeneration, occurring in all variants of the experiment similarly, came after the second moult from the time of the actual amputation. At this phase, the regenerating leg was developing features of a nymphal appendage; a cuticle, being initially thin, transparent, and colourless, became thicker and thicker, and bristle–like hairs and claws appeared on the regenerate. At that time the regenerating leg was considerably shorter than the normal leg (Tab. 1–4).

The third phase (last one) of the regeneration consisted in reaching a normal size by the appendage, which took two to three consecutive moults.

A different regeneration effect was achieved in *Dolomedes* (Bonnet, 1930). In this spider the regenerating leg in most cases did not undergo the stage of a larval appendage, but immediately after the beginning of the regeneration it took shape of a nymphal leg with characteristic equipment with bristle–like hairs and claws. It also happened that the leg did not reach a normal size until the end of postembryogeny. The differences pointed out – as one may suppose – had to do with the fact that in *Dolomedes* postembryogeny followed a different course. In this spider, the development is exceptionally greatly dependent on environmental conditions, mainly on the temperature of surroundings. The development of this spider lasted around 12 months under normal thermal conditions (10–12 months). The abatement of the temperature to below the optimum lengthened the development up to 36 months, that is three times. On the other hand, the superoptimal temperature accelerated the development many times. Individuals reached sexual maturity after 3 to 4 months. The latter situation most probably took place in Bonnet's investigations. The development was so short that repair processes could not keep up with greatly accelerated general morphogenesis.

Another reason of such a course of the regeneration process was the little number of moults. Namely, each moult brings next phases of regeneration (the so–called "stair regeneration"). Therefore, the more moults after the amputation period, the more steadily the regeneration processes proceed and the regeneration becomes more complete.

In investigations on the regeneration in *Tegenaria atrica* C.L. Koch, which was kept in favourable conditions, the repair processes kept up with the general morphogenesis, and the differences in the course of this process mainly depended on the time at which the amputation was carried out. The restitution of legs lasted considerably longer in this spider than in *Dolomedes,* and the number of moults necessary for complete regeneration amounted to four to five as was the case in initial investigations made by Mikulska, Jacuński, and Weychert (1975). Generally, one may state that the regeneration of the leg amputated at the larva stage lasted 77–103 days, at the nymph I stage 88–93 days, and at the nymph III stage around 71 days (Tab. 5, 6).

From the above compared data it appears that the leg cut off at the nymph III stage regenerated most quickly. Judging from the results of the above quoted works one may come to the conclusion that there exist two regeneration strategies in spiders. One of them concerns spiders which live a short time and have relatively little moults, e.g. *Dolomedes.* In this case the earlier the amputation was carried out, the more complete was the regeneration process. In *Tegenaria atrica* C.L. Koch, a spider which lives in laboratory conditions relatively long and has many moults, it was quite different (Jacuński, Wiśniewski, in course of issue). In this species the earlier the amputation took place, the longer the regeneration

Tab. 5. **Mean number of days between consecutive moults and the duration of the complete regeneration in the individuals which completed regeneration after the fourth moult**

Developmental stage at which the amputation was carried out	Number of days				
	between consecutive moults from the time when the sash was tied				until the complete regeneration
	A*–1	1–2	2–3	3–4	
larva	7	21	24	25	77
nymph I	19	21	20	28	88
nymph III	17	16	21	17	71

A* – the sash was tied

Tab. 6. **Mean number of days between consecutive moults and the duration of the complete regeneration in the individuals of *Tegenaria atrica* C.L. Koch which completed regeneration after the fifth moult**

Developmental stage at which the amputation was carried out	Number of days					
	between consecutive moults from the time when the sash was tied					until the complete regeneration
	A*–1	1–2	2–3	3–4	4–5	
larva	7	21	24	34	17	103
nymph I	19	21	20	16	17	93

A* – the sash was tied

process could last. The amputation of a leg in nymph I and III resulted in accelerating the repair process. It allows us to suppose that the regeneration in *Tegenaria atrica* C.L. Koch occurs most actively in the first half of its life and spiders at this time try to make up all defects related to the loss of the appendage. Namely, this acceleration may compensate a shorter time of regeneration, and thus it affords the possibility for the restitution of the leg in a period of intense morphogenetic activity, which occurs in most spiders in the first half of postembryogeny (Tab. 7). The amputation of an appendage in older nymphae is likely to result in much greater acceleration of the regeneration process, but this will be the subject of the next publication.

Tab. 7. **The length of normal appendages and of regenerating ones in particular stages and phases of development (with maximal number of moults necessary for complete regeneration)**

Stage of development	Growth in the following moults (mm) in the forms to which the sashes were tied					
	larva		nymph I		nymph III	
	legs					
	normal	regene-rating	normal	regene-rating	normal	regene-rating
larva	1.24	Amp.	1.24	–	1.24	
nymph I	2.13	1.30	2.13	Amp.	2.13	
nymph II	3.20	2.70	2.40	1.29	2.80	
nymph III	3.84	3.40	4.45	2.32	5.44	Amp.
nymph IV	4.50	3.52	5.12	4.48	5.70	2.08
nymph V	5.76	5.76	6.72	5.76	6.08	5.12
nymph VI		the end of rege-neration	7.25	7.25	7.04	6.40
nymph VII				the end of rege-neration	9.12	9.12

The formation of regenerates in the form of stumps or clublets was – as is supposed – a result of repair initiation (Fig. 5, 6). It appears that in this regard the regeneration processes in invertebrate animals basically do not differ from those in the vertebrates, much better studied in that regard. Structures made of differentiated cells, the so–called regene-ration–bud's are well–known in the vertebrates *(Vertebrata)*. One should also add that they are accompanied by changes of certain metabolic processes.

The development processes, especially in young forms, are characterized by a great dynamic and steadiness. In the case of a repair process, this dynamic is far greater (Tab. 7). This is conditioned by biological reasons – a lost appendage has to repeat main phases of ontogenesis in a shorter time, and the quick restitution of a lost organ or its part increa-ses chances for survival.

The reduction of the time for the development of the lost part of the appendage increases the dynamic of this process in comparison with appendages developing normally. The regeneration processes also disturb the normal rhythm of development in comparison with that observed in normal leg (Fig. 10–12, Tab. 8). An especially large increase in length of the legs with regenerates occur in initial and final phases of repair processes (Tab. 8, Fig. 13). The acceleration and insteadiness of the growth is much more visible if expressed as a percentage of the increase in length in comparison with the previous stage (Tab. 9, Fig. 14).

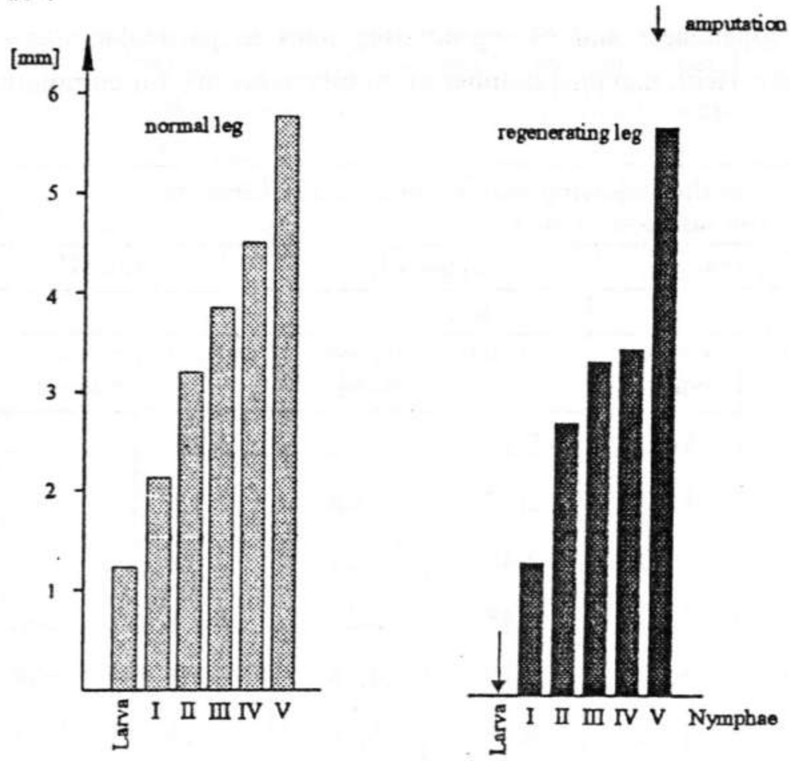

Fig. 10. Dynamic of the growth of the leg in larvae

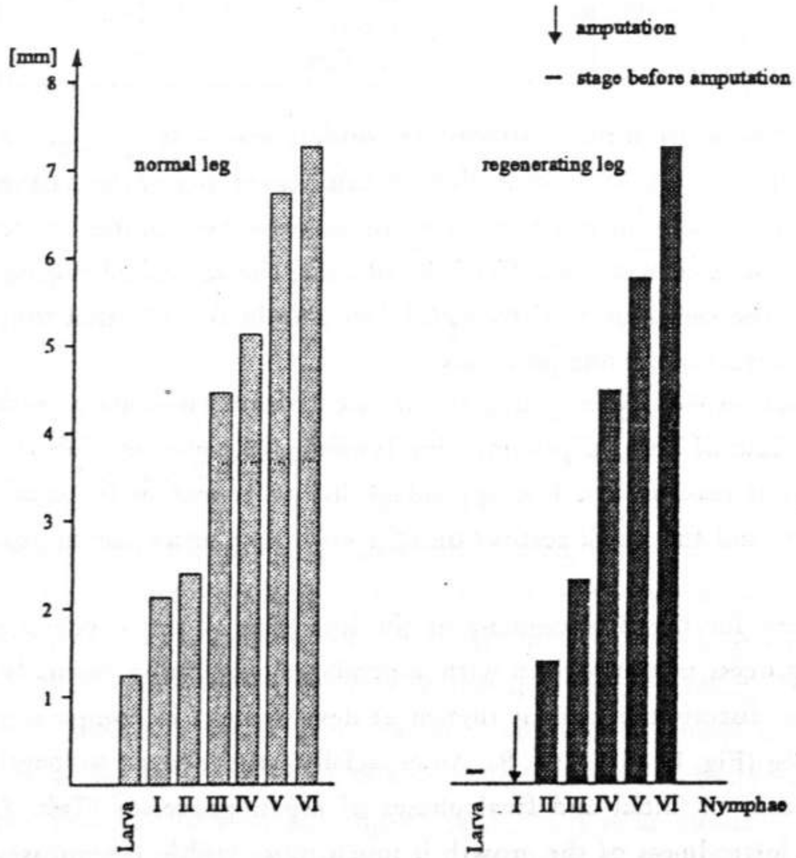

Fig. 11. Dynamic of the increase in length of legs in the forms in which the part of the appendage was amputated at the nymph III stage

Tab. 8. Mean increase in length expressed in mm in comparison with the previous stage

Stage of development	Growth in mm after the following moult in the forms in which the sashes were tied at the stage of					
	larva		nymph I		nymph III	
	legs					
	normal	regen-erating	normal	regen-erating	normal	regen-erating
larva		amputa-tion				
nymph I	0.89	0.64	0.89	Amp.	0.89	
nymph II	1.07	2.06	0.27	0.20	0.67	
nymph III	0.64	0.70	2.05	0.93	2.64	Amp.
nymph IV	0.66	0.12	0.67	2.16	0.76	0.14
nymph V	1.26	2.24	1.60	1.28	0.38	3.04
nymph VI			0.53	1.49	0.96	1.28
nymph VII					2.08	2.72

Tab. 9. Dynamic of the growth of normal legs and of regenerating legs expressed as a percentage of the increase in length of an appendage in comparison with the previous stage (with maximal number of moults necessary for complete regeneration)

Stage of development	Growth in the following moults (%) in the forms in which the sashes were tied at the stage of					
	larva		nymph I		nymph III	
	legs					
	normal	regen-erating	normal	regen-erating	normal	regen-erating
larva		A				
nymph I	71.74	lack of the rege-nerate 96.97		A		
nymph II	50.23	107.68	12.68	16.81		
nymph III	20.00	25.93	85.42	79.85		A
nymph IV	17.19	3.53	13.09	93.10	4.78	7.22
nymph V	28.00	63.64	31.25	28.57	6.17	146.15
nymph VI		the end of regen-eration	7.89	25.87	15.79	25.00
nymph VII		the end of regen-eration		the end of regen-eration	21.55	42.50

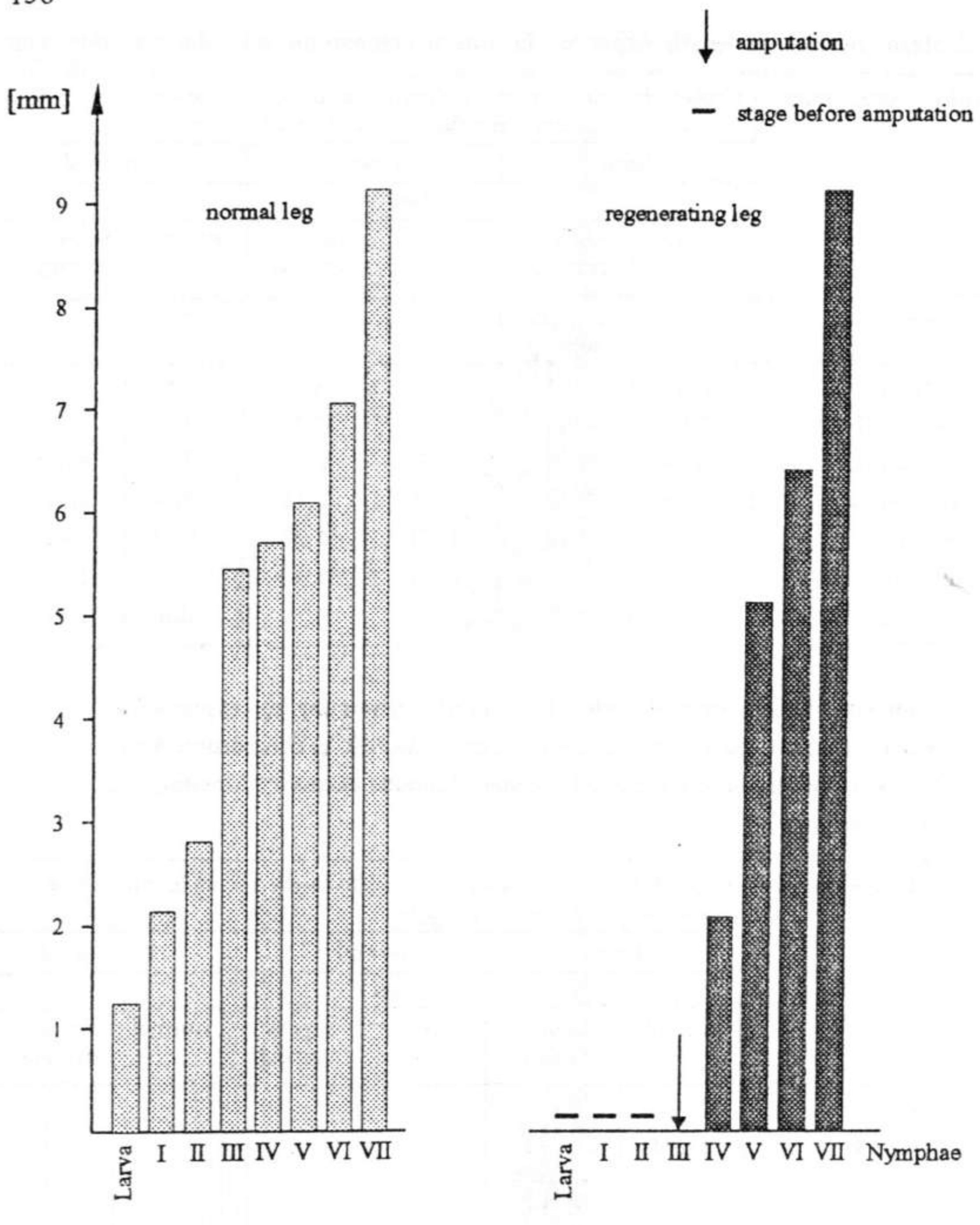

Fig. 12. Dynamic of the increase of the length of legs in the forms in which the part of the appendage was amputated at the nymph III stage

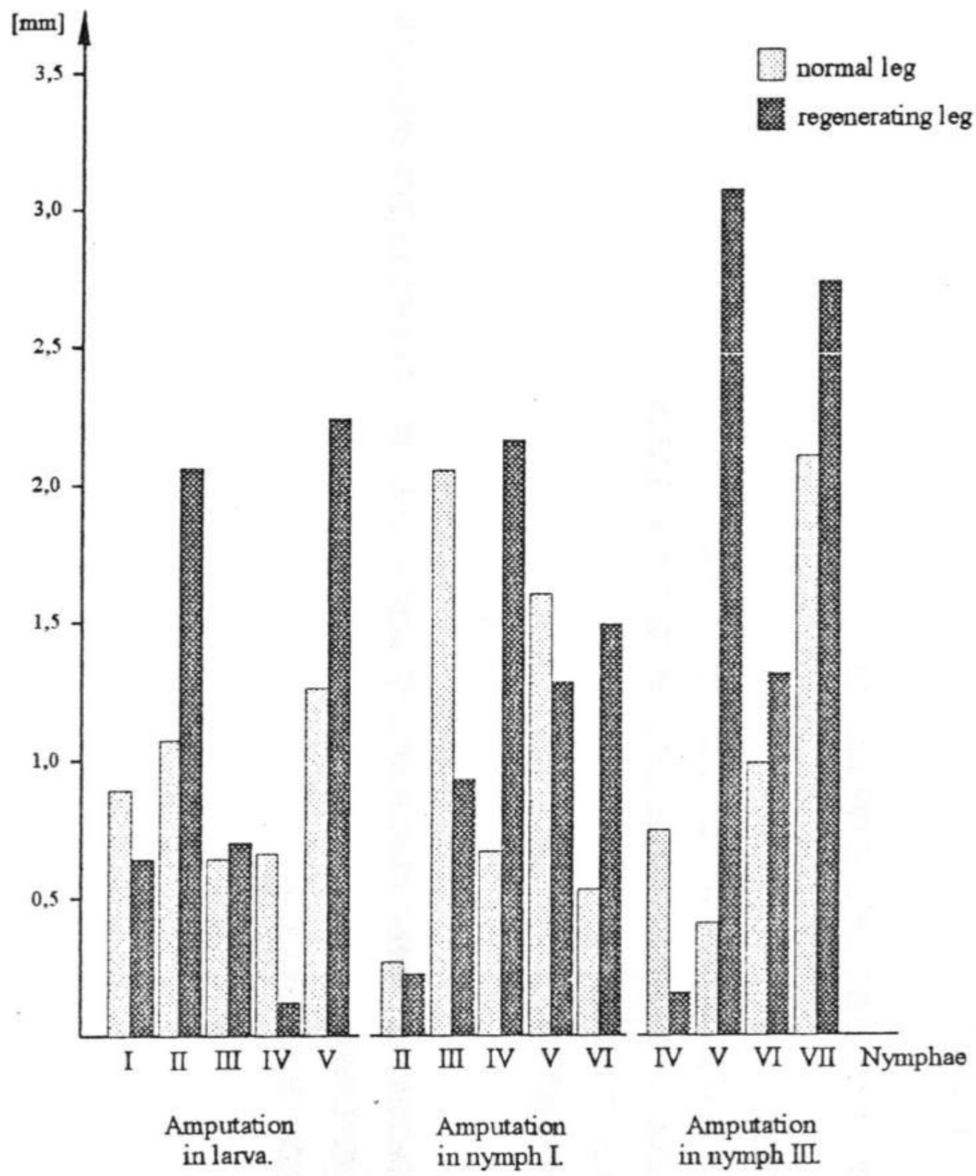

Fig. 13. Mean increase in length expressed in mm in comparison with the previous stage

Despite many benefits, the method used for amputation caused quite high mortality, which ranged from 70 per cent to 46.7 per cent according to the developmental stage at which the operation was carried out. The least mortality was observed in nymphae I, higher – in larvae (Tab. 10).

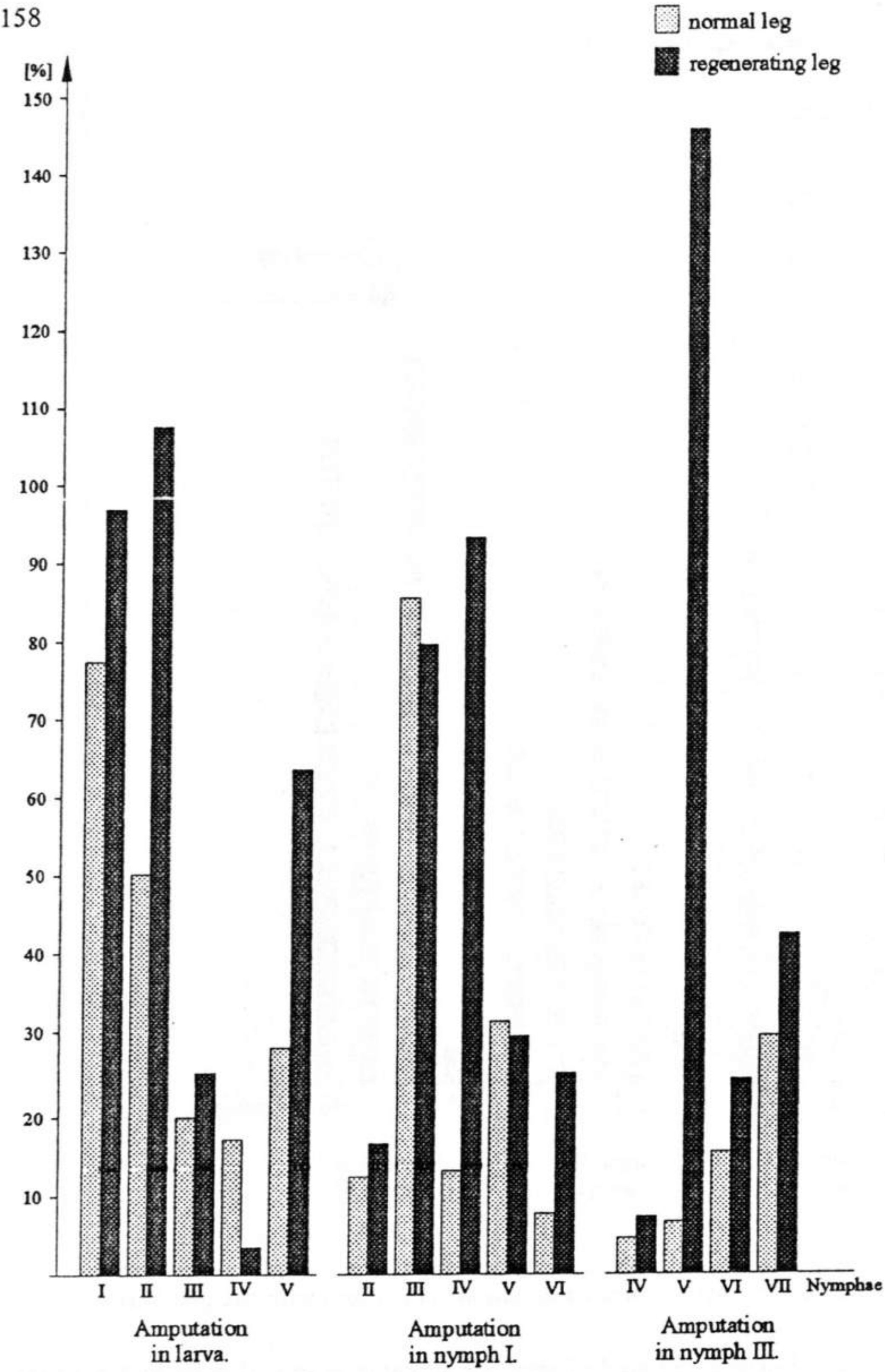

Fig. 14. The percentage of the growth in comparison with the length of the whole leg in particular stages and phases of the development (with the maximal number of the moults necessary for the complete regeneration)

Tab. 10. Death rate of *Tegenaria atrica* C.L. Koch after the amputation carried out in various developmental stages expressed as a parcentage

Developmental stage which the amputation was carried out	Percentage of dead individuals					Survival rate in total (%)
	before 1 st moult	after 1 st moult	after 2 nd moult	after 3 rd moult	in total	
larva	28	32	10	–	70	30
nymph I	47.5	15	–	–	62.5	37.5
nymph III	30	–	–	16.7	46.7	53.3

The relatively low survival rate of larvae and nymphae I at various moments of the regeneration was probably related to great sensitivity of these developmental stages to mechanical manipulating connected with the necessity for immobilizing an animal when tying the sash as well as with cutting off the relatively large fragment of a hemocoel.

The factors influencing the reduction of the survival rate of older developmental stages, i.e. of nymphae III, could have been the means used for anaesthitization and nymphae's restlessness making them carry out autotomy of the tied leg. In order to tie the sash to spiders at this developmental stage, then had to be anaesthitized (with ether or chloroform) or exposed to the action of agents lowering metabolic processes and reducing motion activity. These agents badly influenced the biological form of animals. Besides, nymphae's great mobility and dexterity result in that nymphae bite off the tied legs quite easily and lose much hemolymph, which often leads to death.

Finaly, one should note that the power to regenerate is a very important evolutional achievement for spiders and other arthropoda alike. On the other hand, great powers for regeneration, especially in the species which undergo many moults in postembronic development, may be an evolutional brake, which „freed" arthropoda from seeking an alternative to step growth in the postembroynic development of this group of inverbrate animals.

CONCLUSIONS

1. The experiments confirmed the usefullness of the method of amputation by means of tying sashes in the investigations on the regeneration of walking appendages in spiders in spite of the fact that the death rate observed at the experiment ranged from 70 per cent to 46.7 per cent according to the developmental stage.
2. The studied developmental stages (larvae, nymphae I, nymphae III) are characterized by the capasity to restore the lost part of the appendage thoroughly, both in regards to its

size and to the restitution of the complete segmentation as well as of the equipment with bristle–like hairs and claws.

3. The number of moults necessary for the complete regeneration of the amputated appendage amount to 4 to 5, which expressed as the number of days is respectively 71–88 and 93–100.
4. The duration of the repair process depends on the formation of the regeration field and is shortened (by one moult) in the case of producing the regeneration bud (blastema).
5. The repair processes are characterized by the great dynamic, however, their visible acceleration is observed in the initial and final phases of the regeneration.
6. The regeneration process is the repetition of the ontogenetic way of development of the appendage and occurs in three phases: the formation of the regeneration field, the restitution of the appendage of a nymphal type, the regrowing of the appendage up to its normal size.
7. In *Tegenaria atrica* C.L. Koch, amounted among long–living species (with great number of moults), the acceleration of repair processes is the greater, the older is the developmental stage at which the amputation was carried out. The acceleration compensates the decrease of the time of the regeneration of the appendage.

EPIMORFOZA ODNÓŻA CHODOWEGO WE WCZESNYCH ETAPACH POSTEMBRIOGENEZY *TEGENARIA ATRICA* C.L. KOCH *(ARACHNIDAE, AGELENIDAE)*

STRESZCZENIE

Badano epimorfozę odnóża chodowego w początkowych etapach postembriogenezy u *Tegenaria atrica* C.L. Koch i porównywano ją z normalnym przzebiegiem rozwoju odnóża. Doświadczenia przeprowadzono na larwach, nimfach I i nimfach III, którym amputowano pierwsze lewe odnóża chodowe w połowie ich długości (w połowie goleni). Amputację przeprowadzono metodą przewiązek z nici stylonowej. Odrzucanie odpowiedniej części odnóża następowało podczas najbliższego linienia. Stwierdzono, że przebieg regeneracji odpowiadał etapom ontogenezy odnóża chodowego. Regeneracja następowała po pierwszym linieniu następującym po amputacji i najczęściej zaczynała się od wytworzenia w miejscu cięcia buławkowatego zgrubienia, które odpowiadało morfologicznie przysadce typu embrionalnego. Wspomniana buławka następnie wydłużała się i rozwijała dalej w odnóże typu larwalnego, a następnie nimfalnego. W tym czasie następowało odtworzenie kompletnej 7. członowej przysadki.

Czas pełnej regeneracji odciętych części odnóży u larw i nimf I kończył się po czterech linieniach. Liczba linień niezbędnych do regeneracji odciętego fragmentu odnóża u larw i nimf I była zależna od budowy powierzchni blizny powstałej bezpośrednio po odrzuceniu przewiązanej części przysadki.

Zdolność regeneracji utraconej części odnóża zachowują wszystkie badane stadia rozwojowe *Tegenaria atrica* C.L. Koch, przy czym największe tempo tego procesu stwierdzono u najstarszych z badanych stadiów, tzn. u nimf III.

ЭПИМОРФОЗ ХОДОВОЙ КОНЕЧНОСТИ В РАННИЕ ЭТАПЫ ПОСТЕМБРИОГЕНЕЗА

Tegenaria atrica C.L. Koch (*Arachnidae, Agelenidae*)

РЕЗЮМЕ

Проводились исследования эпиморфоза ходовой конечности в начальные этапы ностембриогенеза у *Тегенария атрика* Ц.Л. Кох и сравнение с нормальным ходом развития конечности.

Исследования проводились на личинках, нимфах I и нимфах III, у которых были ампутированы первые левые ходовые конечности в половине их длины (в половине голени). Ампутания проводилась путем перевязки стилоновыми нитками. Соответственные части конечности были отброшены во бремя ближайшего линяния.

Определено, что процесс регенарации соответствовал этапам онтогенеза ходовой конечности. Регенерация происходила после первого линяния следуемого после ампутации и, чаще вего, начиналась с возникновения на месте разреза булавого утолщения, которое морфологически соответствовало конечности эмбрионального типа. Вышеупомятое утолщение затем удлинялось и развивалось в конечность личиночного типа, а затем инмфального. В течение этого времени происходило восстановление полной членистой конечности.

Полная регенерация ампутированных частей конечностей у личинок и нимф I заканчиваетя после четырех или пяти очередных линяний, а у нимф III - уже после четырех линяний. Число линяний хеобходимых для регенерации ампутированной конечности у личинок и нимф I зависит от структруры поверхности шрама, возникшего непосредственно после того как перевязанная часть была отброшена.

Способность регенерации потерянной части конечности сохраняют все исследуемые стадии развития *Тегенария атрика* Ц.Л. Кох, причем наибольший темп етого процесса был определен у старейших из исследуемих стадий т.е. у нимф III.

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