REGENERATION IN *ARGAS PERSICUS*.

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(With 4 Text-figures.)

General Introduction.

THE present investigation was primarily undertaken by Mr G. Merriman at the suggestion of Professor Nuttall, but was discontinued at a very early stage owing to Mr Merriman's departure for Africa. The object of the investigation was:

(1) To determine if immature stages of ticks whose mouth-parts have been mutilated or torn off by the forcible removal of the parasite from the host possess the power of regeneration. In nature, many immature ticks must be injured in this manner, and it seemed of interest to determine if they could regenerate the lost parts, thus helping them in the struggle for existence.

(2) To determine if the small legs occasionally observed in nymphal and adult ticks are due to mechanical injury followed by regeneration. A very striking example of such a tick is the type specimen of *Amblyomma scaevola* Oudemans (σ), in which four legs are markedly under-developed. In the large quantity of tick material that has been received at the Quick Laboratory, individuals with one or more miniature legs have been observed repeatedly.

In addition, the subject is one possessing considerable interest from a general point of view, as hitherto there have been extremely few

observations upon regeneration in the Arachnida and, so far as we are aware, none in the Ixodoidea.

The present paper contains a short account of the regeneration of the limbs in *Argas persicus*. In a future communication we propose to describe also the regeneration of the mouth-parts.

Historical.

The first author to describe regeneration in an Arachnid was Lepeletier (1812), who noted that in spiders regenerated legs were normally jointed but shorter and darker than the other legs. J. Banks (1829) and Heineken (1829) also observed regeneration of spiders' legs. Heineken noticed that after amputation of a limb the stump remained unchanged until the next moult, and then regeneration processes commenced, extending through two or three moults. The same observer remarks that in fully grown spiders moulting and regeneration do not take place.

Wagner (1887) observed that in the tarantula when a leg was removed at any place other than the coxa, the animal brought the wounded leg to its jaws and bit it off down to the coxa.

In addition to the above, a number of writers have recorded the regeneration of the legs of spiders and the subject has been investigated experimentally by Schultz (1898) and Friedrich (1906).

Schultz, working mainly with the Epeiridae, found that the leg was renewed if cut off at any level; in his experiments he generally removed the leg at the metatarsus, but also at the tibia, and usually at an articulation.

Although these individuals, unlike the tarantula, never attempted to bite off any part of a wounded leg, yet when the leg was cut off at the coxa it regenerated better than when amputated at any other level.

Schultz considers that this is an excellent example of the influence of natural selection, since regeneration takes place best at the point where the leg is most often broken off in nature. On the other hand, regeneration also takes place when the leg is cut off at any other point, thus showing that the power to regenerate is characteristic of all parts of the limbs and not merely a phenomenon of adaptation, for it is unlikely that a spider would ever lose a leg in the middle of an article, the joints being much the weaker. When the leg is cut off between two articles, the formation of the new part takes place somewhat differently

than when the leg is cut off at the coxa. In the latter case, a solid rod is produced from the cut end and this rod increases in length, bending on itself several times. Subsequently the rod becomes segmented and at the next moult is set free as a perfect leg. When the limb is removed nearer the distal extremity a small straight rod is formed, lying inside the last article, and the regenerated part is set free at the next moult.

More recently Friedrich (1906) has given a detailed account of regeneration and autotomy in *Tegenaria domestica*, the common house spider. So far as we are aware the only other observations on regeneration in Arachnida are those of Loeb (1895), who found that if the body of the pycnogonid *Phoxichilidium maxillare* is cut in two, there regenerates from the posterior end of the anterior half a new body-like outgrowth.

Material and methods.

In the following experiments the tick *Argas persicus* Oken has been employed throughout, mainly because of the ease and rapidity with which it can be reared in the laboratory. The ticks were generally maintained at 30° C., at which temperature the life-cycle is briefly as follows:

The adult female, after fertilisation, lays a batch of about 60 eggs approximately 8–12 days after engorgement; these eggs, after an incubation period of about 10 days, hatch out to hexapod larvae.

The latter, when placed upon a fowl, attach themselves and remain feeding for 4–6 days, during which period they increase very considerably in size. Finally the gorged larvae drop off the fowl and seek refuge in any crevice, and after about 7–12 days moult to eight-legged first stage nymphs.

The first stage nymphs, when placed upon a fowl, gorge themselves with blood and drop off within half-an-hour, thus differing markedly from the larvae. About 12 days after engorgement they moult to second stage nymphs. The latter feed in exactly the same way as the first stage nymphs and after a period of about 14–18 days moult again, in the majority of cases giving rise to the adult stage. In certain cases, however, the tick may pass through more than two nymphal stages, but usually the life-cycle of the tick comprises only the stages: egg, larva, first and second stage nymphs, adult. The adult tick does not moult, but will feed at intervals of about a month and a fertilised female generally lays a batch of eggs after each feed.

In our experiments the ticks were kept in glass-topped pill-boxes containing filter paper and were maintained at any desired temperature in incubators. The amputation of the various parts of the legs, a matter of some difficulty in the smaller stages, was best effected by placing the tick, in its normal position, on a piece of card under a binocular dissecting microscope; the limb, or part of the limb, was then removed by means of a fine needle ground to a knife-edge, the tick being kept stationary by means of a camel-hair brush. During this operation it is essential that no considerable pressure should be applied to the body of the tick, as in this case the loss of coelomic fluid is so great that the animal dies.

Larva.

Our experiments with the larvae may be divided into two groups. In the first instance we used ticks that had gorged themselves and dropped off the fowl, but later we adopted the plan of cutting off the legs whilst the larvae were still attached. The results of the first series of experiments were mainly negative, and this is noteworthy since, according to most theories of regeneration, the younger stages should possess greater regenerative power than the older. The results of the first group are as follows:

Experiment 1. A number of larvae, placed on a fowl on May 25th, dropped off gorged on the 29th and 30th, and were kept at a temperature of about 27° C. On June 3rd the second right legs of five of these larvae were amputated and the ticks kept at the same temperature. Two days later one of these larvae (*a*) moulted and possessed a normally-developed second right leg, with the exception that the tarsus was very imperfect. The other four ticks moulted respectively, two on the third and one on each of the fourth and sixth days after the operation; in none of these was there any trace of regeneration. Since in larva (*a*) only two days had elapsed between the amputation and the moult, it seems probable that the immature nymphal leg had already been formed within the skin of the larval leg and partly withdrawn; therefore the needle had only passed through the tarsus, and this portion of the limb was not regenerated.

Experiment 2. A number of larvae were placed on a fowl on November 3rd and dropped off gorged on the 7th. The same day (Nov. 7th) the second right legs of eleven of the gorged larvae were cut off, in every case the four or five distal articles being removed. These ticks were kept in an incubator supposedly at a temperature

of 27° C., but during the experiment the heating apparatus went wrong and for some weeks the temperature varied from 15° C. at night to as much as 35° C. during the daytime. This diurnal variation seems to have had a marked effect on the subsequent development, for although members of the same batch of ticks kept at a constant temperature of 37° C. all became adult after two nymphal stages, in this experiment no less than eight out of the eleven passed through at least three nymphal stages.

Six to ten days after amputation the larvae all moulted to first stage nymphs and in none of them was there any trace of regeneration of the lost appendage. All these ticks were reared through the next two stages, and the results were fairly uniform. In every case, when the animal moulted to the second stage nymph, the second right leg was regenerated. The regenerated appendage was perfectly formed but was slightly smaller than the corresponding appendage of the opposite side. After the next moult, the size of the regenerated limb was practically normal.

Experiment 3. Eleven gorged larvae from the same batch as those used in the preceding experiment had their legs amputated on Nov. 7. In each case, as before, the second right leg was removed between the second and third articles. The ticks were kept at a constant temperature of 37° C. Four days later (Nov. 11) nine moulted, none of them showing any regeneration. The following day (Nov. 12) the remaining two moulted. In one of these there was no regeneration, but in the other the animal had regenerated a short rod-like appendage indistinctly divided into three articles.

All these ticks were raised to the adult state and, apart from the above-mentioned exception, the legs regenerated at the next moult and were perfectly formed, but slightly smaller than those of the other side. The abnormal example however, which had already commenced to regenerate previous to the first moult, after the second, regenerated a limb perfect both in size and form.

After the final moult, the regenerated appendages of all the ticks were practically perfect.

A series of controls kept under identically the same conditions showed that amputation had no marked effect on the duration of the stages.

Experiment 4. In this series the legs of twenty-two gorged larvae were amputated the day they dropped off the fowl and kept at 37° C. Four to five days later they all moulted and in no case was there any trace of regeneration. These first stage nymphs were allowed to remain

without another feed in order to see whether the absence of a limb would stimulate the animals to proceed to another moult, but they all died without proceeding to another stage.

Experiment 5. In all the preceding experiments only the second right leg had been removed and therefore we sought to determine if the removal of other limbs gave corresponding results. The larvae used in this series were all put on a fowl on April 5 and dropped off gorged five days later. After amputation some of these larvae were kept at 37° C. and others at 30° C. For the sake of convenience the results are presented in tabular form.

(a) Larvae kept at 30° C.

- i. First right leg amputated 10. iv. 13 :

16. iv. 13	9 moulted	No regeneration.
17. iv. 13	3 „	„
- ii. Second right leg amputated 10. iv. 13 :

17. iv. 13	8 moulted	No regeneration.
18. iv. 13	4 „	„
- iii. Third right leg amputated, 10. iv. 13 :

16. iv. 13	5 moulted	No regeneration.
17. iv. 13	6 „	„
- iv. Hind pair of legs amputated 10. iv. 13 :

16. iv. 13	2 moulted	No regeneration.
17. iv. 13	6 „	„
18. iv. 13	3 „	In two examples, no regeneration ; in the third, miniature legs one-third the normal size were present.
- v. Two hind pairs of legs amputated 10. iv. 13 :

16. iv. 13	7 moulted	No regeneration.
17. iv. 13	5 „	„
- vi. All three pairs of legs amputated 10. iv. 13 :

17. iv. 13	3 moulted	No regeneration.
18. iv. 13	7 „	No regeneration in eight individuals ; in two cases a two-jointed knob developed in place of the front legs.

With few exceptions the ticks in this series all moulted without showing any signs of regeneration, and it will be noticed that the period elapsing before moulting is only slightly lengthened in the case of those animals which had lost all their limbs. In all the above series the fourth pairs of nymphal legs appeared as usual and were normal both in size and form.

(b) Larvae kept at 37° C.

- i. First right leg amputated 10. iv. 13 :

14. iv. 13	4 moulted	3, no regeneration ; 1, small two-jointed knob regenerated.
15. iv. 13	1 „	Small three-jointed rod developed, with miniature claw.

- ii. Second right leg amputated 10. iv. 13:
14. iv. 13	1 moulted	No regeneration.
15. iv. 13	4 „	2, no regeneration; 2, small pointed knob present.
16. iv. 13	1 „	A two-jointed rod present.
- iii. Third right leg amputated 10. iv. 13:
14. iv. 13	6 moulted	No regeneration.
- iv. Hind pair of legs amputated 10. iv. 13:
14. iv. 13	4 moulted	No regeneration.
15. iv. 13	2 „	„
- v. Two hind pairs of legs amputated 10. iv. 13:
15. iv. 13	3 moulted	2, no regeneration; 1, left side, no regeneration: right side, second appendage, three-jointed rod without claw; third appendage, four-jointed rod, with rudimentary claw.
16. iv. 13	2 moulted	In both cases two pairs of half-size legs regenerated, the articulations somewhat indistinct.
- vi. All three pairs of legs amputated 10. iv. 13:
14. iv. 13	1 moulted	No regeneration.
	4 dead	
15. iv. 13	1 moulted	No regeneration.

In this latter series more of the ticks showed signs of regeneration than in the case of those kept at 30° C., but the results are not definite enough to permit of conclusions.

In both series examples from each of the experiments were fed and allowed to moult to second stage nymphs. In every case the limbs then regenerated and varied from two-thirds to three-quarters the normal size, but were perfectly proportioned.

In all the above experiments the almost total absence of any attempt at regeneration in the larval stage is very striking, but it might be explained as the result of the comparatively short time elapsing between the amputation and the moult.

We therefore performed a second series of experiments in which we cut off the legs whilst the larvae were still feeding, and as will be seen we obtained quite different results.

Experiment 6. A batch of larvae was put on the fowl on April 5. Three days later some of the legs of sixteen ticks were amputated whilst still on the fowl. Three of these ticks dropped off gorged two days later, whilst the remaining thirteen dropped off the following day. Owing to the difficulty of operation, it was impossible to remove any particular articles and therefore the extent of the amputation could only be determined later and varied considerably in different individuals.

The results are presented in tabular form.

Date when ticks dropped off fowl	Number of ticks	Extent of amputation	Date of moult to first stage nymph	Extent of regeneration
10. iv. 13	1	Legs 2 and 3 of left side	17. iv. 13	No regeneration.
„	1	3 right legs	„	First right leg, half-size and imperfect; legs 2 and 3 absent.
„	1	All 6 legs, with exception of 3 articles on left hind leg	„	Right side, 3 perfect legs; left side, leg 1, four-jointed stump; legs 2 and 3 absent.
11. iv. 13	3	3 right legs	„	Perfectly formed limbs, but only three-quarters normal size.
„	7	3 right legs	18. iv. 13	In five, limbs as above; in one, leg 1 three-quarter size, leg 2 half-size, leg 3 a three-jointed stump, with a small terminal hook (Fig. 1, <i>B</i>); in the other, leg 1 half-size, legs 2 and 3 absent.
„	1	Legs 2 and 3 of right side	19. iv. 13	Legs 1, 2 and 3 all half to two-thirds normal size. (Fig. 1, <i>A</i> .)
„	2	Leg 3 of right side	18. iv. 13	Leg absent—no regeneration.

Fig. 1. *Argas persicus*, first stage nymph: (A) tick from Experiment 6, showing almost perfect regeneration of the first three right legs; (B) first three right legs of another individual from the same experiment, showing imperfect regeneration. ($\times 13$.)

From these results it seems that a period of at least nine days must elapse between the amputation and moulting. Even with this period, however, some individuals showed no signs of regeneration, whilst others only developed very imperfect appendages.

We should like to call attention to the case in which, although only the second and third legs of the right side were removed, yet in the nymph the first right leg was reduced in size as well as the regenerated appendages; all the left legs and also the fourth right leg were of normal size.

First Stage Nymphs.

Since all stages of *A. persicus*, excepting the larval stage, usually feed to repletion within half-an-hour, the time between feeding and amputation can be determined exactly, and it will be noticed that the results of this series of experiments are much more uniform than in the case of those with the larvae.

Experiment 7. A batch of first stage nymphs was fed on December 9. The following day the four or five distal articles of the second right leg were removed, the ticks being kept at a uniform temperature of 37° C. At the same time an equal number of the same batch of nymphs were kept as control in order to see whether the amputation delayed the subsequent moulting.

Of the amputated ticks, one moulted on 16. XII. 12, four on 17. XII. 12, and six on 18. XII. 12. In every case, all the articles of the appendage were regenerated and the leg was of normal proportions, but the size was only two-thirds to three-quarters that of the corresponding leg of the opposite side. Of the controls, seven moulted on 16. XII. 12, and four on 17. XII. 12. It seems therefore that amputation may slightly delay the subsequent moulting.

Experiment 8. A batch of first stage nymphs was fed on April 2, and then divided into groups of six, in each of which different legs or different articles were amputated. In every case the ticks were kept at 30° C. The results are given in tabular form.

(a) Claws of second right leg amputated 3. iv. 13.

14. iv. 13	2 moulted	Regeneration perfect.	
15. iv. 13	2 „	„	„
17. iv. 13	1 „	„	„
19. iv. 13	1 „	„	„

This experiment was undertaken especially to determine whether the regenerated claw showed any tendency to revert to the larval type,

which, in *A. persicus*, differs considerably from the nymphal claw, owing to the presence of a large pulvillus. In no case could we detect any sign of a larval pulvillus on the regenerated claws.

(b) First distal article of second right leg amputated 3. iv. 13.

16. iv. 13	1	moulted	Distal article slightly shorter, but same thickness as remainder of leg.
17. iv. 13	1	„	Ditto.
18. iv. 13	1	„	Ditto.
20. iv. 13	1	„	Ditto.
25. iv. 13	1	„	Two distal articles shorter than normal.
29. iv. 13	1	„	Regeneration almost complete.

As might be expected, no marked differences could be detected between the amputated and the normal legs. In most cases the regenerated tarsus was slightly shorter, but approximately of the same thickness as the corresponding one of the opposite side, and the claws seemed to be normal (Fig. 2, *B*). In one case, however, the penultimate was also shortened.

(c) Two distal articles of second right leg amputated 3. iv. 13.

16. iv. 13	2	moulted	Regenerated leg normally jointed, but four distal articles three-quarter size.
19. iv. 13	1	„	Ditto.
21. iv. 13	1	„	Ditto.
25. iv. 13	1	„	Ditto.

The nature and extent of the regeneration were approximately the same in all these cases and will be understood on reference to Fig. 2 (*C*).

It will be noticed that the reduction in size certainly includes articles 4 and 5 and possibly article 3, although only two articles (6 and 7) had been removed.

(d) Three distal articles of second right leg amputated 3. iv. 13.

14. iv. 13	1	moulted	Regenerated leg less than half-size and all six distal articles miniature.
15. iv. 13	2	„	} Nature of regeneration as above, but size varying from half to two-thirds that of normal leg.
19. iv. 13	1	„	
20. iv. 13	1	„	
27. iv. 13	1	„	As above, but leg quite three-quarters normal size.

As in (c), reduction took place in articles other than those which had been removed, for although only three distal articles had been amputated, all the articles were distinctly miniature. As might be expected, the longer the interval between amputation and moulting, the greater was the extent of regeneration.

(e) Four distal articles of second right leg amputated 3. iv. 13.

16. iv. 13	1	moulted	} Regenerated leg about three-quarters normal size; normally jointed; articles down to coxa all reduced in size.
20. iv. 13	2	"	
24. iv. 13	1	"	
30. iv. 13	1	"	} Regenerated leg only very slightly smaller than normal.
1. v. 13	1	"

Fig. 2. *Argas persicus*, second stage nymph from Experiment 8, showing regeneration after removal respectively of—(A) claw, (B) tarsus, and (C) two, (D) three, (E) four and (F) five distal articles of second right leg. In every case the corresponding appendage of the opposite side is shown. (D) represents an abnormal type of regeneration. Magnifications: A, B, C, E, F $\times 22$; D $\times 33$.

On comparing the results of this experiment with those of the preceding (d), although an additional article was removed in (e), yet we find that the extent of regeneration in the latter case is distinctly greater (Fig. 2, E).

(f) Five distal articles of second right leg amputated 3. iv. 13.

13. iv. 13	1	moulted	} Regenerated leg less than half normal size; normally jointed; articles down to coxa all reduced.
14. iv. 13	2	"	
18. iv. 13	1	"	} As above, but size of limb about three-quarters normal size.
20. iv. 13	1	"	
28. iv. 13	1	"	Ditto, but two-thirds normal size.

The small extent of the regeneration in the first three ticks is noteworthy, but is explicable on the comparatively short time elapsing between amputation and moulting (Fig. 2, *F'*).

(*g, h, i, k*) In this series, the first, second, third and fourth right legs respectively were amputated on 3. IV. 13.

The results were uniform; in every case the regenerated appendage was normally jointed but only about three-quarters the size of the corresponding leg on the opposite side. The regenerative power possessed by each limb seems to be approximately the same.

(*l, m, n, o*) In this series, respectively the fourth pair, the third and fourth pairs, the three hind pairs and finally all the legs were amputated 3. IV. 13. The results were uniform; even in those individuals which had lost all their legs, normally jointed limbs were regenerated, but their size varied from two-thirds to three-quarters that of the normal limbs.

Experiment 9. The results of Exp. 8, (*c*) to (*f*), show that no matter how many articles are removed, the regenerated limb is always normally proportioned but reduced in size; in other words, not only those articles which have been removed, but also the remaining articles, partake in the general reduction that ensues. This seemed of such importance that we performed a more extensive series of experiments with larger numbers of ticks. In one batch of nymphs, only the two distal articles were removed, whilst in the other the leg was cut off as near to the coxa as possible. Again we obtained results which thoroughly confirmed our previous observations. In every case the leg regenerated as a whole, not merely renewing the missing articles. Perhaps the regenerated legs were slightly smaller in the case of those ticks which had had more articles removed, but the differences were very insignificant and generally the legs appeared to be of about the size.

Experiment 10. As the period elapsing between feeding and moulting is so important in the larval stage, we tried to determine whether it was equally so in the case of the nymphs. With this object, on 2. IV. 13, a number of first stage nymphs were allowed to engorge themselves and on 4. IV. 13, and each successive day up to 11. IV. 13, the second right legs of a certain number of individuals were removed. As in many cases the moulting was unaccountably delayed, it is necessary, for a clear understanding of the significance of each example, to give the number of days elapsing between feeding and amputation, and between amputation and moulting.

For the sake of convenience we shall refer to these periods as the *pre-* and *post-amputation* periods respectively.

Date of amputation	Date of moult	Number of individuals	Pre-amputation period	Post-amputation period	Nature of regeneration
4. iv. 13	16. iv. 13	3	2	12	$\frac{3}{4}$ - $\frac{3}{4}$ miniature.
"	17. iv. 13	1	2	13	$\frac{3}{4}$ "
"	22. iv. 13	1	2	18	" "
"	29. iv. 13	1	2	25	" "
5. iv. 13	16. iv. 13	1	3	11	$\frac{2}{3}$ miniature, leg rather
"	17. iv. 13	2	3	12	$\frac{3}{4}$ - $\frac{3}{4}$ miniature. [thin.
"	19. iv. 13	1	3	14	" "
6. iv. 13	16. iv. 13	1	4	10	$\frac{3}{4}$ "
"	18. iv. 13	1	4	12	" "
"	20. iv. 13	1	4	14	" "
"	24. iv. 13	1	4	18	" "
"	29. iv. 13	1	4	23	" "
"	30. iv. 13	1	4	24	" "
7. iv. 13	15. iv. 13	1	5	8	Three-jointed stump, with knobbed end.
"	17. iv. 13	1	5	10	$\frac{2}{3}$ miniature.
"	18. iv. 13	2	5	11	$\frac{3}{4}$ "
"	19. iv. 13	1	5	12	$\frac{1}{2}$ "
"	25. iv. 13	1	5	18	$\frac{3}{4}$ "
8. iv. 13	15. iv. 13	1	6	7	None.
"	17. iv. 13	1	6	9	$\frac{1}{3}$ miniature ; normal number of articles.
"	21. iv. 13	2	6	13	$\frac{1}{2}$ - $\frac{2}{3}$ miniature.
"	22. iv. 13	1	6	14	$\frac{2}{3}$ "
"	23. iv. 13	1	6	15	" "
9. iv. 13	13. iv. 13	1	7	4	None.
"	18. iv. 13	1	7	9	$\frac{1}{3}$ miniature.
"	22. iv. 13	1	7	13	$\frac{1}{2}$ - $\frac{2}{3}$ "
"	24. iv. 13	2	7	15	$\frac{2}{3}$ "
"	26. iv. 13	1	7	17	$\frac{3}{4}$ "
10. iv. 13	13. iv. 13	2	8	3	None.
"	14. iv. 13	2	8	4	None.
"	23. iv. 13	1	8	13	$\frac{3}{4}$ miniature.
"	26. iv. 13	1	8	16	$\frac{1}{2}$ "
11. iv. 13	13. iv. 13	1	9	2	None.
"	18. iv. 13	1	9	7	None.
"	20. iv. 13	1	9	9	$\frac{1}{2}$ miniature.
"	23. iv. 13	3	9	12	$\frac{2}{3}$ "

It will be seen that the minimum period in which any regeneration occurred was eight days, but in this particular case only a three-jointed stump appeared. The first tick with a regenerated limb having the normal number of articles required a post-amputation period of nine days.

Second Stage Nymphs.

In all cases the ticks used in these experiments had been reared in the laboratory and their history was known exactly. This precaution was very necessary as it is impossible from their appearance alone to distinguish with certainty the various nymphal stages.

Experiment 11. A batch of second stage nymphs¹ was fed on a fowl on 13. I. 13. The following day (14. I. 13) four to five articles were removed from the second right leg of each individual. Some of the ticks were kept at a temperature of 20–30° C., and others at 37° C.

(a) Second stage nymphs kept at 20–30° C.

19. I. 13	1	moulted ♂	No regeneration.
30. I. 13	2	„ ♀ and 3rd ○	In every case, the regenerated appendage was about half the normal size, but with the usual number of articles.
5. II. 13	2	„ ♀ and 3rd ○	
6. II. 13	1	„ ♀	
8. II. 13	1	„ ♀	
9. II. 13	1	„ ♂	
10. II. 13	1	„ ♀	
12. II. 13	4	„ ♀ and 3 ♂ ♂	
13. II. 13	1	„ ♀	
17. II. 13	1	„ ♂	
1. III. 13	1	„ ♂	

(b) Second stage nymphs kept at 37° C.

23. I. 13	2	moulted ♂ and 3rd ○	Regeneration again about half normal size.
24. I. 13	6	„ 3 ♂ ♂ + 3 ♀ ♀	
25. I. 13	3	„ 2 ♂ ♂ + 1 ♀	
27. I. 13	5	„ 1 ♂ + 3 ♀ ♀ + 3rd ○	

It will be noticed that in one case there was no regeneration, but as this animal moulted within five days after the amputation, it is probable that the formative substance of the adult limb had been removed.

Fig. 3. *Argas persicus*, third stage nymph from Experiment 12, showing regeneration after removal respectively of—(A) tarsus, and (B) two, (C) three and (D) five distal articles of second right leg. A $\times 33$; B, C, D $\times 13$.

¹ The sign ○ denotes nymph throughout.

Experiment 12. As in the case of the first stage nymph, we performed a series of experiments in order to determine whether the regenerative power varied in different appendages, and also after the removal of different articles.

A batch of second stage nymphs was fed on May 5 and the gorged ticks amputated in various ways on the following day.

(a) Tarsus of second right leg amputated 6. v. 13.

20. v. 13	2 moulted	2⊙	} As a rule the three distal articles of the regenerated limb distinctly shortened and whole leg slightly reduced.
21. v. 13	1	„ ⊙	
22. v. 13	2	„ ♂ + ⊙	
24. v. 13	1	„ ♀	

(b) Two distal articles of second right leg amputated 6. v. 13.

21. v. 13	3 moulted	♂ + ♀ + ⊙	} In all cases the regenerated leg was approximately two-thirds normal size; all articles reduced in size.
22. v. 13	2	„ ♂ + ♂	
23. v. 13	1	„ ♀	

(c) Three distal articles of second right leg amputated 6. v. 13.

20. v. 13	1 moulted	⊙	} The regeneration was of the same type as that in (b).
22. v. 13	1	„ ⊙	
23. v. 13	1	„ ♂	
24. v. 13	3	„ 3 ♀ ♀

Fig. 4. *Argas persicus*; (A) third stage nymph from Experiment 12 (c), showing regeneration after removal of all the appendages; (B) normal tick for comparison. ($\times 13$.)

(d) Four distal articles of second right leg amputated 6. v. 13.

23. v. 13	5 moulted	2 ♂, ♂ + 3 ♀ ♀	} Regeneration as in (b).
24. v. 13	1	„ ♂	

(e) All legs amputated 6. v. 13.

27. v. 13	2 moulted	2 ♂ ♂	} In every case the legs were regenerated with the normal number of articles, but were about two-thirds to three-quarters normal size.
28. v. 13	1	„ ○	
29. v. 13	1	„ ♂	
31. v. 13	1	„ ○	

In addition to the above, a few experiments were undertaken in order to find out the minimum time in which second stage nymphs could regenerate their limbs. Owing to lack of material we have been unable to carry out these experiments as completely as we should have wished, but the results obtained were concordant with those obtained with the first stage nymphs.

Summary of results.

In all stages of *Argas persicus*, if the leg is amputated sufficiently long before moulting, regeneration takes place, but the regenerated appendage is practically always less than normal in size, although possessing the usual number of articles. In no case was there any evidence of atavistic regeneration. Moreover, when the legs of a larva were amputated it invariably regenerated nymphal legs.

On comparing the periods elapsing between feeding and amputation, and amputation and moulting, respectively, it will be found that in each stage there is a minimum post-amputation period, beyond which no regeneration takes place. It is rather curious that this period is relatively longer in the case of the larvae than in the other stages.

It should be noticed that amputation of one or two articles causes a reduction in all the remaining articles of the leg. The results were very striking in the experiments with the first stage nymphs, in which we amputated respectively one, two, three, four and five articles, yet in all these cases the regenerated limb was normally proportioned though reduced in size.

We must except from these conclusions those cases in which the appendages were amputated about the end of the pre-amputation period. In these examples the regenerated appendages were frequently much reduced in size and also in the number of articles.

Discussion.

It seems impossible to explain the above-described results on Müller's theory that when regeneration takes place the new portion takes on an atavistic form. This theory was based on his observation (Fritz Müller, 1880) that when the chelae of the Brazilian shrimp, *Atyoida potimirim*, a long clawed form, were amputated, the regenerated limb reverted to the short clawed type of the related genus *Caridina*, the latter genus being phylogenetically older than *Atyoida*.

Similarly Giard (1897) and Bordage (1897-1900) noted that when the tarsus of a pentamerous insect was removed, it regenerated a tetramerous appendage. They considered this as a reversion to the type of limb in the primitive *Apterygota*, in which group tetramery is found. Bordage, however, in his paper on the tetrameric regeneration of the tarsus of the *Phasmidae*, noticed that sometimes a five-jointed tarsus was regenerated. Similar results were also obtained by Brindley (1897) in the case of the *Blattidae*. These results might be explained on the supposition that the power of regeneration was imperfectly developed, and that the four-jointed tarsi were merely examples of incomplete regeneration. Barfurth, however, showed that when the four-toed anterior appendage of an Axolotl was amputated an atavistic five-toed appendage was regenerated.

On the whole we think our results could be best explained by adopting Bonnet's idea of specific substances flowing in definite directions. The hypothesis with some modifications has been applied by Sachs and also Goebel to explain regeneration in plants, and both Loeb and Driesch have also used the same idea to explain the regeneration of *Tubularia*. In this polyp there is present in the hydranth and stem a red pigment in the form of granules in the endodermal cells. This pigment is concentrated in that part of the stem adjoining the hydranth. If a piece of the stem is cut off, it closes its cut ends and in the central cavity a circulation of fluid begins. Globules containing the red pigment granules now appear in this fluid. In the course of twenty hours the new hydranth begins to appear near one end of the stem and in this region the granules are reconcentrated, disappearing from the circulation.

Similarly, we suppose that formative substances are contained in the body of the tick. During the period of growth these substances are continually being distributed to their specific localities in the body.

Thus, the formative substance for the second tarsal claw, during growth periods, is continually passing to that part of the animal, and in the case of the larva the rudiments of the nymphal claw are similarly being deposited. These formative substances might be supposed to be stimulative in their action, merely directing the construction of the nutritive substances into the specific parts of the organism.

We may assume that the amount of nutriment supplied to each part of the body is constant. Accordingly, when two articles of a leg are removed, during the regeneration called forth by the continued deposition of formative substances, food material is used up which ordinarily would have been available for the other articles and only after the missing parts have been replaced can any further growth of the whole limb take place. This would explain the reduction in size of all the articles of the leg even when only one or two distal articles are removed.

The deposition of formative substances seems to cease some time before the animal moults, and beyond this point no regeneration can take place, even though plenty of food material may be available. In the case of the larva, the deposition of stimulative material usually stops before the animal has finished feeding, and as a result no regeneration takes place even when the legs are amputated immediately after the tick has become fully gorged and plenty of food material is available. In the nymphs the deposition ceases at a point about midway between feeding and moulting. This period we have arbitrarily referred to as the pre-amputation period. The extraordinarily short pre-amputation period in the larva suggests that *Argas persicus* is gradually suppressing its free, active larval stage and that most of the formative substance of the nymphal legs is deposited before the larva hatches from the egg. An almost complete suppression of the free larval stage occurs in two other members of the Argasidae, *Ornithodoros savignyi* and *O. moubata*. In the former the larva emerges from the egg and has some (although very slight) power of movement. In the latter species, however, the suppression is carried further; the majority, though not all, of these larvae emerge from the egg-shell, but all are without power of movement. Further, the larvae of both these species pass to the first nymphal stage without feeding.

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