

# REGENERATION OF THE MOUTHPARTS AND LEGS IN TICKS.

*ARGAS PERSICUS*, *AMBLYOMMA HEBRAEUM*  
AND *HYALOMMA AEGYPTIUM*.

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(With 6 Text-figures.)

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## INTRODUCTION.

THE only authors who have concerned themselves with regeneration in Ixodoidea are Hindle and Cunliffe<sup>1</sup>, who, at my suggestion, carried out experiments wherein they mutilated the legs of immature *Argas persicus* and observed the effects of various operations upon the development of the limbs in succeeding stages. I had laid it down as a part of the programme that these authors should also determine if the mouthparts of immature ticks are capable of being regenerated, but they confined their attention to the regeneration of the legs, it being more difficult to deal with the mouthparts especially in small larvae. Ticks are liable to be mutilated in nature by being forcibly removed from the host, whereby their mouthparts are subjected at times to

<sup>1</sup> Hindle, E. and Cunliffe, N. (i. 1914). Regeneration in *Argas persicus*. *Parasitology*, vi. 353-371, 4 text-figures.

considerable injury. These may remain anchored in the host's skin, when the tick's body is torn away, and every grade of injury to the mouthparts is observable in ticks that are collected in the field.

#### METHODS.

In all cases the ticks under experiment were operated upon by me as soon as practicable, *i.e.* an hour or two, after they had fed to repletion and had dropped off the host. A requisite number having been selected for operation, individual ticks were transferred successively to a holder that kept them in a convenient position during the brief operation which took place upon the stage of a Zeiss binocular microscope.

The holder consisted of a glass photographic plate of small size upon which a band of plasticine adhered. The tick was placed on the plasticine with its mouthparts or legs that were to be amputated projecting out over the edge of the plasticine, and a second band of plasticine, covered with a piece of fine linen, was laid upon the first and pressed down sufficiently to fix the tick in position. The tick was therefore held as it were in a vice, a soft one, the gentle grip of which could be regulated during the operation and easily readjusted if the tick shifted. The piece of linen laid on the plasticine prevented its sticking to the operator's fingers during the manipulations.

A polished scalpel was held in the left hand with the blade beneath the tick's capitulum (or legs) so that the steel could be cut down upon as on a plate by means of a fine needle held in the right hand and ground to an oblique chisel edge. Great care was taken to avoid bruising the parts or pulling them about and to make a clean cut expeditiously.

The amputated parts adhered as a rule to the scalpel through the little coelomic fluid that escaped from the tick's wound when the parts were cut, rarely did the amputated parts spring away and get lost. They were collected after each operation and mounted in balsam as follows:

A number of slides were prepared by scratching two rows of small circles (*ca.* 6 mm.) upon them with a writing diamond, a circle punched in a piece of card and held firmly against the slide serving as a guide to the diamond point. The slide was now reversed so that the diamond circles were on the under surface and afterwards clearly visible through the balsam mount on the upper surface, on which, at the margin of the slide and close to each circle, a number was scratched, the number corresponding to that of the tick that was to be operated upon. The slide was cleaned, and a minute drop of water was placed over the centre of a diamond circle and brought into focus upon the stage of a second dissecting microscope. The amputated parts of the tick were transferred on the point of the operating needle to the drop of water and this was allowed to dry. When the series of circles was occupied by amputated parts and these had dried, a small drop of xylol was placed upon them and this was followed by a drop of balsam. Ordinary cover-glasses cut in four yielded squares of a size that sufficed to cover the circles.

These permanent preparations, ten or more per slide, served as accurate checks to graphic notes that were made of each operation. The skins of the ticks as they moulted after operation, or the ticks themselves, were mounted serially in a similar manner, air being excluded from the mounts by taking the usual precautions. The diamond circles greatly facilitate the finding of minute objects mounted within their contours<sup>1</sup>.

The ticks, after operation, were confined separately in numbered tubes standing in racks within a thermostat at 30° C., whence they were taken daily for inspection; the cast skins were periodically removed and mounted in the manner previously described.

The *abbreviations used in the tables* that follow are

R and L denoting Right and Left in respect to the parts that were amputated or left untouched.

— (a dash) denotes that no operation took place on the parts indicated.

The degree of amputation undergone by the several parts is denoted in different ways:

(a) by *fractions* of the length of the part (hypostome, palps) from apex to base, *i.e.* 1/2, 1/3, etc.

(b) by stating the *number of articles* that were removed wholly or partly, reckoning the basal article as 1 (palps have 4 articles 1–4) or by recording the number of distal articles removed (legs have 6 articles including the coxa).

(c) Operations where digits were removed without appreciable injury to the *shaft* of the chelicerae, are indicated by *cut d*; where the shaft was cut across, the amount thereof removed is given in lengths of digit taken as a measure, thus *cut 4 l* denotes that the portion of shaft removed with the digit was four times the length of the digit.

<sup>1</sup> I have devised another simple method of mounting large numbers of small objects in series. The method consists in mounting each small object in a numbered cell in balsam. To do this, cardboard of suitable thickness is cut in oblongs that are shorter (to leave room for a label) and nearly as wide as a slide. Two rows of circular holes (ca. 6 mm.) totalling 10–12 in number, are neatly cut in the card with a sharp hand-punch and serial numbers are written in Indian ink on the card beside the holes. Before use, the holed cards are immersed in xylol for 24 hours and afterwards in balsam for 24 hours; they are then transferred to a slide, the objects are placed in the holes and these are filled with balsam. A coverglass, of a size to match the card, having been dipped in xylol, is at once placed in position, the use of xylol helping to exclude air bubbles. The method is very convenient, economical, and readily lends itself to modifications.

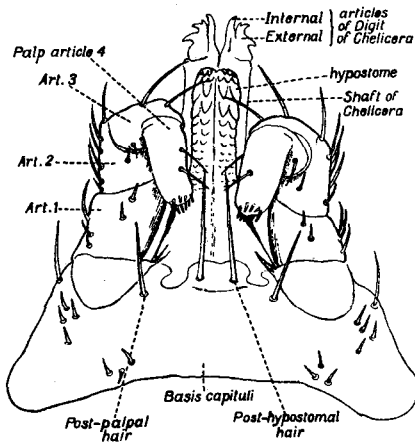


Fig. 1. *Argas persicus* ♂. Capitulum in ventral aspect, giving nomenclature of parts enumerated in the text (Nuttall, 1908).

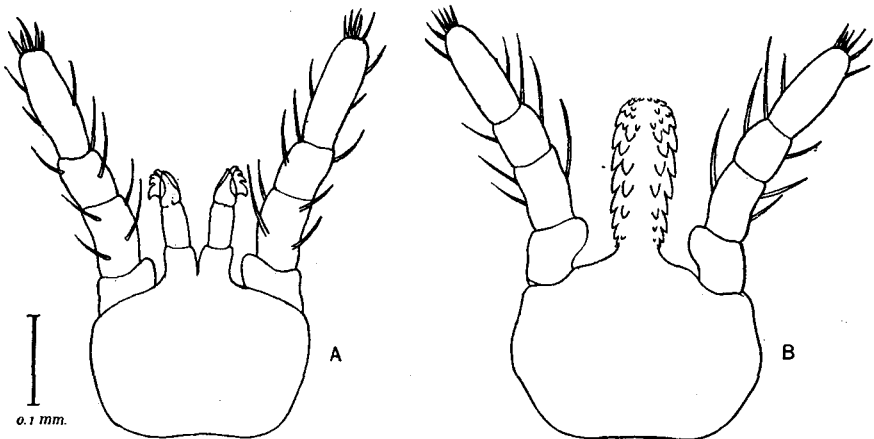


Fig. 2. *Argas persicus* larva. (A) Capitulum in dorsal and (B) in ventral aspect (Orig.).

## I. REGENERATION AFTER AMPUTATION OF THE MOUTHPARTS.

(Experimental Records.)

1. *Argas persicus*.

(a) Operations on the larva and their effect on the first-stage nymph.

Twenty-two larvae were operated upon after feeding on a fowl. Six (Ticks 3, 6, 13, 15, 17, 19) failed to undergo metamorphosis or died soon after operation. Twelve larvae were operated upon on 22. ii. 1915 and ten on the next day. The following record relates to the sixteen specimens that survived and moulted to first-stage nymphs.

Argas No.	Nature of operation on larva			Resultant effect on first-stage nymph		
	Palp articles removed	Hypostome length removed	Chelicerae	Palps showed stumps at article	Hypostome	Chelicerae d = digit
1	R 1-4 L 4	1/3	R — L cut*	R base L 1	perfect	L d perfect
2	R — L 2-4	1/4	R cut L cut	R 2† L 1	perfect	R and L d deformed
4	R 2-4 L 1-4	at base	R — L cut 1 l	R 3 L 3	regen. but deep emarg.	L d deformed
5	R 2-4 L 2-4	2/3	R — L —	R 1 (fig. 4 B) L 1	perfect	—
7	R — L 2-4	at base	R — L —	R deformed† L 1	deformed (see fig. 4 G)	—
8	R 2-4 L —	3/5	R — L —	R 1 L 1	perfect	—
9	R 2-4 L 2-4	1/4	R cut L cut	R and L 1 (fig. 4 D)	perfect	R d deformed (fig. 4 K) L d ?
10	R 2-4 L 2-4	at base	R cut ½ l L cut 1 l	R and L 2 (fig. 4 C)	regen. but some asymmetry	R d distinctly, and L d slightly deformed (fig. 4 J)
11	R 3-4 L 3-4	2/5	R — L —	R 2 L 2	perfect	—
12	R 3-4 L 3-4	1/4	R cut L cut	R and L 3 (fig. 4 E)	perfect	—
14	R — L —	1/4	R cut 4 l L —	—	regen. slight asymmetry	R d not regen. (see fig. 4 M)
16	R — L —	2/5	R cut 1 l L cut 1 l	R deformed but of normal length†	regen. slight emarg.	R d deformed L d deformed
18	R 2-4 L 2-4	at base	R cut 1 l L cut 1 l	R 1 L 1	perfect	R d deformed R d distinctly deformed
20	R — L —	near base	R cut d L cut 1 l	—	perfect	R d deformed L d deformed, useless
21	R — L —	2/5	R cut 1 l L cut 1 l	—	regen. slight asymmetry (fig. 3 A)	R d slightly deformed L d „ „
22	R 2-4 L crushed?	2/5	R cut L cut	R 1 L 2†	do.	R d useless (fig. 4 L) L d slightly deformed

\* Owing to loss of some amputated parts of chelicerae it is impossible in all cases to specify how much was removed.

† The tick must have been unwittingly injured at operation.

*Regeneration in Ticks**(b) Operations on first-stage nymphs and their after effects.*

Twenty-one first-stage nymphs were operated upon on 15-16. iii. 1915, after feeding on a fowl.

Argas No.	Nature of operation on first-stage nymph			Resultant effect on after stages				
	Palp articles removed	Hypostome cut at	Chelicerae digits	Moulted on day and month (1915)	Palps showed stumps at article	Hypostome	Chelicerae digits	Subsequently fed, and developed perfectly, moulted on
28	R — L 2-4	base	R — L —	4. iv.	R L 4	perfect	—	7. v. ♂
29	R 2-4 L —	base	R hurt? L —	29. iii.	R stumpy	perfect	perfect	28. v. ♀
30	R hurt? L —	base	R cut d L —	4. iv.	R 2	regen., emarg.	R perfect	1. vi. ♀
31	R 3-4 L —	base	R — L —	1. iv.	R stumpy	perfect	—	10. v. ♂
32	R — L 2-4	base	R — L —	29. iii.	L short	perfect	—	8. v. 3rd-st. nymph
33	R 3-4 L —	base	R hurt? L —	26. iii.	R 3	perfect	—	8. v. ♀
34	R — L —	base	R cut 1 l L cut	30. iii.	—	perfect	R slightly def. L perfect?	8. v. ♀
35	R — L —	base	R cut d L —	29. iii.	—	regen., emarg.	R. v. slightly deformed	26. v. 3rd-st. nymph, refused to feed
36	R — L —	base	R cut 1 l L cut d	1. iv.	—	perfect	R slightly def. L deformed, small	11. v. ♀
37	R — L —	base	R cut d L —	30. iii.	—	normal?	R (lost)	4. vi. ♀
38	R — L —	base	R cut 1 l L cut 1 l	30. iii.	—	slightly emarg.	R and L (lost)	28. v. ♂
39	R — L —	base	R — L —	30. iii.	—	do.	—	28. v. ♂
40	R — L —	base	R cut 2 l L cut 1 l	2. iv.	—	perfect	R d deformed L d normal	2. vi. ♂
41	R — L —	base	R cut d L —	30. iii.	—	perfect	R d deformed	10. v. ♀
42	R — L —	base	R cut 1 l L cut d	2. iv.	—	perfect	R and L slightly deformed	11. v. ♀
43	R — L 2-4	base	R cut d L —	29. iii.	L regen. but short	perfect	R d perfect	20. vi. ♂
44	R — L 4	base	R — L cut d	30. iii.	do.	perfect	L d deformed	10. v. ♂
53	R 2-4 L —	base	R cut d L —	3. iv.	R few sensory hairs at tip	perfect	R d deformed	22. vi. ♀
55	R — L 2-4	base	R cut 1 l L cut 1 l	1. iv.	L imperfect	perfect	R and L d slightly deformed	1. vi. ♂
59	R 3-4 L 1-4	—	R cut d L —	1. iv.	R 4 L 2	regen., emarg.	R and L slightly small	31. v. ♂
62	R 2-4 L 2-4	base	R cut d L cut d	2. iv.	R and L small?	perfect	R and L deformed	12. v. ♀

## Amputations through the basis capituli.

Ten first-stage nymphs had the basis capituli cut through transversely on 15. iii. 1915. Only four survived this severe operation which was accompanied by a considerable loss of coelomic fluid.

*Argas*

No.

Cut across basis capituli

After effects on 2nd-stage nymph

50 Cut diagonally near base of palps, including a long piece of shaft and digits of chelicerae.

19. iv. 15. Found partly moulted, feeble. Exuviae were carefully removed and found palps absent; hypostome represented by a smooth stump; chelicerae digitless, the tip of one sheath toothed, the other rounded.

51 Cut similarly to No. 50 but more basally.

19. iv. Found partly moulted, feeble, without capitulum, hypostome or palps, whilst two flattened digitless cheliceral sheaths protruded like fingers from a glove.

54 Cut similarly to the last.

5. iv. Moulded and died on 30. iv. Palp absent on the right side and reduced to a thin finger-like process on the left side; hypostome absent; chelicera of left side with its digit very small, the digits on both sides deformed (see Fig. 3 B and B').

60 Cut across anteriorly, a good portion of cheliceral shaft being included.

1. iv. Found moulting, thin and weak; it was helped to free itself. The tick died on 28. iv. Whilst it possessed neither hypostome nor palps, the chelicerae appeared normal.

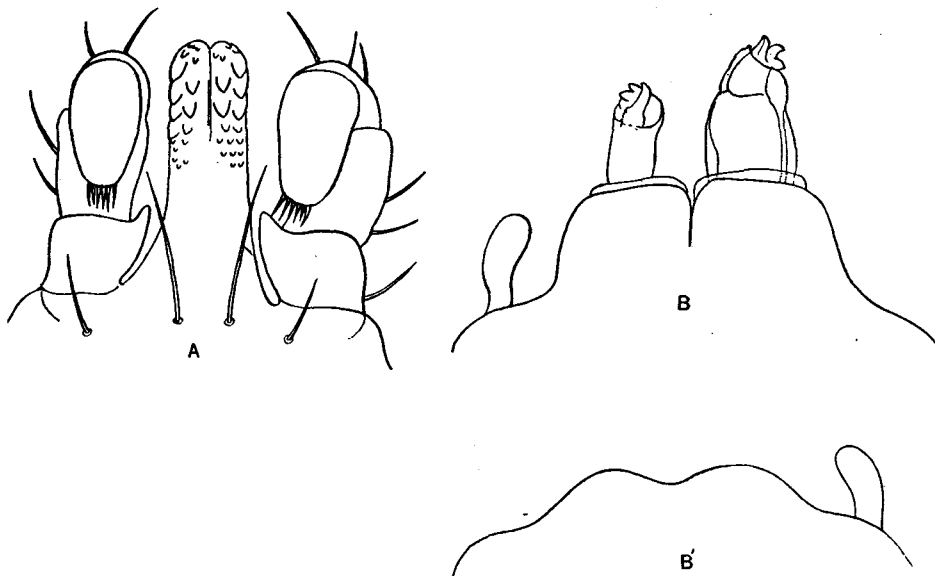


Fig. 3. *Argas persicus* nymphs. (A) Capitulum of 1st-stage nymph which had its hypostome and digits removed when in the larval stage (*Argas* 21); ventral aspect. (B) Capitulum of a 2nd-stage nymph which had its basis capituli cut across when in the first nymphal stage (*Argas* 54); dorsal aspect. (B') Ventral aspect of the same, showing absence of hypostome, one palp reduced to a finger-like process and the other absent, digits deformed.

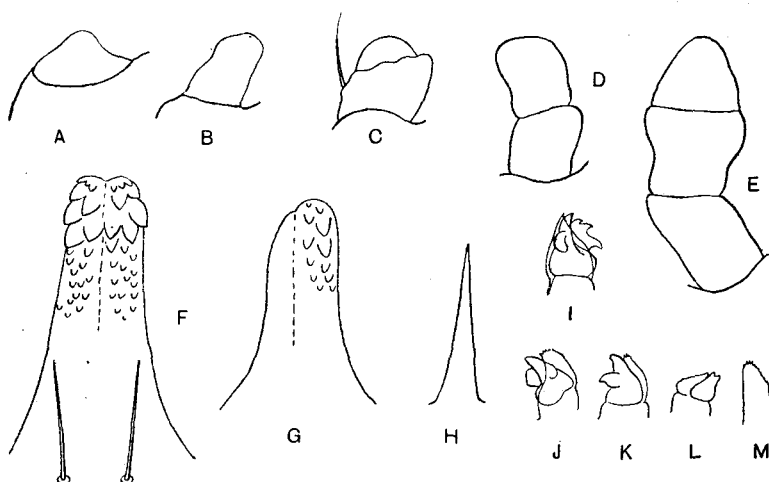


Fig. 4. *Argas persicus* nymphs. Illustrating effects of mutilations inflicted in preceding stages:

- (A) *Argas* 22, right palp in ventral aspect. First-stage nymph operated upon as a larva. Article 1 partly regenerated.
- (B) *Argas* 5, ditto. The left palp closely resembled the one figured at (A). Partial regeneration of article 1.
- (C) *Argas* 10, ditto. The left palp presented a similar appearance in this specimen. Regeneration of article 1 and a stump of article 2.
- (D) *Argas* 9, ditto. The left palp was similarly formed. Articles 1 and 2 regenerated.
- (E) *Argas* 12. Left palp in ventral aspect. First-stage nymph that had been operated upon as a larva. Articles 1 and 2 regenerated but malformed; article 3, partly regenerated, forms the stump.
- (F) Hypostome of normal 1st stage nymph.
- (G) Deformed hypostome of *Argas* 7, a 1st-stage nymph that had been operated upon as a larva.
- (H) Much deformed hypostome of *Argas* 67, a 3rd-stage nymph that had been operated upon as a 2nd-stage nymph. The structure forms a toothless spine.
- (I) Digit of normal 1st-stage nymph in dorsal aspect.
- (J-K-L) Deformed digits of 1st-stage nymphs, in dorsal aspect, as they appeared in *Argas* 10, 9, 22 that had been operated upon as larvae.
- (M) Digitless end of right chelicera in *Argas* 14, a 1st-stage nymph that had been operated upon as a larva.

*(c) Operations on second-stage nymphs and their after effects.*

Fifteen second-stage nymphs were operated upon on 29. iv. 1915 after feeding on a fowl. The following record relates to ten of these that survived and moulted to adults or third-stage nymphs which were subsequently raised to adults.

<i>Argas</i> No.	Nature of operation on second-stage nymph			Moulted on	Resultant effect on after stages
	Palp articles removed	Hypostome cut off at	Chelicerae		
63	R 2-4 L —	base	R and L cut d	18. v. 15	perfect ♂.
65	R and L 2-4	base	R cut d L —	18. v. 15	perfect ♂.
67	R at base L at base	base	R cut d L cut	25. v. 15	A 3rd-stage nymph with palps regenerated, hypostome a toothless spine (fig. 4 H), digits normal. Refused to feed and finally died.
68	R 2-4 L 1-4	base	R and L —	15. v. 15	♀, normal but for asymmetric emarginate hypostome. Fed 28. v.
70	R 2-4 L 1-4	base	R and L cut	17. v. 15	3rd-stage nymph normal but for somewhat asymmetric emarginate hypostome and rather small teeth on the external article of digits. Fed on 28. v.
74	R 2-4 L 2-4	base	R and L cut d	14. v. 15	3rd-stage nymph, perfect; fed 3. vi. and moulted 17. vi. as a normal ♀.
75	R 2-4 L 3-4	base	R cut l l L —	22. v. 15	3rd-stage nymph, perfect.
76	R 2-4 L 2-4	1/2	R cut L —	18. v. 15	3rd-stage nymph, perfect.

*. Amputations through the basis capituli.*

Three second-stage nymphs had the basis capituli cut across on 29. iv. 15, and two survived the operation:

<i>Argas</i> No.	Cut across basis capituli	After effects on third-stage nymph	
72	Cut diagonally from behind one palp to base of other and through digits and pieces of cheliceral shafts.	Tick moulted on 19. v.	Fed on 28. v. The tick was perfectly formed, the external article of the digits was somewhat small.
73	Cut midway across its length and through digits and cheliceral shafts to thrice the length of digit.	Tick moulted on 25. v.	Refused to feed 28. v. Its digits were deformed, the hypostome was absent, the palps merely represented by small protrusions. It survived in this condition until 13. v. 1919 ( <i>i.e.</i> four years unfed) when it was killed and preserved.

**2. Amblyomma hebraeum.***(a) Operations on the larva and their effects on the nymph.*

Thirteen larvae were operated upon on 27. vii. 1915, after they had fed to repletion upon a hedgehog and dropped off the host.

<i>Amblyomma</i> larva No.	Nature of operation on larva			Regeneration observed in nymph
	Palps	Hypostome cut away	Chelicerae digits	
1	R and L —	at base	L cut 4 l	perfect
2	R and L —	at base	R and L cut 3 l	perfect
3	R and L —	at base	R and L cut 3 l	perfect
4	R and L —	1/2	R cut 2 l L cut 1/2 l	perfect
5	R and L —	at base	R cut 3 l	R chelicera shaft 1/4 shorter than normal, otherwise perfect
6	R cut at base L cut near base	1/2	R cut 1/2 l L cut 2 l	perfect
7	R cut at base L —	3/4	R and L cut 2 l	perfect
8	R and L —	3/4	R cut 1 l L cut 2 l	perfect
9	R — L cut 1/2	at base	R cut 2 l L cut 2 l	perfect
10	R — L at base	at base	R cut 2 l L —	perfect

*(b) Operations on the nymph and their effects on the adult.*

Ten nymphs were operated upon on 3. viii. 1915, after feeding to repletion on a hedgehog.

<i>Amblyomma</i> nymph No.	Nature of operation on nymph			Regeneration observed in adult ♂ or ♀
	Palp articles cut away	Hypostome cut across	Chelicerae length of shaft cut in terms of digit length	
1	R and L —	at base	R and L cut 4 l	♂ perfect
2	R and L —	at base	R cut 2 l L cut 3 l	♂ perfect
3	R and L cut 2-4	under base	R and L cut 2 l	♀ perfect
4	R — L cut 2-4	at base	R and L cut 1 l	♀ perfect
5	R and L 2-4	at base	R cut 1 l L cut 3 l	♀ perfect
6	R cut 3-4 L cut 2-4	at base	R — L cut 2 l	♀ perfect
7	R cut 2-4 L cut 1-4	at base	R and L cut 2 l	♂ perfect

3. *Hyalomma aegyptium*.*Operations on the nymph and their effects on the adult.*

Seventeen nymphs were operated upon on 1. vi. 1915, after feeding and dropping from a hedgehog. They all moulted between 17 and 22. vi., except No. 17 which moulted on 24. vi. 15.

Hyalomma No.	Nature of operation on nymph			Regeneration observed in adult ♂ or ♀
	Palp articles removed	Hypostome cut	Chelicerae	
1	R and L —	nr base	R and L cut 2 l	♀ right internal article of one digit slightly deformed, otherwise perfect
2	R and L —	nr base	R cut 1 l	♀ perfect
3	R and L —	nr base	R cut 2 l L cut 1 l	♀ perfect but for deformity of external article of right digit
4	R and L —	nr base	R and L cut 2 l	♀ perfect
5	R and L —	at base	R cut 3 l	♀ perfect
6	R cut 2-4 L —	nr base	R and L cut 2 l	♂ perfect
7	R cut 1-4 L —	nr base	R cut	♀ perfect but for deformed R internal article of digit
8	R and L cut 2-4	at base	R and L cut 4 l	♀ R and L digits badly deformed, rest normal
9	R and L —	nr base	R cut	♀ perfect
10	R and L —	nr base	R and L cut 1 l	♀ perfect

## II. REGENERATION AFTER AMPUTATION OF THE LEGS.

## (Experimental Records.)

The experiments of Hindle and Cunliffe (cited on p. 7) on the mutilation of the legs in *Argas persicus* may be summarized as follows:

When the legs of a freshly gorged larva are amputated, they are not regenerated or are imperfect in the first-stage nymph. If the larva is operated upon whilst on the host, that is 2-3 days *before* it would drop off gorged if left unmolested, the legs may at times be regenerated after the tick abandons the host. This difference in the behaviour of the larva under the two conditions specified is attributed to nymphal development proceeding within the larva whilst it is upon the host and developing nymphal tissues being injured when gorged larvae are operated upon. First-stage nymphs that were mutilated in the larval stage, when raised, without further operative interference to second-stage nymphs, were found to have regenerated the amputated limbs although these were usually of subnormal size whilst perfectly formed; after a further moult, the ticks became normal. Experiments were also made with nymphs. It was found that leg regeneration occurred in all immature stages where amputation took place sufficiently long before moulting, but the legs were usually of subnormal size.

In my experiments the legs were amputated in immature stages of *Amblyomma hebraeum*, *Hyalomma aegyptium*, and in a few *Argas persicus* for purposes of comparison:

### 1. *Argas persicus*.

#### (a) *Operations on the larva and their effect on the first-stage nymph.*

Legs were amputated in three larvae (Nos. 23, 24, 26) on 23. ii. 15. In No. 23 legs I-III on the right side had 4,  $3\frac{1}{2}$ , and 4 distal articles cut off respectively; in No. 24 leg III had  $2\frac{1}{2}$  articles removed; in No. 26 leg I had 4 articles removed.

In none of these larvae were the limbs regenerated in the first-stage nymph, all of the limbs ended in stumps corresponding to the seat of amputation in the larva. No. 26 moulted as a first-stage nymph on 17. iii., it fed on 25. iii., and on 19. iv. it was found to have moulted with all its legs normally formed.

#### (b) *Operations on the first-stage nymph and their effect on the second-stage nymph.*

The legs of the right side were amputated in three first-stage nymphs (Nos. 45-47) on 25. iii. 15. In No. 45 legs I and IV had 5 articles removed; in No. 46 legs I and II had 5 and 3 articles removed respectively; in No. 47 legs I and III had 4 articles removed. The three ticks moulted to second-stage nymphs on 30. iii.-2. iv. and fed normally on 28-30. iv.

In the second nymphal stage, No. 45 had legs I and IV of subnormal size, and No. 46 had legs I and II smaller than the corresponding legs on the side that had not been mutilated. Through an oversight No. 47 was allowed to moult and the moulted legs were lost so that its condition escaped observation.

After further feeding, Nos. 45 and 47 moulted to third-stage nymphs whilst No. 46 emerged as a normal ♂, skipping the third nymphal stage which is more commonly omitted in normal ticks of this species. No. 47 gave rise to a ♀. In all of these stages the ticks appeared perfectly formed, no difference in size between corresponding legs being observable.

### 2. *Amblyomma hebraeum*.

#### (a) *Operations on the larva and their effect on the nymph.*

Legs were amputated in three larvae (Nos. 11-13) on 27. vii. 15 as follows: In No. 11 the greater part of legs II and III was removed; in No. 12 leg I had 5 articles removed; in No. 13 leg III had 5 articles removed.

Examined after they had moulted, the nymphs were found to have regenerated their legs perfectly, the previously mutilated limbs not being smaller than normal.

*(b) Operations on the nymph and their effect on the adult.*

Legs were amputated in three nymphs (Nos. 8–10) on 3. viii. 15 as follows: In No. 8 legs III and IV had 3 and 4 articles removed respectively; in No. 9 legs II and III lost 3 and 4 articles respectively; in No. 10 leg I had 4 articles cut off. These ticks in due course moulted and gave rise to three females.

All of the previously amputated limbs were regenerated in the adults, only in No. 9 were legs II and III (those mutilated) slightly smaller than normal.

**3. *Hyalomma aegyptium*.***Operations on the nymph and their effect on the adult.*

The legs were amputated in seven nymphs (Nos. 11–17) as follows:

<i>Hyalomma</i> No.	Number of articles amputated from legs	Regeneration in adult
11	2 from leg III	perfect
12	2 from leg IV	"
13	4 from legs II and III	" (untouched opposite leg III small)
14	4 from leg I	"
15	3 from leg II	regenerated, distal articles very slightly shorter than normal
16	4 from legs I and II	regenerated, slightly shorter than normal
17	3–4 from legs I, II, III, IV	regenerated, legs II, III, IV somewhat smaller than normal

**SURVEY OF THE RESULTS OBTAINED.**

All the immature ticks in these experiments were operated upon within 1–2 hours of their becoming fully gorged and abandoning the host. Extensive mutilations may cause death through excessive loss of coelomic fluid. Moderate mutilations are well borne by ticks, 78 out of 108 survived the operations herein described; the heaviest loss followed operations affecting the basis capituli. One third-stage nymph (*Argas* 73), in which the basis had been cut across midway in the second nymphal stage, survived for four years in the laboratory although it was unable to feed because of its mutilated mouth-parts<sup>1</sup>. Metamorphosis may be retarded or not according to the severity of the mutilation that has been inflicted.

**REGENERATION OF MOUTHPARTS.*****Argas persicus*.**

Operations on *larvae* wherein the mouthparts are mutilated shortly after the larva has abandoned the host in a fully gorged condition, cause the various structures to be differently affected in the first-stage nymph: the *palps* are not regenerated but appear as stumps which but for their closed and rounded ends correspond mostly in structure to the parts that were left intact in the larva (Fig. 4 A–E); occasionally an additional article or two is

<sup>1</sup> See p. 24.

regenerated. The *hypostome*, when amputated at any point short of its base, is perfectly regenerated, but if cut at its base the regenerated hypostome may be slightly deformed (Fig. 3 A). It should be noted that slight asymmetry and irregularities of dentition occur in all stages normally. The *digits of the chelicerae* are usually deformed to a varying degree (Fig. 4 D, K, L), the greatest deformity or even their non-regeneration may be caused by cutting off any considerable length of the shaft (Tick 14, Fig. 4 M); slightly deformed digits may function well enough for purposes of feeding.

*First-stage nymphs*, when mutilated as described in the case of the larvae, yield second-stage nymphs whose structures are affected as follows: the *palps* may appear like the stumps above described, they may be partly regenerated (*Argas* 28, 33, 53, 59), they may be completely regenerated whilst appearing short but about as broad as normal (*Argas* 32, 43), or they may appear normal. The *hypostome* in most cases is perfectly regenerated, but at times it is emarginated distally, an appearance occasionally met in ticks that are presumably normal. The *digits of the chelicerae* may be regenerated perfectly, or they may be undersized or deformed even when a length of shaft only equal to one or two digit-lengths is removed at operation. Slightly deformed digits do not prevent the mutilated ticks from feeding normally.

The whole series of second-stage nymphs, when left unmolested after the first operation, and adequately fed, gave rise to normal adults (19 specimens) or third-stage nymphs (two specimens) at the next moult.

When the *basis capituli* was cut across in first-stage nymphs only four out of ten of the ticks survived, the operation being accompanied by a great loss of coelomic fluid. None of the survivors moulted normally. In one case the basis capituli was not regenerated (*Argas* 51), in only one case was there an abortive attempt to regenerate a palp (*Argas* 54, Fig. 3 B and B'); the hypostome was not regenerated, at most, in one case it was represented by a smooth stump (*Argas* 50); the sheaths of the chelicerae were regenerated, they terminated either with apparently normal digits (*Argas* 60), small or deformed digits (*Argas* 54, Fig. 3 B), or they were devoid of digits (*Argas* 50, 51), these differences depending no doubt upon the degree of mutilation to which the parts had been subjected according as they were more or less protruded when amputated.

*Second-stage nymphs*, when mutilated, yielded adults (three specimens) or third-stage nymphs (five specimens) at the succeeding moult. In these the *palps* were all perfectly regenerated, in one tick the palp had been amputated basally. The *hypostome*, cut across basally, was perfectly regenerated in five cases, it appeared emarginated and asymmetrical in two, and in only one case did it subsequently develop to a toothless spine (*Argas* 67, Fig. 4 H). The *digits of the chelicerae* were regenerated perfectly in all but one case (*Argas* 70) wherein the external article of the digits appeared somewhat small. Only one of these ticks refused to feed (*Argas* 67 above referred to) and those that emerged as third-stage nymphs were subsequently raised to perfect adults.

When the *basis capituli* was cut across in second-stage nymphs, as was done successfully in two instances, almost perfect regeneration followed in one case (*Argas* 72), whilst in the other, where the basis was cut across half-way, the palps only reappeared as short stumps, the hypostome was absent and the digits deformed.

The results obtained with *Argas*, in respect to the power possessed by various immature stages of regenerating their mouthparts, are best elucidated by the following summary relating to the milder operations described in the foregoing pages:

OPERATIONS ON LARVAE		RESULT IN NEXT STAGE
Palps amputated	21	2 regenerated partly 19 not regenerated
Hypostomes amputated	16	16 regenerated 9 perfect 5 slightly deformed 2 deformed
Digits amputated	18	17 regenerated 5 slightly deformed 8 deformed 4 much deformed 1 <i>not</i> regenerated
OPERATIONS ON FIRST-STAGE NYMPHS		
Palps amputated	13	11 regenerated 2 perfect 5 short or imperfect 4 partially regenerated 2 <i>not</i> regenerated
Hypostomes amputated	20	20 regenerated 15 perfect 5 slightly deformed
Digits amputated	17	17 regenerated 5 perfect 6 slightly deformed 6 deformed or small
OPERATIONS ON SECOND-STAGE NYMPHS		
Palps amputated	15	15 regenerated 15 perfect
Hypostomes amputated	8	8 regenerated 5 perfect 2 slightly deformed 1 badly deformed
Digits amputated	11	11 regenerated 9 perfect 2 slightly deformed

It should be noted, at the outset, that operations on *larvae* which have dropped off in a replete state from the host yield, on the whole, unfavourable

results in respect to regeneration for the reasons specified on pp. 17 and 23. It is noticeable that after operations on larvae the hypostome is regenerated better than the digits, whilst mostly no attempt at regeneration takes place in the palps.

When the *first-stage nymph* has its mouthparts amputated, the hypostome is best regenerated, then follow the digits, whilst the palps are least well regenerated.

This order changes, however, when operations are made in the *second-stage nymphs*, for the succeeding stage (adult or third-stage nymph) shows regeneration to have taken place best in the palps and digits and least well in the hypostome. The cause of this phenomenon requires elucidation.

The foregoing tabular summary shows, moreover, that as the tick develops toward maturity, the power to regenerate the palps and digits increases, there being no distinct difference in respect to the hypostome, although the proportion of perfect to slightly imperfect hypostomes in second-stage nymphs after operation is highest, *i.e.* 15 : 20.

#### ***Amblyomma hebraeum.***

Operations on *larvae*, consisting of basal amputations of palps and hypostome, were followed in the nymph by perfect regeneration in all cases (ten operations); amputations of digits and twice their length of cheliceral shaft had no effect, but in one case, where a longer piece of shaft was removed (*Amblyomma* 5), the shaft appeared shorter than normal in the nymph.

Operations on *nymphs*, consisting of basal amputations of palps and hypostome or of digits with four times their length of shaft, were followed by complete regeneration of all parts in the adult.

#### ***Hyalomma aegyptium.***

Operations on *nymphs* (ten specimens) wherein the palps and hypostome were amputated at or near the base, were followed by perfect regeneration of these parts in the adult. Amputations of the digits and 2-3 times their length of cheliceral shaft, only resulted in deformity of the digit in one case (*Hyalomma* 3), whereas, when more of the shaft was removed (*Hyalomma* 8), the digits appeared badly deformed in the adult.

The results obtained after amputations of mouthparts in the Ixodid ticks are therefore in strong contrast to those in *Argas*. Regeneration takes place equally well after operations on larvae and nymphs. Perfect regeneration followed on almost all operations, *i.e.* 27 hypostome amputations, 23 palp amputations and 40 amputations of digits; slight deformity of digits followed three operations, great deformity in two, and in but one case was the cheliceral shaft shortened through mutilation.

## REGENERATION OF LEGS.

*Argas persicus*. It was found that when freshly gorged larvae had their legs amputated, the corresponding limbs were not regenerated in the first nymphal stage, but when these nymphs were fed and allowed to undergo a further moult without operative interference, they regenerated these mutilated limbs perfectly. When first-stage nymphs, under like conditions, were similarly mutilated, they gave rise to second-stage nymphs with well-formed legs of subnormal size. These results are in conformity with those of Hindle and Cunliffe (cited on p. 7) who explain the difference in the results obtained with larvae and nymphs by an interesting observation on larvae subjected to operations made at an earlier stage whilst still feeding on the host. When replete larvae abandon the host, they are well advanced in their nymphal development, and, consequently, if they are operated upon at this stage, they do not regenerate their legs, the first-stage nymphal formative tissues having been injured.

*Amblyomma hebraeum*. My experiments on this species show that when the legs are mutilated in replete larvae or nymphs immediately after the ticks have abandoned the host, the limbs are regenerated in the succeeding stage; in one adult that developed from a nymph which had been mutilated, the regenerated limbs were slightly smaller than normal, in the remaining six ticks the regenerated limbs were of normal size.

*Hyalomma aegyptium*. Similar experiments of mine upon replete nymphs of this species show that the regenerated limbs in the adult may be either perfect or of subnormal size.

Therefore 21 leg amputations in Ixodid ticks were followed in all cases by regeneration, 13 legs being normal and 8 slightly reduced in size.

## SUMMARY AND CONCLUSIONS.

Amputation experiments upon immature stages of *Argas persicus* (Oken 1818), *Amblyomma hebraeum* Koch 1844, and *Hyalomma aegyptium* (Linnaeus 1746) show that the mouthparts and legs of these ticks may be more or less regenerated when mutilated shortly after the ticks have abandoned the host in a fully engorged condition.

## Regeneration of Mouthparts.

In immature *Argas persicus* the mouthparts are regenerated more or less perfectly according to the structure affected and the stage of development of the tick. Freshly gorged larvae regenerate their mouthparts badly compared to first and second-stage nymphs because during their period of parasitism upon the host (5-6 days usually) the larvae have advanced well along the road to becoming nymphs, therefore the operation largely affects the formative tissues of the developing nymph. The nymphs feed rapidly, usually in

15–20 minutes, and proceed with their metamorphosis after abandoning the host, consequently, when they are mutilated soon after feeding, they regenerate their mouthparts (and legs) better than do the larvae. After operations on larvae and first-stage nymphs regeneration takes place best in the hypostome, next in the chelicerae and least well in the palps. This order was reversed after similar operations on second-stage nymphs. The power to regenerate palps and chelicerae grows as the tick approaches maturity, but it remains fairly constant for the hypostome. Amputations made through the basis capituli usually render the tick incapable of feeding after it has moulted to the next stage, the mouthparts not having been adequately regenerated. In one case (*Argas* 72), however, the amputation of the anterior part of the capitulum with its appendages in a second-stage nymph was followed by almost perfect regeneration of the part in the succeeding third-stage nymph. Where regeneration did not take place adequately after severer operations on the basis capituli, as exemplified in another case (*Argas* 73), the mutilated tick lived on without its capitulum for four years without a meal. This experiment explains the origin of the anomalous “headless female” of *Ixodes ricinus*, referred to and figured by Wheler<sup>1</sup>, which lived four years and was lost. Lesser mutilations, *i.e.* those affecting the appendages only, are frequently followed by perfect regeneration if the injury is slight. Imperfectly regenerated parts are often capable of functioning so that the tick can feed. Such a tick, if not interfered with, will acquire perfectly normal mouthparts at the next moult.

The immature stages of the Ixodid ticks *A. hebraeum* and *H. aegyptium* behave differently to *Argas persicus* when injured soon after abandoning the host, in that they possess much greater powers of regeneration. Basal or partial amputations of the hypostome and palps, or moderate mutilations of the chelicerae, are followed by perfect regeneration in most cases.

The greater power of regeneration possessed by Ixodid ticks bears directly upon their parasitic habits. The slowly feeding larval stages of Argasids (*A. persicus*, *A. vespertilionis*, for example) possess relatively more dentate hypostomes than do the adults which are rapid feeders. With the exception of *Haemaphysalis concinna* (vide Brumpt's observations recorded by me in *Parasitology*, VII. 434) all known Ixodids are slow feeders and an examination of genera like *Ixodes*, *Amblyomma* and *Hyalomma* proves that their hypostomes are most efficient organs for anchoring the tick to the host. The accompanying illustrations (Figs. 5 and 6) of the capitulum of *A. hebraeum* and *H. aegyptium* females show that they possess long, highly dentate hypostomes broadening distally and liable to break where they narrow toward the base when violence is done to the tick that is fixed thereby to the skin. The examination of such Ixodid ticks collected in the field proves how frequently their long hypostomes get broken off near the base, and consequently how vital it is that they should be readily regenerated. Whilst a tick may save

<sup>1</sup> Wheler, E. G. (iii. 1906). British Ticks. *Journ. of Agricult. Sci.* 1. p. 401, Plate X, fig. 38.

its digits by moving and retracting them, it cannot do so with its hypostome which is a rigid structure whose chitin is continuous with that forming the basis capituli. The hypostome is the most readily regenerated element of the mouthparts because it consists almost entirely of chitin, its formative tissues lying beneath the stout chitinous base whilst it is developing in the

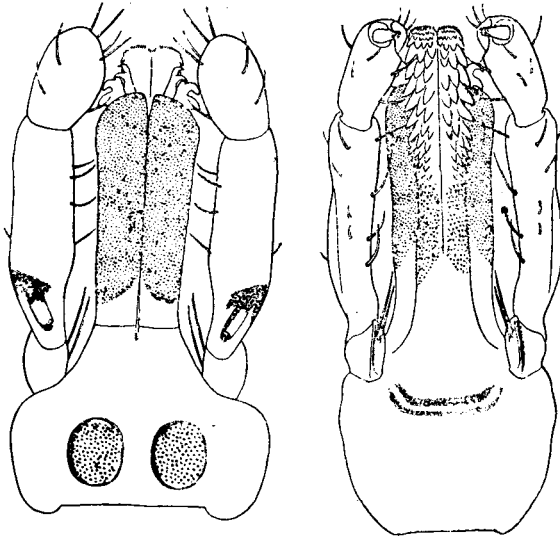


Fig. 5. *Amblyomma hebraeum* ♀. Capitulum in dorsal and ventral aspect (Original, ca.  $\times 16$ ).

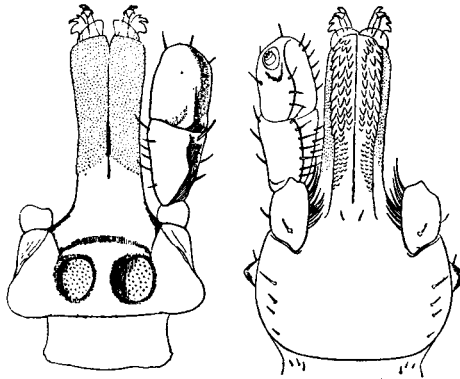


Fig. 6. *Hyalomma aegyptium* ♀. Capitulum in dorsal and ventral aspect (Nuttall, 1911 ca.  $\times 16$ ).

immature stages prior to a moult. In this position, the formative tissues are not liable to be hurt, whereas in the case of the palps and chelicerae as with the legs, the formative tissues are more directly accessible to injury.

The power of regenerating mutilated mouthparts possessed by immature ticks is of paramount importance in connection with their maintenance in

nature where they are frequently injured through their forcible removal from the host, the hooked hypostome and digits of the chelicerae being broken off to a varying degree because they are so firmly anchored in the host's skin. Such mutilation of the mouthparts is much more likely to occur in Ixodid than in Argasid ticks because the mouthparts of the latter are as a rule less effective anchoring organs, the structure of the mouthparts in the two groups being correlated with their feeding habits upon the host.

#### Regeneration of legs.

The experiments with *Argas persicus* herein recorded confirm those of Hindle and Cunliffe (*loc. cit.*). If the legs of the larva are amputated shortly after the tick has abandoned the host in a fully gorged condition, the first-stage nymph usually shows stumps corresponding in length to the portion of limb that was left intact in the larva. The authors cited found, however, that if the larva had its legs amputated whilst attached and feeding on the host, *i.e.* 2-3 days prior to its dropping off in a replete state, that the legs may at times be regenerated in which case they are usually of subnormal size. When the legs of first and second-stage nymphs are amputated soon after they have fed, the limbs are regenerated but are usually small. Immature ticks with small or stumpy legs, if not subjected to further interference, develop normal limbs after a further moult.

My experiments with *A. hebraeum* and *H. aegyptium* show that these Ixodid ticks possess greater powers of regeneration than *A. persicus* in respect to the legs, this being in harmony with the results above described in connection with the mouthparts. The number of legs amputated from the two Ixodid species in the larval and nymphal stages was 21 and all of them were regenerated; 13 limbs were of normal size and 8 slightly smaller than normal.