# ANTENNAL REGENERATION IN DAPHNIA MAGNA

BERTIL GOTTFRID ANDERSON,
Biological Laboratory, Western Reserve University

Antennal regeneration in Cladocera has been found to be rather variable. Przibram (1896, 1899) noted considerable variation both in amount and type of antennal regeneration in various species of *Daphnia* and *Simocephalus*. Kuttner (1913) found that antennal regeneration in *Daphnia magna* was extremely diverse. She noted that fully amputated segments never regenerated. Sciacchitano (1925) also pointed out that in *Ceriodaphnia pulchella* new segments of the antenna never formed. Setae were regenerated but never became as numerous, although often as large as the normal.

Recently Agar (1930, 1931) published some extensive researches on antennal regeneration in *Daphnia carinata* and *Simocephalus gibbosus*. His data show that the degree of variation in the amount of regeneration was high in spite of the fact that he limited the amputation to a specific level on a certain segment in each species and that the experimental conditions were very well controlled.

## GENERAL OBSERVATIONS ON DAPHNIA MAGNA

When a ramus of an antenna is cut the distal end of the remaining stump turns brown within a few hours after the operation. The brown area is quite distinct from the rest of the stump. (Fig. 1.) The brown color is usually deepest at the proximal edge of the area. Late in the instar, provided the amputation was performed within the first half of the instar, a cleavage occurs between the brown area and the remainder of the stump. At ecdysis the brown material is cast off. During the next instar the injured ramus corresponds closely to that portion which was proximal to the brown area during the instar of amputation. A ramus may be cut in one segment and the brown area may extend down into the next. In such instances as well the ramus during the following instar always corresponds to that part which was proximal to the brown area.

A similar situation is found after the carapace is injured (Anderson and Brown, 1930; Anderson, 1933). When a small

area of the carapace is crushed with a ball-pointed needle a ring of brown material forms in the outer edge of the crushed area. At ecdysis this area is shed with the old chitin. During the next instar the wound corresponds closely to the area enclosed within and including the brown ring during the instar of injury. No part of the carapace need be removed to produce this phenomenon.

Further, no part of the antenna need actually be cut to cause the formation of brown material and the subsequent shedding of the portion of the antenna distal to and including

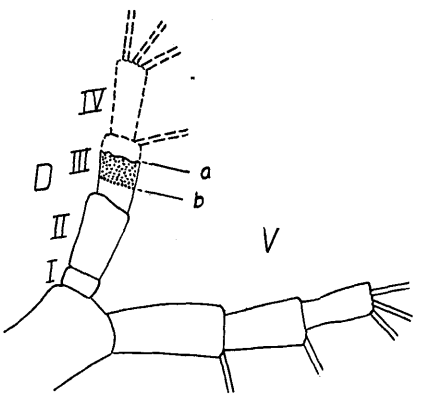


FIG. 1. An injured left antenna during the latter part of the first instar. A portion of the dorsal ramus was amputated early in the same instar. Line *a* denotes the level of amputation. The dotted lines represent the part removed. Line *b* denotes the level of injury. The stippled area between *a* and *b* is the brown portion—that injured by crushing in the amputation procedure.

the brown area. This was found by crushing part of the antenna sufficiently to injure the tissues but not break the chitinous cuticle. The area injured turns brown. The brown area together with the distal end of the antenna is cast with the rest of the old chitin at the next ecdysis. During the next instar the injured ramus corresponds closely to that part which was proximal to the brown area during the instar of injury.

Any amputation procedure involves the crushing of a part of the antenna before actual section is accomplished. Tissues proximal to the level of amputation are thereby injured. That part which is injured turns brown shortly after amputation. The brown color may be due to oxidized tyrosine in the clot of blood which forms in the injured portion (Pinhey, 1930).

The brown area probably corresponds to the necrotic area described by Agar (1930).

From the foregoing observations one may conclude that the injury is not limited to the level of amputation but extends to the proximal edge of the brown area. In making a quantitative study of the amount of regeneration the actual extent of the injury should be taken into consideration.

In order to determine the relation between the level of injury and the amount and type of regeneration the following series of experiments was performed.

## MATERIALS AND METHODS

Females from a single clone of *Daphnia magna* Straus. were used. These were isolated within three hours after their release from the mothers and placed in a watch glass together with a few drops of culture medium. Just enough of a saturated solution of chloretone was added to render the animals immobile. Each animal was placed on its left side with the left antenna stretched out in front of the body. A specially ground steel needle was brought against the dorsal branch with enough pressure to sever it. The level of amputation was varied in each instance. After amputation each animal was placed in a vial containing approximately fifty cubic centimeters of fresh manure-soil medium (Banta, 1921). These were then allowed to remain at room temperature (18°–23° C.).

At least twelve hours after the time of amputation, but before the end of the instar, each animal was removed from its vial and placed in a watch glass together with a few drops of culture medium. Again just enough chloretone solution was added to bring about cessation of movement. After placing the animal on its left side with the injured antenna stretched out in front of the body, a camera lucida drawing of the amputated member was made. Care was taken to denote exactly the extent of the pigmented area. Immediately after making the drawing the animal was returned to its vial and allowed to remain until primiparous.

When the animal became primiparous the injured antenna was again drawn by following the same procedure as outlined above. Usually females bear their first clutch of eggs from six to eight days after their release from the mother when maintained at room temperature (Anderson, 1932). Those

which were not primiparous on the eighth day were discarded. This probably limited the data to those animals which passed through seven pre-adult instars or less.

From the first drawing the level of the injury was determined, i. e., the segment injured and the distance from the base of the segment to the proximal edge of the brown area. The obliquely bounded surfaces of the segments render measurement difficult. In each case the length was taken on the central axis of the segment. Uniformity of measurements was thus secured. From the second drawing, the length of the regenerated segment, the number of setae, and the length of the setae were determined. The length of the segment was taken in the same manner as in the first drawing.

Agar (1930) observed that the antennae change in size and shape only at ecdysis and immediately thereafter—they remain the same throughout the remainder of any instar. Drawings and measurements for any instar can therefore be made at any time during that period.

## RESULTS AND DISCUSSION

The nature of regenerated antennae has been adequately described by Przibram (1896, 1899), Kuttner (1913), Sciacchitano (1925), and Agar (1930, 1931), and need not be detailed here. Regeneration is limited to the completion of the most proximal segment injured and the formation of new setae.

Agar (1930) considered only three measures of the amount of regeneration, namely: length of the antennal stump, number of setae, and length of setae. Of these he employed number and length of setae. In his estimation the stump was a poor criterion since regeneration involved only a slight growth of the operated segment. However, in *Daphnia magna* the volume of the regenerated material in the stump is much greater than that of the setae in most instances. On this basis one might conclude that the regenerated part of the stump would be a better criterion than that of the setae.

The amount of regeneration in the stump is not measurable directly. The segment in which regeneration has taken place may be looked upon as a product of both normal growth and regeneration. Normally a segment is about three times as long when an animal is primiparous as it is during the first instar. The difference between the length of the segment when an animal is primiparous and three times the length of the

uninjured portion during the first instar would then represent the amount of regeneration.

TABLE I

SUMMARY OF DATA ON LEVEL OF INJURY AND AMOUNTS OR REGENERATION
(Lengths are in $\mu$)

| LEVEL OF INJURY | | REGENERATION | | | | |
|---|---|---|---|---|---|---|
| Segment | Level in Segment | Length of Segment | Calculated Segmental Regeneration | Number of Setae | Total Length of Setae | Number of Cases |
| I | Base | 0 | ..... | 0.0 | 0.0 | 4 |
| II | Base | 0 | ..... | 0.0 | 0.0 | 4 |
| | 1- 6 | 59 ± 15 | 50 | 1.25 ± .15 | 407 ± 44 | 4 |
| | 7–13 | ..... | ..... | ..... | ..... | 0 |
| | 14–19 | 136 ± 7 | 85 | 2.40 + .15 | 1020 ± 13 | 5 |
| | 20–25 | 127 ± 7 | 60 | 2.13 ± .16 | 770 + 60 | 8 |
| | 26–32 | 143 ± 6 | 53 | 2.33 ± .13 | 847 ± 64 | 6 |
| | 33–38 | 183 ± 10 | 77 | 2.50 ± .14 | 1127 ± 87 | 6 |
| | 39–45 | 169 ± 3 | 43 | 2.25 ± .28 | 910 ± 116 | 4 |
| | 46–51 | 199 ± 8 | 54 | 2.60 ± .15 | 1082 ± 15 | 5 |
| | 52–57 | 216 ± 14 | 51 | 3,00 ± .00 | 955 ± 83 | 3 |
| | 58–64 | ..... | ..... | ..... | ..... | 0 |
| | 65–70 | 229 ± 5 | 29 | 3.75 ± .27 | 1394 ± 141 | 4 |
| | 71–76 | 216 ± — | 0 | 4,00 ± — | 1210 ± — | 1 |
| III | Base | 0 | ..... | 2.50 ± .13 | 1094 ± 72 | 12 |
| | 1- 6 | 0 ± — | 0 | 2.00 ± — | 738 ± — | 1 |
| | 7–13 | 73 ± 6 | 43 | 2.00 ± .00 | 1062 ± 36 | 7 |
| | 14–19 | 93 ± 5 | 45 | 2.14 ± .14 | 1196 ± 91 | 7 |
| | 20–25 | 112 ± 6 | 53 | 2.40 ± .15 | 1275 ± 53 | 5 |
| | 26–32 | 140 ± 10 | 53 | 3.33 ± .37 | 1553 ± 146 | 3 |
| | 33–38 | 134 ± 6 | 26 | 2.70 ± .18 | 1375 ± 95 | 7 |
| | 39–45 | 135 ± 11 | 6 | 3.00 ± .00 | 1566 ± 3 | 2 |
| | 46–51 | 172 ± 6 | 25 | 2.00 ± .00 | 1146 ± 35 | 2 |
| | 52–57 | 169 ± 8 | 0 | 2.00 ± .00 | 1177 ± 31 | 2 |
| IV | Base | 0 | ..... | 2.47 ± .14 | 1273 ± 79 | 15 |
| | 1- 6 | 20 ± 5 | 11 | 1.80 ± .30 | 802 ± 153 | 5 |
| | 7–13 | 69 ± 6 | 39 | 1.67 ± .13 | 815 ± 64 | 6 |
| | 14–19 | 93 ± 4 | 45 | 2.50 ± .12 | 1299 ± 55 | 8 |
| | 20–25 | 108 ± 10 | 39 | 2.75 ± .15 | 1458 ± 31 | 4 |
| | 26–32 | 96 ± — | 9 | 3.00 ± — | 1476 ± — | 1 |
| | 33–38 | 99 ± 8 | 0 | 2.50 ± .24 | 1247 ± 79 | 2 |
| | 39–45 | 127 ± — | 0 | 1.50 ± — | 1081 ± — | 1 |
| Terminal Setae | ..... | ..... | ..... | 2.75 ± .10 | 1578 ± 70 | 8 |

Table I summarizes the results. The level of injury is the level of the proximal edge of the brown material. Segmental

regeneration was calculated as proposed in the above paragraph. In those cases where the injury extended to the base of a segment that segment never regenerated. When the injury extended to the base of segment III or IV new setae were formed, but not when the injury reached the base of segment I or II. Removal of the terminal setae alone was followed by their regeneration. Whenever a part of segment II, III, or IV remained intact that segment exhibited regeneration and new setae were formed. Segment I was never regenerated nor were setae formed when the injury extended to that segment.

In general the length of the segment in which the regeneration occurred varied directly with the level of injury in the segment. The coefficients of correlation between the level of injury and the length of the segment were $+.76 \pm .04$, $+.80 \pm .04$, and $+.73 \pm .06$ for segments II, III and IV, respectively. The amounts of regeneration varied inversely with the level of injury except when the injury reached the base of a segment or only a very small part of the segment remained intact.

Setal regeneration appears to fall into a different category. Each segment seems to present a specific problem. In the case of segment II the number of setae formed appears to be directly related to the level of injury. The coefficient of correlation was $+.67 \pm .05$. The total length of the setae was not so well correlated, $+.34 \pm .09$. For segments III and IV the maximum number of setae and also maximum total length of setae occurred when the level of injury was just distal to the middle of the segment. The coefficients of correlation were $+.08 \pm .11$ and $+.39 \pm .11$ for number of setae and $+.31 \pm .10$ and $+.46 \pm .10$ for total length of setae for segments III and IV respectively.

The variations in the amount of regeneration reported by Agar (1930, 1931) are readily explainable. They are in all likelihood due to variations in the level of injury even though the level of amputation was kept constant. On the basis of the evidence presented in this paper one may alter Agar's (1930) statement from "the length of the segment left after operation is probably the most important single factor in determining the amount of regeneration" to—the length *of the uninjured portion* of the segment left after operation is probably the most important single factor in determining the amount of regeneration.

SUMMARY

The antennae of 150 female *Daphnia magna* were amputated at various levels during the early part of the first instar. In each case, within a few hours after amputation a brown pigment was deposited immediately proximal to the level of the cut. The extent of the brown area varied considerably. At the end of the instar the part of the antenna distal to and including the brown area was cast with the old chitin. During the next instar the antennal stump corresponded closely to that part of the antenna which was proximal to the brown area during the instar of amputation.

The amount of regeneration, as measured during the instar when the animals were primiparous, is qualitatively and quantitatively related to the segment in which the proximal edge of the brown area was located and to its level within that segment during the instar of amputation.

CITATIONS

**Agar, W. E.** 1930. A statistical study of regeneration in two species of Crustacea. Journ. Exp. Biol., Vol. 7, pp. 349–369.

1931. A Lamarkian experiment involving a hundred generations with negative results. Journ. Exp. Biol., Vol. 8, pp. 95–107.

**Anderson, B. G.** 1932. The number of pre-adult instars, growth, relative growth, and variation in *Daphnia magna*. Biol. Bull., Vol. 63, pp. 81–98.

1933. Regeneration in the Carapace of *Daphnia magna*. I. The Relation between the Amount of Regeneration and the Area of the Wound during Single Adult Instars. Biol. Bull., Vol. 64, pp. 70–85.

**Anderson, B. G.** and **L. A. Brown.** 1930. A Study of Chitin Secretion in *Daphnia magna*. Physiol. Zool., Vol. 3, pp. 485–493.

**Banta, A. M.** 1921. A convenient culture medium for daphnids. Science N. S., Vol. 53, pp. 557–558.

**Kuttner, O.** 1913. Über Vererbung und Regeneration angeborener Missbildungen bei Cladoceren. Arch. f. Entw. mech. d. Organ bd. 36, s. 649–670.

**Pinhey, K. G.** 1930. Tyrosinase in crustacean blood. Journ. Exp. Biol., Vol. 7, pp. 19–36.

**Przibram, H.** 1896. Regeneration bei niederen Crustaceen. Zool. Anz. bd. 19, s. 424–425.

1899. Die regeneration bei den Crustaceen. Arbeiten a. d. Zool. Inst. d. Univ. Wien. Tom. 11, s. 163–194.

**Sciacchitano, I.** 1925. Die Regenerationsfähigkeit der *Ceriodaphnia pulchella* Sars. Zool. Anz. bd. 19, s. 173–177.