REGENERATION AND THE MOULTING CYCLE IN
BLATTELLA GERMANICA L.

II. SUCCESSIVE REGENERATION OF BOTH METATHORACIC LEGS

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Summary

At 29°C, 70 per cent. relative humidity, removal of the left and right metathoracic legs in succession, at intervals of 1, 2, or 3 days, in first instar Blattella germanica of known ages, results in the appearance at the first moult of:

(a) Two asymmetrical papillae, the timing of the moult being undisturbed; or
(b) A regenerate on the left and a papilla on the right side, the time of moulting being determined by age at the first operation and independent of the second; or
(c) Two asymmetrical regenerates, the time of moulting being then determined by age at the second operation and independent of the first.

In (b) and (c) the rather constant size of regenerates from the first operation exceeds that of regenerates derived from either unilateral or simultaneous operations, while the smaller regenerates from the second operation in (c) show wide but erratic variation in size, not regularly related to the timing of the operations or the moult.

In about 2 per cent. of the material, the second operation only gives rise to a structure intermediate between a papilla and a complete regenerate. Such rare exceptions to the “all or nothing” principle can appear only when ecdysis occurs during the exceedingly rapid process of differentiation of the blastema into a regenerated leg. This proceeds proximodistally in these individuals, in contrast to animals producing a regenerate and a papilla at their first moult, where the rapid differentiation of the blastema contained within the papilla and its coxa occurs, early in the second instar, in the opposite (i.e. distoproximal) direction.

These results conform closely to predictions derived from the initial hypotheses put forward regarding the relationship between regeneration and the moulting cycle. New information on the regeneration process has also been obtained.

I. INTRODUCTION

Possible interrelations between regeneration and the endocrine balance controlling moulting in Blattella germanica have been discussed in earlier papers of this series (O'Farrell and Stock 1953; Stock and O'Farrell 1954). In these discussions it was suggested that regeneration, in certain conditions, produces a sort of physiological “putting back the clock,” which involves a fresh start in the rhythm of the moulting cycle, dating from the time of removal of a metathoracic leg. Such a secondary moulting cycle, once established, should

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then be capable of modification, and even perhaps subjected to a further fresh start, by the onset of a second regeneration process appropriately timed. The postulated modification, however, would not be fully manifested unless the moulting cycle were relatively little influenced by the extra quantity of tissue to be produced in completing the regeneration of two appendages instead of only one. Since it has been shown (Stock and O'Farrell 1954) that simultaneous regeneration of both metathoracic legs during the first instar influences the first moult only very slightly more than unilateral regeneration of a single metathoracic leg, this condition appears to be fulfilled.

The present work, then, is an investigation of the nature of the secondary cycle set up by regeneration, and of the types of regenerate produced as a result of successive operations. It has been assumed that satisfactory evidence on these matters will be produced by examining the consequences of removing the two metathoracic legs in succession, at suitably varied intervals.

II. Material and Methods

The general cultural and experimental methods used were those described by O'Farrell and Stock (1953), but the animals were kept throughout in a constant-temperature room at 29°C, 70 per cent. relative humidity (R.H.) (± 0.5°C, and approx. 8 per cent. R.H.). Experimental material was obtained by subdivision of the batches used in work on simultaneous regeneration (Stock and O'Farrell 1954) so that the results of the two series of experiments would be directly comparable with one another and with the same population of controls.

Removal of one metathoracic leg at the proximal autotomy plane was carried out on animals of known age on each of the first 4 days of post-embryonic life, and followed at intervals of 1, 2, or 3 days by removal of the other metathoracic leg, in accordance with the schedules in Table 1. In the early stages, the first operation was performed alternately on the right and left side. This procedure was abandoned after results from about 300 experimental animals had confirmed the expectation that the overall nature of the regenerates and the timing of the first two mouls were unaffected by the side on which the first operation was performed. Thereafter the standard practice adopted was to remove the left leg at the first and the right leg at the second operation.

Representative series of preparations of the metathorax, bearing regenerates produced from each combination of treatments, were fixed in alcoholic Bouin's fluid, stained in alcoholic light green, and mounted whole for detailed examination, measurement, and photography.

III. Experimental Results

(a) Morphology of Regenerates

The headings of columns 4-7 in Table 1 briefly describe the four types of combinations of regenerated structures observed at the first moult, after successive removal of the metathoracic legs in accordance with the nine operating
schedules shown in columns 2 and 3 and allotted reference letters in column 1 of Table 1. These abbreviated descriptions and reference letters will be used throughout this paper.

### Table 1

OPERATING SCHEDULES IN RELATION TO NUMBERS OF ANIMALS PRODUCING VARIOUS COMBINATIONS OF REGENERATES AT THE FIRST MOULT

<table>
<thead>
<tr>
<th>Operating Schedule</th>
<th>Age (days) at First Operation</th>
<th>Numbers of Animals Producing Regenerate and Two Regenerates</th>
<th>Total Numbers Surviving First Moul</th>
<th>Experimental Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>0-1</td>
<td>2-3</td>
<td>0-0-0</td>
<td>24</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>0-1</td>
<td>3-4</td>
<td>0-33</td>
<td>58</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>1-2</td>
<td>2-3</td>
<td>0-0-0</td>
<td>85</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>1-2</td>
<td>3-4</td>
<td>0-21</td>
<td>113</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>1-2</td>
<td>4-5</td>
<td>0-73</td>
<td>25</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>2-3</td>
<td>3-4</td>
<td>19-3</td>
<td>108</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>2-3</td>
<td>4-5</td>
<td>70-6</td>
<td>81</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>2-3</td>
<td>5-6</td>
<td>11-47</td>
<td>18</td>
</tr>
<tr>
<td><strong>J</strong></td>
<td>3-4</td>
<td>4-5</td>
<td>125-0</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total numbers observed</strong></td>
<td></td>
<td></td>
<td>225-183-22-540</td>
<td>970</td>
</tr>
</tbody>
</table>

* The second operation in schedule H occurs after the majority of fast-growing animals have already moulted and have to be discarded, hence the very low proportion of “two papillae.”

A detailed account of the four combinations of regenerates follows.

(i) **Two Papillae.**—This condition occurred only in association with operating schedules F, G, H, and J. Usually (Plate 1, Fig. 1) the side of the first operation (hereafter called “side I”) showed a perfectly healed, smoothly rounded papilla, without trace of external differentiation or of clotted blood, replacing the original trochanter. On the side of the second operation (“side II”) there was a blood-clotted, partly healed trochanter, showing neither the smooth rounding-off nor the associated reduction in size sometimes observed on side I. At the first moult, dedifferentiation of the coxal musculature and the beginning of blastema formation were often recognizable on side I, but not on side II (Plate 1, Fig. 1).

A less common form of “two papillae” occurred with a longer time lapse between the second operation and the first moult. Rounding-off, smoothness, reduction in size, and completeness of healing, denoted by the absence of any trace of clotted blood, were apparent in the trochanters of both sides (Plate 1, Fig. 2). They were usually asymmetrical, but the complexities of the healing process and subsequent events of dedifferentiation and blastema formation...
appeared to be such that the larger papilla was sometimes on side I and sometimes on side II, presumably according to the precise time relations between operation and moulting in the individual concerned. Internal changes visible at the first moul of such animals usually included some blastema formation, but internal asymmetry was not so clearly recognizable as in the material with a persistent blood-clot on side II.

Marked asymmetry in the size and differentiation of the blastemata on the two sides became apparent during the second instar, but tended to diminish towards the time of the second moul. At the second moul, two completely regenerated legs, with the usual tetramerous tarsi, appeared in place of the papillae. These regenerates were usually nearly symmetrical in size, and apparently completely so in their level of differentiation (Plate 1, Fig. 3).

Preparations of these papillae and of the regenerates which followed them were carefully compared with equivalent preparations from the experiments on simultaneous regeneration. Apart from the asymmetry of the two sides, the types and sizes of papillae resulting from the successive operations did not appear to differ from those produced after simultaneous operation. The regenerates appearing at the second moul showed a slight size asymmetry, usually detectable only by measurement, but otherwise appeared similar to those observed in similar circumstances in the work on simultaneous regeneration.

(ii) Regenerate and Papilla.—Occurring mainly in association with schedules B, D, E, and H, rarely with F and G, and never with A, C, or D, this condition was the least symmetrical observed. A large, highly differentiated complete regenerate with tetramerous tarsus appeared on side I, and a completely healed, smoothly rounded papilla, without external differentiation and showing no sign of clotted blood, replaced the trochanter on side II. This papilla seldom showed much size reduction by comparison with a normal trochanter, and occasionally even exceeded it in length; but such variations were sporadic and irregular, not being clearly related to the timing of operation or moulting, or both. At the first moul, the papilla usually showed considerable blastema development, extending into the distal musculature of the coxa (Plate 1, Fig. 4). The timing of the operations in relation to the moul had no recognizable or regular effect on the size or the differentiation of the regenerate on side I, which was rather consistently larger in size than comparable products of either unilateral or simultaneous regeneration.

Rapid differentiation of the blastema on side II was seen early in the second instar (Plate 2, Figs. 7 and 8), all essential features of a complete regenerate being apparently fully formed within 2-3 days after the first moul (Plate 2, Fig. 9). The numerous preparations studied suggested that the differentiation sequence was mainly distoproximal, with the tarsus being formed first and the femur last; but it is not yet clear to what extent departures from such a sequence may occur. Associated with differentiation was a certain amount of sculpturing of the cuticle of the papilla by the pressure of the growing blastema within. No evidence was obtained to suggest that true cuticular differentiation of the papilla ever occurred during the intermoult period,
although a *spurious* appearance of segmentation, easily recognized as a fixation artefact, was observed in some preparations, fixed a day or two after the moult. No examination of the regenerates appearing at the second moult was made, but the observations on papillae suggested that they would be more or less symmetrical in size and in differentiation.

(iii) *Regenerate and Intermediate.*—A total of 524 animals, in 50 batches subjected to six different operating schedules, constituted the fraction of the experimental population in which every batch included one or more individuals producing "regenerate and papilla" at the first moult. This population included 22 animals, of which five came from one batch, two each from another four batches, and single examples from a further nine batches, in which an "intermediate" appeared on side II at the first moult. The regenerate on side I in these animals was normal, large, and complete, but that on side II ranged in appearance from an elongate, blastema-filled papilla with incipient segmentation visible on the cuticle (Plate 2, Fig. 11), quite different from the fixation artefacts mentioned above, to the almost complete regenerate with imperfect pretarsus seen in Plate 2, Figure 18. The absence of a properly formed pretarsus with claws formed a good criterion for distinguishing such "advanced" intermediates from the small and sometimes poorly differentiated, but essentially complete, regenerates occasionally found on side II in material of the "two regenerates" category (see below).

A surprisingly complete range of possibilities in the *proximodistal* differentiation of a blastema-filled papilla towards a complete regenerate was observed among the intermediates. Representative stages are shown in Plate 2, Figures 10-18. Most of these were represented by two or more examples, but the stage in Plate 2, Figure 16, was unique. Differentiation of the intermediates seemed to be very rapid, as shown especially by the history of the batch in which five intermediates were recorded from nine animals undergoing operations of schedule D. The first to moult did so on the eighth day of post-embryonic life, with a normal "regenerate and papilla" (Plate 2, Fig. 10). In the ensuing 24 hr the series of stages shown in Plate 2, Figures 11, 14, 15, 17, and 18, appeared. The sequence in which moulting occurred was readily deduced from the condition of the cuticle, ranging from very fresh, white, and soft in the animal of Plate 2, Figure 18, to completely hardened and darkened in the specimen of Plate 2, Figure 11. Intermediate stages in darkening corresponding to the sequence of differentiation of the intermediates were clearly recognizable in the animals of Plate 2, Figures 14, 15, and 17. The remaining animals moulted on the 10th day of post-embryonic life with normal pairs of "two regenerates" showing only mild asymmetry. Extremely rapid differentiation must have been in progress on side II during the ninth day of post-embryonic life, and the whole process of conversion of a fully organized (but not visibly differentiated) blastema into a complete regenerate cannot have occupied more than a few hours.

The rarity and theoretical interest of intermediates made it necessary to fix them as soon as seen, i.e. within a few hours of the first moult, and it has consequently been impossible to trace their differentiation and growth during
the second instar, or to compare it with the distoproximal differentiation occurring in the side II papilla during the second instar of animals moulting with "regenerate and papilla."

Unpublished observations on teratological specimens indicated that many intermediates might be lost by autotomy early in the second instar—a further discouragement against attempting to follow their differentiation with the very limited number available. In an attempt to increase this number, and to test a hypothesis on the time relations of operations needed to secure the appearance of intermediates, a random assortment of animals aged between 0 and 2·5 days was subjected to successive operations separated by roughly 65 hr. The age at the first operation not being accurately known, this material has been excluded from the tables. It consisted of 74 individuals; 54 moulted with "regenerate and papilla," eight with "regenerate and intermediate," essentially similar in types to the stages shown in Plate 2, and 12 with "two regenerates." The proportion of intermediates, although increased, was not high enough for the attempt to be pursued further at this stage.

(iv) Two Regenerates.—This condition (Plate 1, Figs. 5 and 6) involved the appearance on each side, at the first moult, of a complete regenerate with tetrumerous tarsus and fully formed pretarsus. The regenerate of side II was smaller in size and sometimes inferior in minor details of cuticular and muscular differentiation to that of side I. Combined asymmetry of size and differentiation sometimes gave rise to a rather "lop-sided" appearance (Plate 1, Fig. 5), but the presence of a fully formed pretarsus on each side readily distinguished all such examples from "regenerate and intermediate" material. At the other extreme (Plate 1, Fig. 6) were individuals in which the two regenerates were apparently identical in differentiation, and differed so little in size that measurement was necessary to establish that they were in fact asymmetrical.

Representative preparations of "two regenerates," derived from each of the operating schedules used, were examined in detail and a selection of them measured. They were also compared with preparations derived from animals undergoing unilateral or simultaneous regeneration. Evidently an adequate biometrical study of the symmetry of successive regenerates would require a more elaborate experimental design than the present work, and no firm conclusions were reached on the time relations of the varying degrees of symmetry observed. The size of the regenerate on side I, however, appeared to be rather constant for all operating schedules (except possibly J) and to exceed considerably the size of regenerates resulting from either unilateral or simultaneous operations. The regenerate of side II was much more variable in size, although always smaller than that of side I, but no consistent relationship was recognized between its size or differentiation and the time relations of the operations and the moult. In general, the sum of the lengths of the two successively produced regenerates was of the same order (5·5-6·5 mm) as that of two regenerates produced simultaneously. Occasional higher values were observed, up to 7 mm. Insufficient data are available for critical assessment of the importance of these exceptions.
O'Farrell and Stock

Plate 1

Regeneration and Moulting in Blattella. III

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REGENERATION AND MOURTING IN BLATTELLA. III

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The second moult of "two regenerates" material was observed only in a few individuals, from which no preparations were made. The symmetry of their regenerated metathoracic legs at the second moult appeared to be fairly complete, probably resembling that observed in the second moult of "two papillae" animals.

(b) Influence of Successive Operations on the Time of Moult

The production of "two papillae" had no effect on the timing of the first moult, but the second moult was delayed significantly in comparison with that of the corresponding controls (Table 2). The magnitude of this delay was less than the age of the animals at the first operation, but was decidedly greater for schedule J than for F or G. The difference between the moult of the material of schedules F and G was not significant, and the difference between either of them and schedule J was only doubtfully significant. Insufficient material of schedule H was available to permit their second moult to be investigated.

<table>
<thead>
<tr>
<th>Operating Schedule</th>
<th>Numbers of Animals Surviving at First Moult</th>
<th>Observed Delay in Moult of Experimental Animals (days)*</th>
<th>Numbers of Animals Surviving at Second Moult</th>
<th>First Moult</th>
<th>Second Moult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiments</td>
<td>Controls</td>
<td>Experiments</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>19</td>
<td>85</td>
<td>17</td>
<td>60</td>
<td>No delay</td>
</tr>
<tr>
<td>G</td>
<td>70</td>
<td>144</td>
<td>59</td>
<td>100</td>
<td>No delay</td>
</tr>
<tr>
<td>H</td>
<td>11</td>
<td>40</td>
<td>N.r.†</td>
<td>N.r.†</td>
<td>No delay</td>
</tr>
<tr>
<td>J</td>
<td>125</td>
<td>137</td>
<td>92</td>
<td>78</td>
<td>No delay</td>
</tr>
</tbody>
</table>

* Computed by the method of Simpson and Roe (1939, pp. 192-3).
† Denotes "not recorded."

Where a "regenerate and papilla" appeared at the first moult, the moult was delayed (except in schedule B) by a period approximating the age of the animal at the first operation, irrespective of the timing of the second (Table 3). The schedule B animals behaved in a similar way to simultaneously regenerating animals undergoing operation on the first day of post-embryonic life (Stock and O'Farrell 1954), their moult being delayed to about the same extent as would have been expected had the first operation been performed on the second day of post-embryonic life.

The number of animals mouling with "regenerate and intermediate" was too small, and their distribution over the several schedules too scattered, for satisfactory tabulation of their mouling. In general, they tended to moult distinctly later than the animals producing "regenerate and papilla," so that
the delay in their moulting was intermediate between the age at the first and at the second operation.

Production of "two regenerates" was associated, in all the schedules, with a delay in moulting approximating the age of the animal at the second operation, regardless of its age at the first (Table 4). Operation during the first day of post-embryonic life gave rise to no anomaly, the time of moulting being determined by the second operation in all "two regenerates" material of schedules A and B.

<table>
<thead>
<tr>
<th>Operating Schedules</th>
<th>Numbers of Animals Surviving at the First Moul</th>
<th>Observed Delay in First Moul of Experimental Animals (days)*</th>
<th>Age of Experimental Animals at the First Operation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Experimental: 33  Controls: 90</td>
<td>1.28±0.15</td>
<td>0-1</td>
</tr>
<tr>
<td>D and E</td>
<td>Experimental: 94  Controls: 185</td>
<td>1.20±0.10</td>
<td>1-2</td>
</tr>
<tr>
<td>F, G, H</td>
<td>Experimental: 56  Controls: 124</td>
<td>2.21±0.11</td>
<td>2-3</td>
</tr>
</tbody>
</table>

* Computed by the method of Simpson and Roe (1939, pp. 192-3).

<table>
<thead>
<tr>
<th>Operating Schedules</th>
<th>Numbers of Animals Surviving at the First Moul</th>
<th>Observed Delay in First Moul of Experimental Animals (days)*</th>
<th>Age of Experimental Animals at the Second Operation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A and C</td>
<td>Experimental: 109  Controls: 129</td>
<td>2.37±0.10</td>
<td>2-3</td>
</tr>
<tr>
<td>B, D, F</td>
<td>Experimental: 279  Controls: 383</td>
<td>3.39±0.06</td>
<td>3-4</td>
</tr>
<tr>
<td>E, G, J</td>
<td>Experimental: 134  Controls: 235</td>
<td>4.20±0.10</td>
<td>4-5</td>
</tr>
<tr>
<td>H only</td>
<td>Experimental: 18   Controls: 54</td>
<td>4.81±0.20</td>
<td>5-6</td>
</tr>
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</table>

* Computed by the method of Simpson and Roe (1939, pp. 192-3).

IV. DISCUSSION

Omitting for the time being any consideration of the clearly exceptional animals moulting with "regenerate and intermediate," the bulk of the results reported can be discussed in the light of expectations derived from the "fresh start" hypothesis proposed by O'Farrell and Stock (1953), and on their assumption that regeneration interacts with the endocrine system controlling moulting, in such a way that there is a "critical period" in the moulting cycle, before which it can be subjected to a fresh start by regeneration and after which it
cannot be so modified. On these assumptions, then, the following results are to be expected from successive operations:

Should the first operation occur after the critical period, neither it nor the second operation should influence the timing of the first moult, at which papillae should appear on both sides. The difference in the time available between operation and moult for the healing of the cuticle etc. on the two sides should result in asymmetry of the two papillae, and that of side II should often show incomplete rounding off, with clotted blood indicating the presence of imperfectly healed cuticle on the trochanter at the moment of ecdysis. The “two papillae” results reported above accurately conform to these expectations. (A significant delay in the second moult of these animals cannot yet be clearly related to the general hypothesis under discussion, but might be expected in the light of observations on simultaneous regeneration (Stock and O'Farrell 1954). Such a delay, somewhat greater than in simultaneously regenerating animals, but not clearly related to the nature of the operating schedule, appears in Table 2. The resulting increase in the time available for growth and differentiation should enable side II to approximate towards symmetry with side I, as was in fact observed.)

If the first operation occurs before the critical period, it should set up a new moulting cycle dating from the day on which it is performed (except in animals undergoing operation during the first day of post-embryonic life, whose cycle will suffer an unexplained “excess” delay, as described in previous papers). Should the second operation then occur after the “secondary” critical period pertaining to this new cycle, it should not further influence the time of moulting. At the moult, a regenerate should appear on side I, and a papilla on side II, ecdysis being delayed by a period determined entirely by the age of the animal at the first operation. Table 3 shows the precise conformity with these expectations of the present results, even to the “excess” delay in moulting of schedule B material.

Where the second operation takes place before the onset of the “secondary” critical period set up by the first, a third or “tertiary” cycle should arise, associated with the appearance of two complete but more or less asymmetrical regenerates at the first moult. Assuming that the time relations of the “secondary” cycle are essentially similar to those of the original or “primary” cycle, the moult should then be delayed by a period approximating to the age of the animal at the second operation and uninfluenced by the timing of the first. Table 4 demonstrates the correctness of these expectations.

“Excess delay” in moulting, already observed in animals undergoing unilateral or simultaneous operations during the first day of post-embryonic life, appears in the present work only in “regenerate and papilla” material of schedule B, and not elsewhere. Apparently the “excess delay” does not persist through the “secondary cycle” to influence the “tertiary” one. This suggests a physiological equivalence, rather than a mere resemblance, between the effects on the moulting cycle of operations performed on the first and those done on the second day of post-embryonic life. Such an equivalence might result from the regeneration process failing to begin, or at least to take effect, until the second day.
Thus far, the results support the view that the "secondary cycle" not only exists, but represents a fresh start in time, a physiological "putting back the clock" such as can be explained most satisfactorily in terms of regeneration modifying the secretory rhythms of the neuro-endocrine complex controlling moulting.

After unilateral operation, the production of a regenerate requires a period approximating the normal duration of the instar (O'Farrell and Stock 1953). Successive operations leading to the appearance of "two regenerates" provide a longer time than this for development of the regenerate on side I, but those resulting in the production of "regenerate and papilla" clearly do not. In both categories, however, the rather constant size of the regenerate on side I is distinctly greater than that of regenerates resulting from either unilateral or simultaneous operations. Almost all the obvious variation observed is confined to side II in "two regenerates" material, where it is not recognizably related to the operating schedule nor to the time of moulting. These results suggest that neither of the successive regeneration processes exerts any inhibitory influence on the other. It further seems likely that the extra growth on side I may be related to physiological conditions associated with the early stages of the regeneration process on side II, since it is manifested in "regenerate and papilla" as well as in "two regenerates" material.

The foregoing discussion concerns the 98 per cent. of the experimental population conforming in the regeneration of both legs to the "all or nothing" principle of O'Farrell and Stock (1953). Exceptions to this principle occurred only on side II in the remainder of the experimental population. This provides the only justification for suggesting the existence of a real difference between the "primary" and the "secondary" moulting cycles. If the "secondary" critical period were somewhat less sharply defined than the "primary" one, a few individuals might undergo the second operation during a physiologically unstable phase. These animals would then moult with intermediates on side II, and at an age intermediate between the two expectations based on a strict application of the "all or nothing" principle. Table 1 suggests that this "secondary" critical period, although variable in its incidence, usually falls between 48 and 72 hr after the first operation. With a 65-hr interval between operations the proportion of intermediates obtained rose to 11 per cent., thus supporting this general interpretation. Even here, however, the occurrence of intermediates is rarer and less regular than would be expected if modification of the moulting cycle were the sole factor concerned. This leads to the suggestion that the extreme rapidity of conversion of the organized blastema into a visibly fully differentiated regenerate also reduces the probability of ecdysis occurring during the few hours in which the differentiating regenerate is in an intermediate condition. Such a hypothesis was put forward in partial explanation of the "all or nothing" principle by O'Farrell and Stock (1953), in preference to the idea that physiological (not necessarily visible) discontinuities arising in the hypodermis during a rapid differentiation process might inhibit moulting and hence prevent the appearance of intermediates. In the present work, a complete range of intermediates has appeared, such as could hardly arise if the process of differentia-
tion were associated with hypodermal disturbances sufficient to prevent moulting. A fairly satisfactory explanation of the absence of intermediates on side I and their rarity on side II seems to be provided by the interdependence between the moulting cycle, with its short critical period, and the regeneration process, with its brief period of differentiation.

There is still no explanation, however, of the observation that differentiation of the regenerate on side II proceeds proximodistally when it occurs before the moult, but distoproximally in animals moulting with "regenerate and papilla," where differentiation on side II is postponed until early in the second instar. The existence of this reversal suggests the possibility that different phases of the moulting cycle may have different effects on the same stage of the regeneration process. Further elucidation of such relationships depends on knowledge of the sequence of events involved in the normal regeneration process and in the moulting cycle.

The present work seems to provide experimental evidence for the suggestion that the process of regeneration falls into three distinct phases. The first stage, occupying probably 2-4 days under the conditions of the present work, is that of dedifferentiation, mobilization of material, and organization of the blastema at the biochemical, but not at the visible, level. A few hours of very rapid visible differentiation constitute the second phase, at the end of which the essential cuticular structure and probably most of the muscular differentiation of the regenerate is complete. The last phase of 2-3 days duration seems to be occupied only by minor differentiation and by growth.

These three phases closely resemble the three phases in the moulting cycle postulated from experiments on unilateral and simultaneous regeneration, viz. pre-critical period (2-4 days), critical period proper (very short, occurring usually on the third or fourth day of post-embryonic life) and post-critical or pre-moult period, ending with the first moult 2-3 days after the critical period. Moreover, it has now been shown (Wickham 1954) that the prothoracic glands in Blattella undergo a sharp burst of mitotic activity, lasting at most a few hours, immediately before the appearance of mitoses in the hypodermis signals the physiologically irreversible onset of the moult. This burst of mitotic activity in the prothoracic glands approximately coincides in timing with the experimentally deduced primary critical period, and is preceded and followed by periods of 2-3 days of inactivity. This parallelism between the observed phases of regeneration as such, the phases of the moulting cycle deduced from experiments on regeneration, and the observed mitotic cycle of the prothoracic glands, demonstrates the complex possibilities available for interaction between regeneration and the neuro-endocrine system controlling moulting.

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VI. REFERENCES


EXPLANATION OF PLATES 1 AND 2

All preparations are photographed from the anterioventral aspect. Scales indicate 0.5 mm.

PLATE 1

Fig. 1.—"Two papillae" from operating schedule J, showing: (A) rounded, blastema-filled papilla of side I, with blastema extending into distal part of coxal musculature; (B) coxal musculature on side I showing distinct signs of disorganization; (C) trochanter on side II, angular, imperfectly healed with clotted blood and no clearly defined blastema; (D) coxal musculature on side II; disorganization has scarcely begun.

Fig. 2.—"Two papillae" from operating schedule J, showing complete healing on both sides. Asymmetry of dedifferentiation less marked than in Figure 1.

Fig. 3.—Pair of regenerates appearing at the second moult after operating schedule J had led to production of "two papillae" at the first. Note the nearly complete symmetry of the regenerates.

Fig. 4.—"Regenerate and papilla" from operating schedule H. Fixed at ecdysis, aged 8 days. Note (X) rounded, blastema-filled papilla on side II; coxal musculature modified, especially in distal region; (Y) unmodified coxal musculature of regenerate on side I; (Z) reestablished musculature of trochanter on side I.

Fig. 5.—"Two regenerates" from operating schedule E. Regenerates markedly asymmetrical.

Fig. 6.—"Two regenerates" from operating schedule H. Regenerates almost completely symmetrical.

PLATE 2

Figs. 7-9.—Sequence of differentiation on side II during second instar of "regenerate and papilla" material from operating schedule E. Moulted at 7-8 days old, fixed on second day after moult (Figs. 7 and 8), third day after moult (Fig. 9). Distal-proximal differentiation apparent.

Figs. 10-18.—Representative series of stages in proximodistal differentiation of intermediates appearing on side II at the first moult of "regenerate and intermediate" material. Figure 10 moulted at 7·5, Figure 13 at 9·5 days old, the rest during the ninth day of post-embryonic life. Figures 10, 11, 14, 15, 17, and 18 all come from one batch undergoing operation schedule D. Figure 12 is from schedule D in another batch, Figure 13 from E, and Figure 16 from F.