REGENERATION AND THE MOULTING CYCLE IN *BLATTELLA GERMANICA* L.

II. SIMULTANEOUS REGENERATION OF BOTH METATHORACIC LEGS

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Summary

At 29°C, 70 per cent. relative humidity, simultaneous removal of both metathoracic legs at the proximal autotomy plane in first instar *Blattella germanica* results in the appearance at the first moult of either a symmetrical pair of differentiated regenerates or a symmetrical pair of undifferentiated papillae, but not of intermediate stages in differentiation. Papillae at the first moult are followed by differentiated regenerates at the second. Simultaneously produced regenerates are not distinguishable from those arising from unilateral operations.

The first moult of simultaneously regenerating animals is not delayed when a pair of papillae is produced, but a delay somewhat greater than the age of the animal at operation is associated with appearance of a pair of regenerates at the first moult. The second moult of simultaneously regenerating animals producing papillae at the first is delayed by about one day. Comparison with unilaterally regenerating animals shows a slightly but significantly increased delay in moulting related to simultaneous regeneration. This is probably due to increased demands for metabolites in producing two regenerates, added to the disturbance of endocrine balance already postulated from experiments on unilateral regeneration.

I. INTRODUCTION

Regeneration of one metathoracic leg during the first instar in *Blattella germanica* has been shown to follow an "all or nothing" principle, related to the existence of a "critical period" during the instar. Removal of the leg before this period leads to the appearance at the first moult of a highly differentiated regenerated leg with a tetramerous tarsus. Loss of the leg apparently subjects the instar to a fresh start, so that the moult is delayed by a period roughly equal to the age of the animal at the time of removal of the leg. No such delay results from removal of the leg after the "critical period," when an undifferentiated papilla appears at the first moult, and a regenerate not until the second.

In reporting these results, O'Farrell and Stock (1953) tentatively interpreted them in terms of a postulated interaction between regeneration and the endocrine balance involved in moulting. An alternative hypothesis discussed was that competition between regenerative and normal growth for metabolites other than hormones (e.g. structural or energy-producing materials) might be responsible for the observed delay in moulting, but this was considered less satisfactory.

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Assuming that both metathoracic legs can be regenerated simultaneously, further evidence should be obtainable by simultaneous removal of both of them, which would double the quantity of tissue to be regenerated. The present paper describes experimental work along these lines.

II. Material and Methods

The experimental methods used in this work were substantially those described by O'Farrell and Stock (1953). Since it was thought necessary to work on large numbers of animals under one set of physical conditions such that the first instar of controls would be completed in about 6 days from hatching, all cultures and experimental animals were kept 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.).

Simultaneous removal of both metathoracic legs at the proximal autotomy plane was carried out on each of the first 4 days of post-embryonic life, using a total of about 1000 experimental animals and 1100 controls. A further 200 animals subjected to removal of only one metathoracic leg provided a direct comparison between the effects of unilateral and simultaneous operation in the same material and under the same conditions.

III. Results

Locomotion, although modified, was not found to be apparently impaired by the absence of both metathoracic legs, at least during the first and second instars, the period covered by these experiments. It appeared that simultaneous regeneration of both metathoracic legs took place during the first instar in much the same way as the regeneration of one. Operations late in the instar gave rise to a symmetrical pair of undifferentiated papillae, appearing at the first moult (Plate 1, Fig. 1), while those early in the instar led to the appearance at the first moult of a symmetrical pair of well-differentiated regenerates with tetramerous tarsi (Plate 1, Fig. 2). In only two instances did any structure suggesting an intermediate condition between papilla and regenerate appear externally at the moult, taking the form on both occasions of a pair of rather elongate and irregularly shaped papillae with what may have been signs of incipient segmentation. Such rare exceptions to the "all or nothing" principle were regarded as probably due to accidental injury and did not seem to warrant the suggestion that the principle is not operative in simultaneous regeneration.

Variations between individual animals in the size and minor morphological detail of simultaneously produced regenerates were observed, but did not seem to be consistently related to the timing of the operation or the moult. Further, no consistent morphological or size difference was noticed between the simultaneously produced regenerates and those resulting from unilateral operation, an example of which is shown for comparison in Plate 1, Figure 3. Similar general statements apply to the symmetrical pair of highly differentiated regenerates appearing at the second moult (Plate 1, Fig. 4), following production of a sym-

metrical pair of undifferentiated papillae at the first moult. Symmetry in size and differentiation appeared to be characteristic of all simultaneously produced pairs of regenerates or papillae.

TABLE 1

FIRST MOULT OF ANIMALS PRODUCING REGENERATES AFTER SIMULTANEOUS REMOVAL OF BOTH METATHORACIC LEGS, COMPARED WITH THAT OF THE CORRESPONDING CONTROLS AND OF UNILATERALLY REGENERATING ANIMALS

Age at Operation (days)	Mean Age (days) and Numbers Surviving at First Moult of		Mean Age Difference (days) at First Moult Between	
	Controls	Animals Undergoing Simultaneous Operation	Controls and Simultaneously Regenerating Animals*	Simultaneously and Unilaterally Regenerating Animals*
0-1	6·38±0·06 (114)	7·58±0·05 (77)	1·20±0·08	0·04±0·12 (27, 39)
1-2	6·37±0·04 (348)	8·26±0·03 (339)	1·89±0·05	0·29±0·07 (110, 103)
2-3	6·30±0·04 (415)	9·03±0·04 (331)	2·73±0·05	0·64±0·11 (82, 55)
3-4	6·48±0·11 (51)	10·52±0·12 (27)	4·04±0·16	No data

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

The time relations observed between unilateral and simultaneous operation, regeneration, and moulting are summarized in Tables 1 and 2. The appearance of regenerates at the first moult, whether resulting from unilateral or from simultaneous operation, was associated with a delay in the moult approximating the age of the animal at operation (Table 1). Operations performed on the first day of post-embryonic life, however, produced a delay in the moult exceeding 1 day; this recalls the similar results reported for unilateral regeneration by O'Farrell and Stock (1953). On the fourth day of post-embryonic life, operations resulted in the appearance at the first moult (which was not delayed) of papillae in the great majority of animals (Table 2); only a few exceptionally slow-growing batches produced regenerates at a delayed first moult (Table 1). Similarly, the majority of batches undergoing operation on the third day of post-embryonic life produced regenerates at a delayed first moult (Table 1), and papillae appeared at an undelayed first moult only in exceptionally fast-growing material (Table 2).

It is clear from Table 1 that there was a considerable similarity between the moulting of unilaterally and simultaneously regenerating animals, apparently amounting to identity for those undergoing operation on the first day of post-embryonic life. Operations performed on the second and third days of post-

embryonic life, however, gave rise to the apparently highly significant differences between singly and simultaneously regenerating animals shown in Table 1, column 5, where figures in parentheses show, for each day of operation, the respective numbers of simultaneously and unilaterally regenerating animals which were available for the purposes of this comparison.

No direct comparison between singly and simultaneously regenerating animals producing papillae at the first moult appeared to be necessary. Table 2 shows that the first moult in animals producing papillae, after undergoing simultaneous operations on either the third or fourth day of post-embryonic life, was not delayed in comparison with that of the corresponding controls. Their second moult, at which regenerates appeared, was significantly delayed by comparison with that of the controls (Table 2, column 6).

TABLE 2

FIRST AND SECOND MOULTS OF ANIMALS PRODUCING PAPILLAE AT THE FIRST AND REGENERATES AT THE SECOND MOULT, AFTER SIMULTANEOUS REMOVAL OF BOTH METATHORACIC LEGS, COMPARED WITH THOSE OF THE CORRESPONDING CONTROLS

Age at Operation (days)	Mean Age (days) and Numbers Surviving				Mean Difference (days) at Second Moult, Between Controls and Experimental Animals*
	At First Moult		At Second Moult		
	Controls	Experimental Animals	Controls	Experimental Animals	
2-3	5·85±0·05 (122)	5·76±0·06 (60)	11·56±0·09 (102)	12·41±0·14 (45)	0·85±0·17
3-4	6·29±0·06 (137)	6·09±0·05 (128)	11·47±0·16 (78)	12·57±0·08 (81)	1·10±0·13

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

IV. DISCUSSION

The results here described demonstrate the possibility of regeneration, within one instar, of both metathoracic legs after their simultaneous removal at the proximal autotomy plane. Such simultaneous regeneration conforms to the general pattern of the "all or nothing" principle and associated "critical period," already described for unilateral regenerations by O'Farrell and Stock (1953). Further, the regenerates produced do not differ in any obvious respect from those arising from unilateral operations, and the members of a pair are symmetrical with respect to one another, so that they conform *as a pair* to the "all or nothing" principle. Hence there seems to be some justification for concluding that the pair of regenerates appearing after a simultaneous operation represent approximately twice as much expenditure of structural material and metabolic energy as would be required by regeneration of a single member of the pair at the same time in the instar.

If this conclusion be accepted, and it is postulated that the delay in the first ecdysis imposed by production of a regenerate is primarily due to the draining off by regeneration of certain quantities of material otherwise available for general growth, then simultaneous regeneration might be expected to produce something approaching twice as great a delay in moulting as results from unilateral regeneration. In fact, as Table 1 shows, simultaneous regeneration produces a significantly greater delay in moulting than unilateral regeneration, but the magnitude of this difference is small in comparison with the postponement of moulting, in both cases, by a period approximating the age of the animal at operation. In short, the increased delay in moulting resulting from simultaneous regeneration is not proportionate to the doubling of the quantity of tissue to be produced. Such a proportion, however, may possibly be realized in the delayed second moult of animals producing papillae at the first moult. This delay is significant for simultaneously regenerating animals (Table 2, column 6), but was not found to be statistically confirmable in singly regenerating material by O'Farrell and Stock (1953).

Except for operations performed on the first day of post-embryonic life then, the present work suggests that simultaneous regeneration always results in a slight but definite increase in the delay in moulting observed for unilateral regeneration. Increased blood loss and/or a more profound wound shock resulting from the loss of two legs instead of one may possibly be responsible for this. However, this interpretation is difficult to justify for the results of operations performed after the critical period (when the delay in moulting is transferred to the next instar) or on the first day of post-embryonic life (when both singly and simultaneously regenerating animals show an identical but unexpectedly prolonged delay in moulting). Moreover, the data as a whole appear rather more internally consistent than would be expected if the time of moulting were being seriously affected in experimental animals, but not controls, by uncontrolled variables such as blood loss or wound shock.

On the whole, the present results appear to support an interpretation in which a major part is played by disturbance of the endocrine balance on the lines already suggested by O'Farrell and Stock (1953). A minor part, represented by the slightly increased delay in moulting of simultaneously regenerating animals, may be ascribed either to blood loss and wound shock, or, perhaps more plausibly, to the effects of successful competition by regeneration for the available limited supplies of structural or energy-producing materials, or both.

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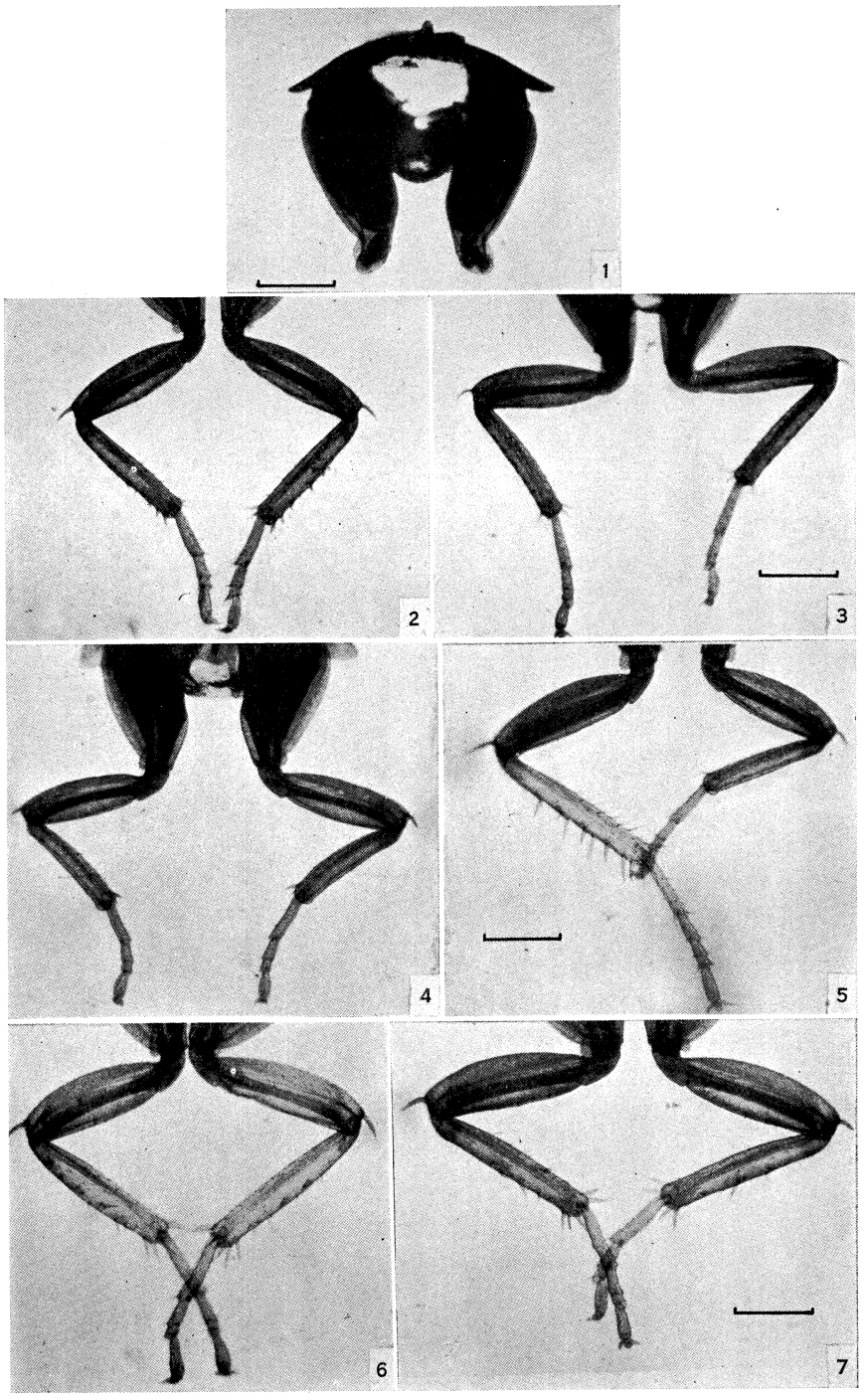
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VI. References

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Explanation of Plate 1

Blattella germanica L.

All preparations fixed in alcoholic Bouin, stained in alcoholic light green, and photographed from the anteroventral aspect. Scales indicate 0·5 mm.

Fig. 1.—Symmetrical pair of smoothly rounded papillae appearing at the first moult after simultaneous removal of both metathoracic legs on the fourth day of post-embryonic life. Fixed on the fourth day of the second instar to show advanced differentiation within the old coxae, and extending into the papillae, of the regenerates due to appear externally at the second moult.

Figs. 2, 3.—Symmetrical pairs of regenerates appearing at the first moult after simultaneous removal of both metathoracic legs (Fig. 2) on the second, and (Fig. 3) on the fourth day of post-embryonic life.

Figs. 4, 5.—Regenerates appearing at the first moult after operations performed on the third day of post-embryonic life, (Fig. 4) after simultaneous removal of both metathoracic legs; (Fig. 5) after removal of the left metathoracic leg only.

Figs. 6, 7.—Symmetrical pairs of regenerates appearing at the second moult, following the appearance of papillae at the first moult, in animals subjected to simultaneous removal of both metathoracic legs on (Fig. 6) the third, and (Fig. 7) the fourth day of post-embryonic life.