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

I. SINGLE REGENERATION INITIATED DURING THE FIRST INSTAR

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Summary

The effects of removal of the left metathoracic leg of *Blattella germanica* at the proximal autotomy plane, i.e. between trochanter and femur, are described from operations on animals at known ages in the first instar, at temperatures of 25 and 30°C, 70-80 per cent. R.H. Either a completely differentiated regenerate or an undifferentiated papilla, but *never* an intermediate, appears at the first moult after operation. Where a papilla is produced the complete regenerate appears at the second moult after operation. Ecdysis is not delayed by production of a papilla, but is delayed by production of a complete regenerate. The interval between the operation and the first moult then approximates to the duration of the first instar of controls, being greater with operations very early in the instar and less with later ones.

There is a "critical period" in the first instar, before which operation gives rise to a regenerate with delay in the first ecdysis, and after which it results in production of a papilla without delay in ecdysis. Delay in the first ecdysis resulting from regeneration appears to be compensated by a subsequent speeding up of the moulting cycle, the ages of operated animals at their fourth ecdysis being approximately the same as those of controls. Hypotheses are discussed relating these results to endocrine control of the interaction between regeneration and the moulting cycle. The possibility of using regenerating *Blattella germanica* in experimental work on moulting and metamorphosis is suggested.

I. INTRODUCTION

Although one of the earliest studies on regeneration of an insect leg was made by Brindley (1897, 1898) with the cockroach *Blatta orientalis*, the Blattidae have since received little attention from workers in this field. Morphological features and comparative growth rates of regenerating and normal legs have been studied by Woodruff and Seamans (1939) in *Blattella germanica,* and by Voy (1949, 1951) in *Blatta orientalis.* A general, but irregular, increase in the total number of moults undergone by regenerating animals was suggested by Zabinsky (1936) for *Periplaneta,* and confirmed by Seamans and Woodruff (1939) for *Blattella.* Much work on Phasmidae and Mantidae, e.g. Przibram (1931), and the recent study by Luscher (1948) on *Rhodnius* (Hemiptera) suggest that, at the first ecdysis after an operation leading to regeneration of a leg or part of a leg, the regenerate reaches a level of differentiation largely dependent on the time elapsing between the operation and the next ecdysis.

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Voy (1951), however, reported that *Carausius* will produce a differentiated but incomplete regenerate if operated on before a certain critical stage in an instar, but at most a blastema within the old stump if operated on after this stage. However, little attention seems to have been paid to the relationship between regeneration and the moulting cycle. The present paper describes some investigations on *Blattella germanica* indicating the existence of such effects.

II. Materials and Methods

Cultures were kept at 25°C in a constant-temperature room or at 30°C in an incubator, in both of which the relative humidity was controlled approximately within the limits 70-80 per cent. R.H. Each culture container was provided with a vial of water loosely plugged with cotton wool. Crumpled filter papers provided shelter for the animals, which were fed on a mixture of equal weights of cane-sugar and "Bemax" (a proprietary wheat germ preparation) ground up together. The food was placed in a cone of filter paper, since Woodruff and Seamans (1939) found that operated animals had difficulty in climbing out of glass food receptacles. Cultures were cleaned regularly and food, water, and shelter were renewed.

Small groups of females bearing oothecae were isolated at intervals from the mass cultures and observed daily; at each observation any newly hatched animals were counted out in as random a fashion as possible into groups of approximately 10 individuals, each such group being placed in an experimental container consisting of a muslin-covered glass cup of about 200 ml capacity, provided with shelter, food, and a water-tube as described above. From one-third to one-half of the groups from a given batch were taken at random as control material, and the rest were used for operations performed on one or more of the first few days of post-embryonic life. Single oothecae produced from 12 to 42 individuals, the usual number being about 30. Several batches treated as units for experimental purposes, however, included animals hatched on the same day from two or more oothecae.

The operation consisted of removal of the left metathoracic leg at the proximal autotomy plane, i.e. between trochanter and femur, by gentle pulling of the femur with fine forceps. This technique gives remarkably uniform results, which would probably be difficult to achieve by cutting the leg. Each operated group, with the control animals of the same batch, was then examined daily until all had moulted at least once. Operations were performed on each of the first 4 days of post-embryonic life at 30°C on several groups of different origin. A similar procedure was adopted to cover the first 6 days of post-embryonic life at 25°C. Only a few operations were performed after the sixth day at 25°C, as preliminary work had shown that for the present purpose little additional information was gained by operations very late in the instar, which had a duration in the controls of about 5 days at 30°C and 10 days at 25°C.

Blattella germanica is too active at ordinary room temperatures for easy handling and accurate observation, especially of young animals. Hence, pairs of cups, one containing operates and the other the corresponding controls, were put together into a desiccator in which they were exposed to ether vapour for

a few minutes before operation or observation, or both. This method was found preferable to carbon dioxide anaesthesia for the rapid and convenient handling of large numbers of individual small containers arranged in pairs. Although Chauvin (1945) stated that ether causes very high mortalities in *Blattella germanica,* there was no indication of this in the present work. The mean duration of the first four instars of controls examined daily under ether did not differ significantly from that observed in animals in another investigation (Monro, unpublished data), which were not anaesthetized at all. The intervals between moults were similar and the etherized animals were no less viable than the others.

At 25°C, but not at 30°C, however, certain hatchings, whether examined under ether or not, showed considerable mortality in both operates and controls. In a given hatching, such mortality seldom appeared random, but usually affected all or most animals in a particular container. Death was often preceded by a characteristic "dwarfish" appearance, usually recognizable within a day or two of hatching, if not at the time of hatching; this might persist for weeks before death occurred, but no moulting took place. A few recoveries were observed, but the affected animals were so delayed and erratic in their first ecdysis as to be useless for the purpose of the present work. Hence it took a considerable time to assemble a sufficient body of reliable data for animals at 25°C in which none of the operated or control groups showed signs of "dwarfism," which may be due to some form of infection affecting growth and metabolism.* The high death rate affecting both operated and control animals, occurring in some but not all of their hatchings, reported by Woodruff and Seamans (1939), may perhaps have been due to a similar phenomenon.

III. Experimental Results

(*a*) *Morphological Appearance of Regenerating Legs at the First and Second Ecdyses*

Although the operation performed involved detachment of the leg at a natural "plane of weakness" across the articulation between the trochanter and femur, this articulation is traversed by a muscle attached distally in the femur and proximally to the median wall of the trochanter (Plate 1, Fig. 1). Detachment of the femur involves tearing out this muscle. The muscle always breaks free at its proximal end, and this presumably occurs also in the frequent instances of loss of a leg without experimental interference.† The amount of damage produced in the trochanter by the operation seems to be quite uniform, although a somewhat variable amount of bleeding seems to occur before the initial closure of the wound by clotted blood. The missing region of the leg

* We are indebted to Professor O. W. Tiegs, F.R.S., for drawing our attention to the presence of a microsporidian infection in the fat-body in material from our mass cultures.

† Woodruff (1937) referred to "autospasy" in *B. germanica.* This term was used by Wood (1926) for the casting of a limb when pulled by some outside agent, as distinct from the reflex shedding of the appendage implied in the term "autotomy." Our observations leave us uncertain which term is strictly applicable to the mechanism operating in *B. germanica,* and the more familiar term "autotomy" is therefore retained.

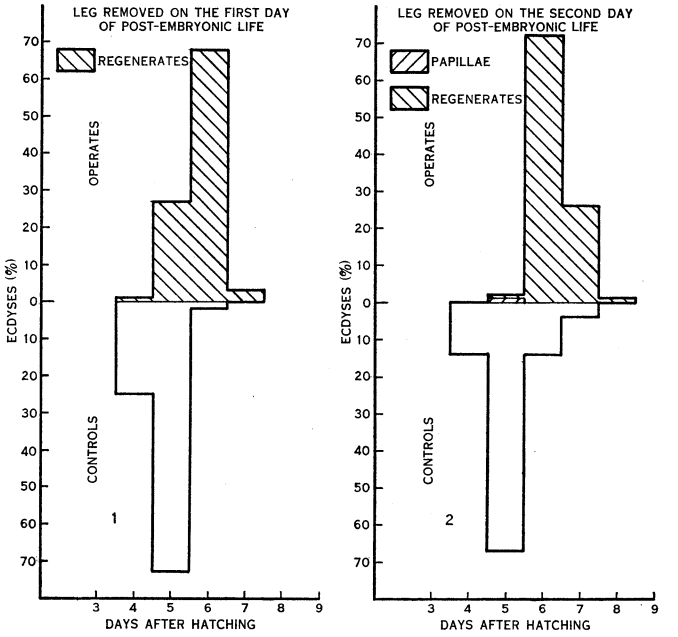
is regenerated within the coxa, and when fully developed the regenerated tarsus extends into the empty space within the trochanter, coming into contact with the blood clot (Plate 1, Fig. 2).

When ecdysis occurs within a few hours after operation at 30°C, the blood-clotted trochanter persists more or less unchanged in outward appearance through the next instar, and a fully differentiated regenerate appears at the second moult after the operation. Lapse of a longer period between operation and ecdysis permits a varying amount of closure of the wound by newly formed cuticle, apparently at the expense of some resorption of tissue, so that animals moulting more than 24 hr after operation have the trochanter replaced by a more or less well-rounded papilla, typically smaller than the normal trochanter, with little or no trace of clotted blood (Plate 1, Fig. 3). This condition persists through the ensuing instar, and at the second ecdysis after operation a fully differentiated regenerate appears. Such papillae occur at 30°C when the interval between the operation and the next ecdysis does not exceed 3 days (exceptionally 4 or 5 days), and at 25°C when this interval does not exceed 6 (exceptionally 7-9½ days. At both temperatures, the papillae were observed to vary considerably in size, and slightly in general shape and in the number and arrangement of setae present. These variations were not recognizably related to the timing of operation, except insofar as some of the papillae appearing an unusually long time after operation, mentioned above as exceptional occurrences, were rather larger than usual and seemed to contain a somewhat more differentiated blastema. They did not, however, resemble an intermediate stage in differentiation between the papilla and the fully formed regenerate, being simply large papillae without any external differentiation.

Fully differentiated regenerates (Plate 1, Fig. 4), consisting of femur, tibia, and four tarsomeres with a pretarsus, appear at the first ecdysis after operation when the interval between the operation and the ecdysis exceeds 4 days at 30°C, or 7-8 days at 25°C. Such regenerates are obviously smaller than the corresponding normal leg of the same animal, although in our material they were seldom less and often considerably more than two-thirds of its length. (Biometrical information on this point was provided by Woodruff and Seamens (1939), who also showed that the regenerate approximates more closely to the size of the normal leg at successive ecdyses after its first appearance.) While a detailed account of the morphology and histology of regenerates and papillae will be given in a future paper, it is necessary to mention here that the regenerates observed by us varied appreciably in morphological detail (e.g. arrangement of musculature, size and number of spines and setae) as well as in size. But the differences from the normal leg were confined to these points and to the presence of only four instead of five tarsomeres. All the gross features of the normal leg were well differentiated and clearly recognizable even in the rare instances of gross abnormality, e.g. reduplication. Nothing resembling an intermediate structure between the papilla and the fully differentiated regenerate was ever observed, and there seemed to be no relationship between the size or differentiation of regenerates and the timing of operation. These general

statements apply also to regenerates appearing at the second ecdysis after operation, following production of a papilla at the first.

In short, there appears at the first ecdysis after operation *either* a papilla *or* a fully differentiated regenerate, but *never* an intermediate condition, and an "all or nothing" principle appears to be involved.



Figs. 1, 2.—Daily frequency of first ecdysis in regenerating *Blattella germanica* and in the corresponding controls, expressed as percentages of total number of operates or controls used in each series of experiments, 30°C, 70-80 per cent. R.H.

(*b*) *Effect of Regeneration on the First Post-operational Moult*

Both at 25 and 30°C, production of a regenerate at the first ecdysis after operation leads to this ecdysis being delayed, in comparison with that of the corresponding controls, by a period roughly equal to the age of the animals at the time of operation. No such delay occurs when only a papilla is produced at the first post-operational ecdysis. At both temperatures, operations on the second day of post-embryonic life or later, if they result in production of a regenerate at the next ecdysis, are separated from that ecdysis by a mean time interval slightly less than the mean duration of the first instar in the corresponding controls. This time interval is, however, distinctly greater than the duration of the control first instar for operations performed on the first day of post-embryonic life. These results are summarized for 30°C in Figures 1-4 and Table 1, and for 25°C in Table 2. The data are based on daily counts of the number of animals which had moulted at any time during the 24 hr preceding each such count. In recording the data, no distinction was made

TABLE 1

MOULTING OF REGENERATING ANIMALS AFTER OPERATIONS AT VARIOUS AGES IN THE FIRST INSTAR AT 30°C, 70-80 PER CENT. R.H.

Age at Operation (days)	Number of Animals Used		Numbers and Mean Ages (days) at First Ecdysis of Animals Producing Papillae and Regenerates, and of the Corresponding Controls			
	Operates	Controls	Papillae	Controls	Regenerates	Controls
0-1 (Fig. 1)	81	80	0	—	81 6·03±0·06	80 5·26±0·05
1-2 (Fig. 2)	147	135	1 (5·5?)	—	146 6·77±0·04	135 5·57±0·06
2-3 (Fig. 3)	126	120	90 5·13±0·07	120* 5·27±0·06	36 7·72±0·07	89* 5·52±0·07
3-4 (Fig. 4)	96	91	88 5·22±0·09	91* 5·27±0·10	8 8·63±0·21	56* 5·57±0·12

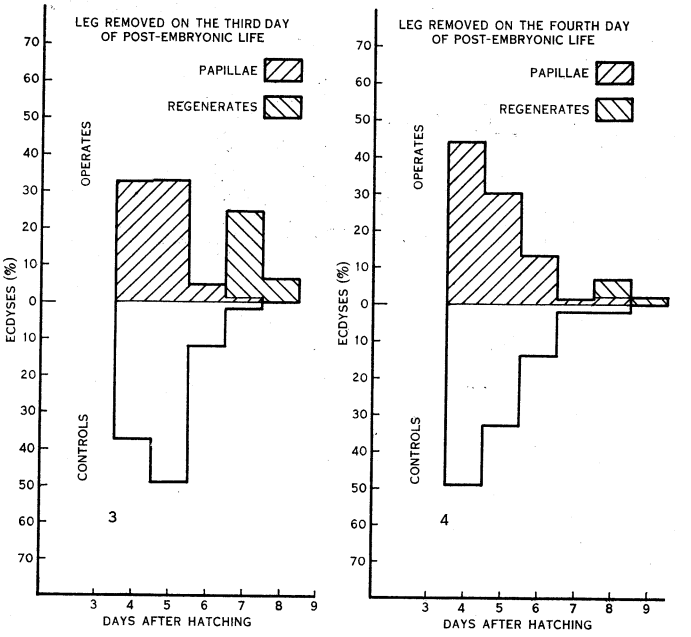
TABLE 2

MOULTING OF REGENERATING ANIMALS AFTER OPERATIONS AT VARIOUS AGES IN THE FIRST INSTAR AT 25°C, 70-80 PER CENT. R.H.

Age at Operation (days)	Number of Animals Used		Numbers and Mean Ages (days) at First Ecdysis of Animals Producing Papillae and Regenerates, and of the Corresponding Controls			
	Operates	Controls	Papillae	Controls	Regenerates	Controls
0-1	22	21	0	—	22 12·0±0·2	21 10·3±0·2
1-2	76	60	0	—	76 11·4±0·1	60 11·2±0·15
2-3	65	67	0	—	65 11·6±0·15	67 9·8±0·15
3-4	85	99	4 9·8±0·5	—	81 13·0±0·1	99 10·2±0·1
4-5	61	49	37 9·4±0·2	49* 9·6±0·2	24 14·7±0·2	34* 10·4±0·1
5-6	70	66	64 9·8±0·2	66* 9·6±0·1	6 15·7±0·3	34* 10·3±0·1

* These figures in Tables 1 and 2 refer to the number of control animals originating from the same hatchings as the operated animals. Since all batches of experimental animals operated at or after the "critical period" produced papillae in at least a proportion of individuals, the figure appearing for controls is equal to the total number of controls used. Regenerates, however, were produced only by a proportion of the late-operated batches, and the figures in the last column refer only to the controls of these batches, which also contribute to the total given in the fifth column of the tables.

between pale, freshly moulted individuals and those which had already hardened and darkened completely at the time of observation. Since the process of hardening and darkening occupies some hours, such a distinction would have necessarily been very arbitrary and would scarcely have improved the precision of the information obtained. The variability of the material was such that it is also doubtful whether any gain would result from shortening the interval between counts to less than the 24 hr here adopted.



Figs. 3, 4.—As for Figures 1 and 2.

Re-examination of these results shows that very few operated animals moulted on the fourth day after operation at 30°C, or on the sixth to eighth days after operation at 25°C, irrespective of the timing of the operation in relation to the duration of the instar in the controls, or of whether a regenerate or a papilla was produced. Figures 5 and 6 clarify this point, by presenting all the available data for 30°C and 25°C respectively, plotted, regardless of age at operation, with the day of operation as the zero of the time scale. Thus the "all or nothing" regeneration appears to be the result of an inhibition of ecdysis during the period in which differentiation of the regenerate from the initial blastema is taking place. A preliminary study of whole-mount preparations suggests that this period of differentiation is of fairly short duration, and the experimental data indicate that it occupies about 1 day at 30°C and 2 or 3 days at 25°C. It further appears that there is a brief "critical period," rather less than half-way through the instar. Operations performed before this period lead to appearance at the next ecdysis of a fully differentiated regenerate, the ecdysis being inhibited until differentiation of the regenerate is complete.

Operations after the "critical period" result in the appearance of a papilla at the next ecdysis, which is not delayed.

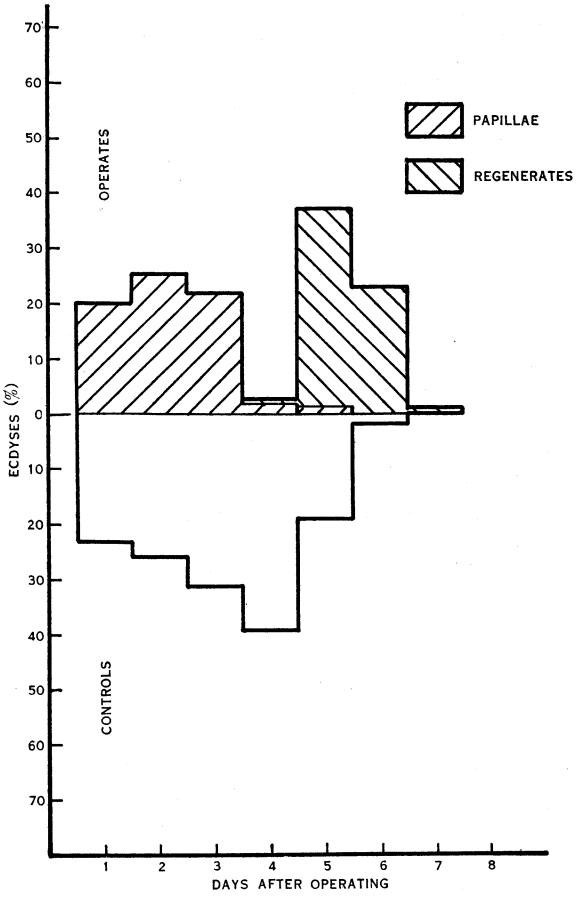


Fig. 5.—Daily percentage frequency of first ecdysis of all operated and control animals at 30°C, 70-80 per cent. R.H., plotted with day of operation as zero on the time scale.

One cannot, of course, predict by inspection whether a given batch, undergoing operation at or about the estimated time of the "critical period," will have controls moulting earlier or later than the average. Early moulting by the controls will indicate that the "critical period" was already over at the time of operation in most of the operated animals, which will therefore produce papillae. This explains the apparent association in Tables 1 and 2 between the production of papillae by operates and early moulting of the corresponding controls, which may be treated as spurious for the purpose of interpreting the present results. The most satisfactory results obtained were those in which the operation coincided with the "critical period." The natural variability of the material then resulted in a clear-cut split in the timing of ecdysis in the

operated animals, some of which moulted simultaneously with the controls and produced papillae, while others moulted much later than the controls and produced fully differentiated regenerates. Such a division within a single batch of uniform origin was observed at 30°C in 10 batches and at 25°C in seven batches. The few observed instances of moulting at an abnormal time (see above) occurred mainly in these batches, as might be expected if the critical period is itself of very short duration and primarily responsible for the "all or nothing" regeneration.

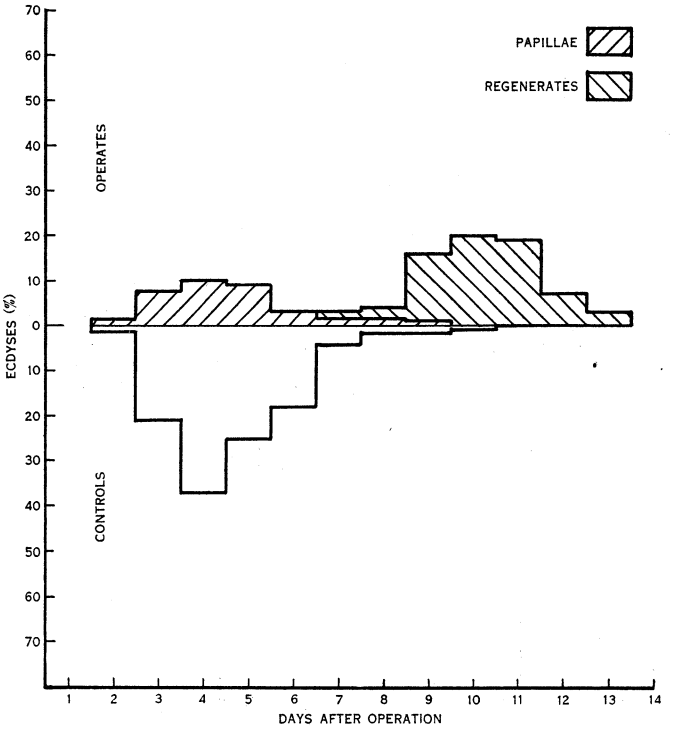


Fig. 6.—Daily percentage frequency of first ecdysis of all operated and control animals at 25°C, 70-80 per cent. R.H., plotted with day of operation as zero on the time scale.

(*c*) *Effect of Regeneration on Ecdyses After the First*

Figure 7 summarizes the data obtained from 12 batches, comprising 160 operates and 156 controls, in which operations were performed on either the first or second day of post-embryonic life, and daily observations continued until all surviving animals (118 operates and 114 controls) had completed their fourth ecdysis. All the operates produced fully differentiated regenerates at the first ecdysis, which was significantly delayed in comparison with that of the controls. The fourth ecdysis, however, although spread over a rather long period, seemed to be almost identical in timing for operates and controls.

Similar data are presented in Figure 8 for five batches (seven oothecae) including 102 operates and 103 controls, in which operations on the third and fourth days of post-embryonic life resulted in production of papillae at the first ecdysis by all but two of the operated animals. These ecdyses with papillae were simultaneous with those of the corresponding controls; regenerates appeared only at the second moult, which was slightly, but not significantly, delayed in comparison with that of the controls. The fourth ecdysis, survived by 43 operates and 43 controls only, was apparently identical in timing for both groups.

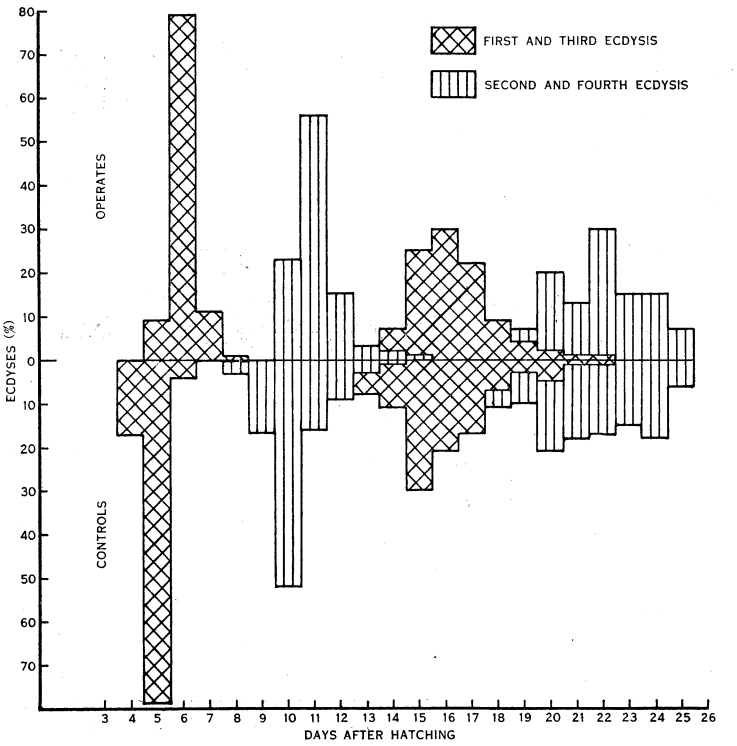


Fig. 7.—Daily percentage frequency of the first four ecdyses of operated animals producing regenerates at their first ecdysis, and of the corresponding controls. 30°C, 70-80 per cent. R.H.

Table 3 interprets these data in terms of mean duration of the first four instars. The wide spread and erratic incidence of the third and fourth ecdyses in particular make it impossible to confirm these figures statistically, since the data do not provide an adequate indication of the statistical identity of the operated and control populations at the fourth ecdysis. The experience of the authors with the rearing of *B. germanica,* together with some indications derived from preliminary work on a limited scale at 25°C, leads them to the provisional interpretation of the results in terms of a speeding up of the moulting cycle in operated animals whose ecdysis has been delayed by production of a regenerate. This would lead to the moulting of the operates "catching

up" with that of the controls. Larger numbers of animals, with less erratic moulting, would be required to permit statistical evaluation of this suggestion.

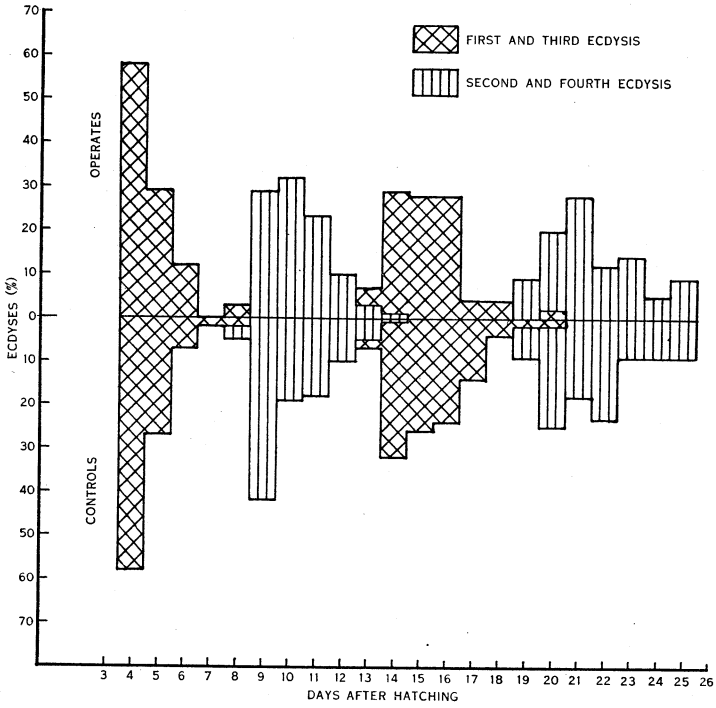


Fig. 8.—Daily percentage frequency of the first four ecdyses of operated animals producing papillae at their first ecdysis, and of the corresponding controls. 30°C, 70-80 per cent. R.H.

IV. Discussion

(*a*) *"All or Nothing" Regeneration*

In most arthropods so far studied, including insects, intermediate stages in the differentiation of regenerating appendages can be observed. In Crustacea, this often seems to occur without the intervention of a moult, the onset of differentiation being determined more or less independently of ecdysis, perhaps by re-innervation of the blastema (Needham 1945). But in insects the successive stages in differentiation usually appear at successive moults, the details of the process varying with the time of operation, and with the capacity for regeneration of the species concerned. This gradual type of regeneration is best known in *Carausius* (e.g. Heldmann 1929; Friedrich 1930), and probably occurs in many other insects, e.g. in *Rhodnius* (Luscher 1948). In such insects, an undifferentiated structure, with or without a blood clot, appears at the first post-operational moult when the operation takes place so late in the instar that the cuticle of the next instar has already begun to form, as shown in *Carausius* by Voy (1951) and in *Rhodnius* by Luscher (1948). But an "all

or nothing" type of regeneration like that of *B. germanica* does not seem to have been described in arthropods, except perhaps for the observations of Cameron (1926) on the myriapod *Scutigera*.

It is worth noting that Brindley (1897, 1898), in describing regenerates obtained from operations on many individuals of *Blatta orientalis* of unknown ages, does not seem to have encountered anything that could be regarded as intermediate between the papilla and the fully differentiated regenerate as defined here. If such intermediates do not occur in *B. orientalis* it would dispose of the suggestion that the peculiarities of regeneration in *Blattella germanica* are due to its high growth rate and brief intermoult periods under the conditions of our experiments; for *B. orientalis* grows far more slowly and has far longer intermoult periods than *B. germanica* (Laing 1946).

TABLE 3

DURATIONS OF FIRST FOUR INSTARS IN OPERATED AND CONTROL ANIMALS AT 30°C, 70-80 PER CENT. R.H.

		Mean Duration of Instar (days)				Mean Age at Fourth Ecdysis (days)
		I	II	III	IV	
Operated animals	Producing regenerates at first ecdysis	6·5	5·1	5·2	5·8	22·6
	Producing papillae at first ecdysis	5·1	5·6	5·0	6·4	22·1
All controls		5·25	5·4	5·6	6·05	22·3

It is possible, however, that the "all or nothing" effect results from the ability to produce a fully differentiated regenerate in something less than the duration of an instar. Here, the processes of histolysis and histogenesis involved are likely to be much more drastic than in regenerations taking several instars to complete (irrespective of the actual lapse of time involved). If so, the cuticle of the old coxa may play a part analogous to that postulated by Hinton (1948) for the pupal cuticle of Holometabola, and the inhibition of ecdysis may arise partly from epithelial discontinuities (Wigglesworth 1950, p. 45) produced by the extreme activity of the regenerating hypodermis, especially in the formation of new muscle attachments and apodemes. But this explanation is not wholly satisfactory in view of Luscher's (1948) observation that moulting in regenerating *Rhodnius* seems to depend upon re-establishment of cuticular continuity, but appears to be independent of the stage of differentiation attained by the regenerate. Moreover, the results here described suggest that production of a fully differentiated regenerate at the first ecdysis after operation subjects the whole moulting process to a fresh start, the ecdysis being delayed by a period roughly equal to the age of the animal at the time of operation. There is also some evidence that subsequent ecdyses are affected. No such

effects are associated with the production of a papilla at the first post-operational ecdysis, whether the cuticular continuity over the papilla has been re-established or not. Finally, the regular and predictable relationship of papillae and regenerates to the "critical period," in contrast to the fact that their variation in size and structure is unrelated to the timing of the operation, render an interpretation solely in terms of temporary epithelial discontinuity during regeneration rather unsatisfactory, and it seems desirable to consider possible relationships between regeneration and the endocrine balance controlling moulting.

(*b*) *Regeneration and the Endocrine Balance Involved in Moulting*

Current theories on the control of moulting and metamorphosis in insects (e.g. Scharrer 1946, 1948, 1952; Wigglesworth 1948*a*, 1948*b*, 1950, 1952*a*, 1952*b*; Novak 1951*a*, 1951*b*; Williams 1952) postulate the operation of an interacting hormone system under the control of the brain. Major roles are played by the "juvenile" hormone from the corpora allata, and by the "moulting" or "growth and differentiation" hormone. The latter originates from thoracic glands, under the influence of the brain, in Lepidoptera (Williams 1947), in *Rhodnius* (Wigglesworth 1952*a*), and in the cockroach *Periplaneta* (Bodenstein, quoted by Wigglesworth 1952*a*). Thoracic glands are present in the cockroach *Leucophaea* (Scharrer 1948) and have been observed in *Blattella germanica* in our laboratory (Wickham, unpublished data 1952) but their function has not been demonstrated experimentally.

Much of the experimental work on which these theories are based involves "all or nothing" responses, e.g. in extirpation of the corpora allata in *Leucophaea* (Scharrer 1946). The corpora allata are necessary to successful differentiation of regenerates in *Carausius* (Pflugfelder 1939). It therefore seems reasonable to assume that the "critical period" here described for regeneration in *B. germanica* bears some relation to the critical period found in experimental studies of the functions of the corpora allata and thoracic glands. The secretory cycles of these organs are usually minimal about the time of ecdysis, and reach their peak somewhere about the time in the instar at which the changes in the hypodermis leading to formation of a new cuticle are initiated. The presence of both hormones (or hormone complexes) in adequate concentration seems to be necessary for moulting to take place (e.g. Scharrer 1946). If regeneration imposes a drain on the supply of one or both hormones, the following theoretical picture results:

Operations late in the instar, after the critical period, initiate regeneration when the demand of the tissues in general for hormones has been met, the changes leading to ecdysis have been irreversibly established, and the secretory cycle of the endocrine organs is declining towards its minimum. No delay in ecdysis is likely under these conditions, and the low level of hormones in the blood cannot provide for extensive differentiation. Production of a papilla, without delay in ecdysis, is therefore to be expected. With operations before the critical period, however, regeneration, with the "priority" over normal growth often postulated by Przibram (e.g. 1919), competes successfully for

hormone supplies with tissues whose response has not yet been determined. The competence of the tissues in general to respond to the moulting hormone is thus reduced, and is not restored until the hormone demands of the differentiating regenerate have been met; rapid differentiation, as seen in *B. germanica,* is likely to impose a much more severe drain on hormones, so that tissue competence is not merely reduced but abolished, and no ecdysis occurs until the normal endocrine balance has been restored. Wigglesworth (1952*b*) has observed that in *Rhodnius* the maximum secretion of juvenile hormone probably occurs after the critical period, which coincides rather with the secretory peak of the thoracic glands. The postponement of ecdysis in regenerating *B. germanica* by a period equal to the age of the animal at operation, however, suggests that the hormone demands of the regenerate have a more profound effect on the overall endocrine balance with operations near the critical period than with those performed early in the instar. This leads to the suggestion that the secretory cycle disturbed must be one which has its peak about the critical period, rather than after it, or else that the critical period for regeneration is in fact slightly different from the critical period for the endocrine system. So far as it goes, the above theoretical picture is compatible with the results described in the present paper for the effects of regeneration on the first post-operational moult, and conforms with the view that the non-appearance of intermediate stages in differentiation of the regenerate is due to an inhibitory influence of differentiation on the moulting process.

Other findings here described also conform with such an interpretation. Operations on the first day of post-embryonic life lead to an unexpectedly prolonged delay in ecdysis, but this is not surprising if it is assumed that a disturbance of endocrine balance is involved. Such a disturbance, beginning at or before the initiation of secretory activity in the endocrine organs, might well have a greater effect than one initiated when the secretory cycle was well under way, but short of its peak. The similarity of the results for all animals undergoing operation on the first day, whether still pale and soft or fully hardened and darkened, renders this explanation at least as satisfactory as the suggestions that the apparent anomaly may be due either to a specially severe "operational shock" in very young animals, or to their failure to ingest any food before operation. Again, the evidence here presented for a speeding up of the moulting cycle in later instars to compensate the delay in the first ecdysis is not incompatible with an interpretation in terms of hormone effects. If the delayed ecdysis is due to abolition of tissue competence towards the moulting hormone, it is possible that an excess of this hormone may accumulate in the blood, and Williams (1952) has shown that the moulting hormone in lepidopterous pupae may activate the prothoracic glands to produce further supplies. Hence there is a theoretical possibility that the rate of production of moulting hormone in instars following a delayed ecdysis in *B. germanica* may be increased, its threshold concentration may be reached abnormally early in the instar, and one or more accelerated ecdyses may result.

It is of course impossible to exclude the hypothesis that some of the effects observed result from preferential utilization by the differentiating regenerate

of food materials and metabolites other than hormones. The general conformity of the results with a theoretical picture based on current views of hormone action, however, seems fairly satisfactory. There is as yet no confirmation of the belief induced by the results of Pflugfelder (1939) that the hormone mainly concerned is the juvenile hormone, which would tend to support the view expressed by Novak (1951*b*) that the moulting hormone has little, if any, direct effect on growth or differentiation. The possibility of using regenerating *B. germanica* in studies on moulting and metamorphosis, as well as on regeneration itself, seems to be sufficiently established to justify further experimental and histological work on all three aspects, which it is hoped to publish in future papers of this series.

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VI. References

Brindley, H. H. (1897).—On the regeneration of the leg in the Blattidae. *Proc. Zool. Soc. Lond.* **1897**: 903-16.

Brindley, H. H. (1898).—On certain characters of reproduced appendages in Arthropoda, particularly the Blattidae. *Proc. Zool. Soc. Lond.* **1898**: 924-58.

Cameron, J. A. (1926).—Regeneration in *Scutigera forceps*. *J. Exp. Zool.* **46**: 169-79.

Chauvin, R. (1945).—Notes sur la physiologie comparée des Orthoptères. V. L'effet de groupe et la croissance larvaire des Blattes, des Grillons, et du Phanéroptère. *Bull. Soc. Zool. Fr.* **71**: 39-48.

Friedrich, H. (1930).—Zur Kenntnis der Regeneration der Extremitaeten bei *Carausius* (*Dixippus*) *morosus*. *Z. wiss. Zool.* **137**: 578-605.

Heldmann, G. (1929).—Die Gewebsentwicklung bei der Regeneration der Beine von *Dixippus morosus*. *Arch. EntwMech. Org.* **115**: 852-75.

Hinton, H. E. (1948).—On the origin and function of the pupal stage. *Trans. R. Ent. Soc. Lond.* **99**: 395-409.

Laing, F. (1946).—The cockroach, its life history and how to deal with it. *Econ. Ser. Brit. Mus.* **12**: 1-28.

Luscher, M. (1948).—The regeneration of legs in *Rhodnius prolixus* (Hemiptera). *Brit. J. Exp. Biol.* **25**: 334-43.

Needham, A. E. (1945).—Peripheral nerve and regeneration in Crustacea. *Brit. J. Exp. Biol.* **21**: 144-6.

Novak, V. J. A. (1951a).—New aspects of the metamorphosis of insects. *Nature* **167**: 132-3.

Novak, V. J. A. (1951*b*).—The metamorphosis hormones and morphogenesis in *Oncopeltus fasciatus* Dal. *Mém. Soc. Zool. Tchécosl.* **15**: 1-48.

Pflugfelder, O. (1939).—Beeinflussung von Regeneration bei *Dixippus morosus* Br. *Z. wiss. Zool.* **152**: 159-81.

Przibram, H. (1919).—Tierische Regeneration als Wachstumsbeschleunigung. *Arch. Entw-Mech. Org.* **45**: 1-38.

Prizibram, H. (1931).—Fuehler- und Beinregeneration bei Phasmiden, I-V. *Anz. Akad. Wiss., Wien* **68**: 160-5.

Scharrer, B. (1946).—The role of the corpora allata in the development of *Leucophaea maderae* (Orthoptera). *Endocrinology* **38**: 35-45.

SCHARRER, B. (1948).—The prothoracic glands of *Leucophaea maderae*. *Biol. Bull.* **95**: 186-98.

SCHARRER, B. (1952).—Neurosecretion. XI. The effects of nerve section on the intercerebralis-cardiacum-allatum system of the insect *Leucophaea maderae*. *Biol. Bull.* **102**: 261-72.

SEAMANS, L., and WOODRUFF, L. C. (1939).—Some factors influencing the number of molts of the German roach. *J. Kans. Ent. Soc.* **12**: 73-6.

VOY, A. (1949).—Sur la croissance des pattes de la Blatte (*Blatta orientalis* L.). *C.R. Acad. Sci., Paris* **228**: 207-8.

VOY, A. (1951).—Étude de la croissance chez deux espèces d'Orthoptères: *Blatta orientalis* L. (Blattidae); *Carausius morosus* Br. (Phasmidae). *Bull. Biol.* **85** (2): 1-30.

WIGGLESWORTH, V. B. (1948*a*).—The functions of the corpus allatum in *Rhodnius prolixus* (Hemiptera). *Brit. J. Exp. Biol.* **25**: 1-14.

WIGGLESWORTH, V. B. (1948*b*).—Le contrôle humorale de la mue et de la metamorphose chez les insectes. *Bull. Biol.* Suppl. **33**: 19-26.

WIGGLESWORTH, V. B. (1950).—"Principles of Insect Physiology." 4th Ed. 544 pp. (Methuen: London.)

WIGGLESWORTH, V. B. (1952*a*).—The thoracic gland in *Rhodnius prolixus* (Hemiptera) and its role in moulting. *Brit. J. Exp. Biol.* **29**: 561-70.

WIGGLESWORTH, V. B. (1952*b*).—Hormone balance and the control of metamorphosis in *Rhodnius prolixus* (Hemiptera). *Brit. J. Exp. Biol.* **29**: 620-31.

WILLIAMS, C. M. (1947).—Physiology of insect diapause. II. Interaction between the pupal brain and prothoracic glands in the metamorphosis of the giant silkworm, *Platysamia cecropia*. *Biol. Bull.* **93**: 89-98.

WILLIAMS, C. M. (1952).—Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the Cecropia silkworm. *Biol. Bull.* **103**: 120-38.

WOOD, F. D. (1926).—Autotomy in Arachnida. *J. Morph.* **42**: 143-95.

WOODRUFF, L. C. (1937).—Autospasy and regeneration in the roach *Blattella germanica*. *J. Kans. Ent. Soc.* **10**: 1-9.

WOODRUFF, L. C., and SEAMANS, L. (1939).—The rate of regeneration in the German roach. *Ann. Ent. Soc. Amer.* **32**: 589-99.

ZABINSKI, J. (1936).—Inconstancy of the number of moults during the post-embryonic development of certain Blattidae. *Ann. Mus. Zool. Polon.* **11** (12).

EXPLANATION OF PLATE 1

Blattella germanica L.

Fig. 1.—Trochanter and adjacent regions of the normal metathoracic leg in the first instar, showing the plane of autotomy at the trochantero-femoral articulation (*A*), traversed by a muscle (*M*). Whole mount stained with eosin and photographed by polarized light.

Fig. 2.—Coxa and trochanter of an operated metathoracic leg, photographed just before the first post-operational ecdysis, showing the dark blood clot (*B*) closing off the old trochanter, and the newly formed regenerate folded within the stump of the old leg.

Fig. 3.—Bases of the normal and operated metathoracic legs of an animal, operated on the third day of post-embryonic life at 30°C, after its first post-operational moult at 5 days old, showing a typical smoothly rounded papilla on the operated side.

Fig. 4.—Regenerated and normal metathoracic legs of an animal, operated on the day of hatching at 30°C, after its first post-operational moult at 6 days old. Note the completeness of differentiation of the regenerate, which differs from the normal leg mainly in size and in the presence of only four tarsomeres.

REGENERATION AND MOULTING IN *BLATTELLA*. I