Extent and properties of the regeneration field in the larval legs of cockroaches (*Leucophaea maderae*)

I. Extirpation experiments

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**SUMMARY**

Extirpation experiments have been performed on the larvae of cockroaches (*Leucophaea maderae*) to determine the extent and properties of the regeneration field of the legs of these insects. The distal segments, including the coxa, may all be removed without loss of regenerative capability; but regenerative capability eventually disappears if more proximal parts are removed. There are two regions adjoining the coxa anteriorly and posteriorly which are both important for leg regeneration. The anterior region consists of the sclerotized basal parts of the leg mainly formed by the trochantin and the praecoxa. The posterior region is an unsclerotized membranous area extending from the posterior border of the coxa to the anterior border of the next segment. This membrane is called 'leg-inducing membrane'. If only one of these two regions is present, no leg regeneration will occur. The interaction of both parts is necessary to allow the formation of a complete leg. An extra leg with reversed anterior–posterior polarity is formed when the 'leg-inducing membrane' of one segment is brought into contact with the sclerites of the following segment after extirpation of the membranous area which normally separates them. This membranous area, which represents the most anterior part of a segment, is called 'sclerite-inducing membrane', for if the basal sclerites or part of them are combined with this membrane only sclerites are formed, either in normal or reversed polarity depending on the experimental conditions.

**INTRODUCTION**

Many animals possess the capacity to replace lost appendages by regenerates. Very often the capacity to regenerate is not restricted to the tissues of the appendage itself but may extend for some distance around the appendage. The area within which it is possible to elicit, for instance, a leg regenerate is called limb territory or the regeneration field of the leg.

In amphibians (*Triturus*), this regeneration field includes large parts of the shoulder region up to the dorsal crest (Guyénot, Dinichert-Favarger & Galland, 1948). There are several investigations dealing with this problem in the Crustacea. The appendages of most of the Crustacea which have been studied also

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have a highly extended regeneration field. This is true not only for the different types of legs (Audoinot, 1956; Montel, 1957; Giordano, 1961; Patanè, 1961; Glazial, 1962; Hoarau, 1969) and the antennae (Bossuat, 1958; Patanè, 1961; Varèse, 1961; Hoarau, 1969) but also for eyes (Pastre, 1960; Vernet, 1967).

In insects, the regeneration capacity is much more reduced. Mostly it is restricted to the appendage itself. In *Tachycines* (Saltatoria), the most proximal level from which regeneration of the missing parts may occur is the femuro-tibial joint (Krause & Geisler, 1968); in *Rhodnius*, it is the coxa-trochanter joint (Lüscher, 1948). Some animals still show regeneration from a wound surface in the coxa (Megusar, 1910; *Gryllus*), but the regeneration capacity is eventually abolished when the whole coxa has been removed (Schaxel & Adensamer, 1923; *Caraustius*). Nevertheless, there are a few species which may regenerate complete legs even after partial or complete extirpation of the base of the leg (Bodenstein, 1933; Bourdon, 1937; Labour, 1966). The situation in the Blattaria, which normally show considerable capacity for regeneration, is not completely clear. Penzlin (1963), working with *Periplaneta*, got regenerates after extirpation of most of the basal sclerites (episternum and epimeron). According to the results of the other authors, the proximal limit for leg regeneration in *Blabera* lies more distally, namely between coxa and trochantin (Bullière, 1967) or between trochantin and praecoxa (Urvoï, 1963). Because of these differences it seemed desirable to investigate this problem once more.

The present experiments on *Leucophaea maderae* consist of three series of extirpation experiments in which various parts of the integumental tissues separating two adjacent legs had been removed. The results show that the regeneration field consists of two different parts: the basal sclerites lying anteriorly and the membranous area behind the coxa. Neither of the two parts alone is able to produce distal leg structures. A leg only develops when the two areas are allowed to come into direct contact and to interact.

**MATERIALS, METHODS, AND EXPERIMENTS**

The experiments were performed in third instar larvae of the African cockroach, *Leucophaea maderae*, during the first 2 or 3 days after molting. The animals were anaesthetized with carbon dioxide and fixed on a plate of paraffin. The operations were done with very fine scissors under a stereomicroscope. The wound was covered with a drop of Nobecutan (Bastianwerke München) which immediately hardened to a thin film covering the whole amputation area and thus prevented the animal from losing too much of its haemolymph. When a leg of *Leucophaea* is cut at the coxa or at a still more proximal level, normally segmented regenerates, in most cases, appear only after the second moult. The operated animals therefore had to be observed over a period of two or more molts, or as long as necessary to get unequivocal results (usually three or four molts).
Regeneration field of cockroach legs

Fig. 1. Survey of the ventral structures of the second and third thoracic segment of a larval cockroach. Longitudinally hatched area: pronotum and wing Anlagen; dotted area: membranous parts; clear area: skeletal elements. The left midleg had been removed at the proximal border of the coxa; the cut surface is indicated by the cross-hatched area. At the right: the different kinds of tissues of one thoracic segment (zones 1–4). The segment border coincides with the boundaries between zones 1 and 4. Interpretation of the results: leg regeneration needs the contact between basal sclerites (zone 2) and the membranous tissues of zone 4, the ‘leg-inducing membrane’. After the extirpation of a leg (zone 3) the basal sclerites and the ‘leg-inducing membrane’ of the same segment come into contact; a normal leg is regenerated. When the leg is removed together with the adjoining ‘leg-inducing membrane’ the sclerites come into contact with the ‘sclerite-inducing membrane’ (zone 1) of the next segment; sclerites are regenerated but no leg. When only the ‘sclerite-inducing membrane’ is removed the ‘leg-inducing membrane’ of one segment contacts the basal sclerites of the following segment; an extra leg is formed.

The segments of the cockroach leg and its basal parts are shown in Fig. 1. The basal parts of the leg (i.e. the parts of the leg which follow proximally to the coxa) consist of several sclerites which are more or less integrated into the ventral surface of the animal. Anteriorly adjacent to the meron there is a small sclerite, the epimeron; adjoining the coxa one finds a triangular sclerite, the trochantin. The three most proximal sclerites, the episternum, praecoxa and laterosternite, are fused to one chevron-like sclerite, which, for simplicity, will be referred to as praecoxa. A little more anteriorly lies the spiracle of the same segment. Adjoining the coxa posteriorly there are no sclerites but a membranous area which reaches to the basal sclerites of the following leg. Medially and laterally the leg is enclosed by the sclerites of the sternum and by the wing Anlagen, respectively.

Three series of experiments have been performed. In series A, all segments
distal to the trochantin-coxa joint (i.e. coxa, trochanter, femur, tibia, and tarsus) of the left midleg were removed. Various parts of the basal sclerites were also extirpated (Table 1). In series B, the distal segments of the left midleg including the coxa were removed and the membranous parts adjoining the leg posteriorly were extirpated to a varying extent (Table 2). In series C the distal segments of mid- and hind-legs were left intact; parts of the basal sclerites, together with various amounts of the membranous field between the mid- and the hind-legs, were extirpated (Table 3).

RESULTS

Series A

The results of the experiments of series A are shown in Table 1. Despite the removal of large parts of the basal sclerites, regeneration of a normal leg may occur (Expts A1-5, A8-9). After the extirpation of the coxa (A1), coxa plus trochantin (A2), or of coxa, trochantin, and half of the praecoxa (A3), regeneration takes place in all operated animals. But the frequency of regeneration decreases relative to the area of praecoxal sclerite removed (A4-5, Fig. 2). If all sclerites are extirpated almost no leg regeneration occurs (A6, 7).

Regeneration did not seem to be completely absent in those cases of experiments A4 and A5 in which no leg had been regenerated. The sclerites often had become symmetrical by longitudinal reduplication of the remaining skeletal elements (Fig. 3). Similar structures can also be found in those animals of experiments A4 which had regenerated legs; a normal leg with all basal sclerites is lying just behind such a symmetrical praecoxal sclerite (Fig. 4).

Experiments A8-9 were performed to determine if leg regeneration takes place when only the medial or lateral half of the basal sclerites is present. The results show that leg regeneration occurs at a more (A8) or less (A9) high rate. The regenerated legs of experiment A3 are completely normal. Those of experiment A9 have nearly always (10 out of 11) abnormal basal structures: the remaining sclerites had been reduplicated towards the median; the reduplicated structures are followed medially by a complete leg with all basal sclerites of a normal leg. Thus those sclerites which had not been removed by the operation had tripled (Fig. 5). In all cases of the two experiments in which no legs are regenerated, there had been a lateral reduplication of the sclerites, either of the median half (A8) or of the lateral half (A9, Fig. 6) of the basal sclerites.

Series B

This series was performed to determine if the presence of the basal sclerites is a sufficient prerequisite for leg regeneration; or if it is necessary to have, in addition, some other tissues normally surrounding the leg, for instance, the membranous area behind the coxa (Table 2).

In experiment B1 (which is the same as A1), only the coxa has been removed,
Regeneration field of cockroach legs

Table 1. Experimental series A; types of operation and results

The figures in the second column show the basal parts of the left midleg, including its surroundings; in the bottom figure the hind-leg is also drawn. (For comparison see Fig. 1.) Lightly dotted area: membranous parts; clear area: skeletal elements, at the left the sternites, followed (to the right) by the basal sclerites; longitudinally hatched area: wing Anlagen; cross-hatched area: amputation surface of the excised midleg; heavily dotted area: additionally excised tissues in front of the leg.

<table>
<thead>
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<th>Experimental series A number</th>
<th>Kind of operation</th>
<th>No. of operated animals</th>
<th>Surviving</th>
<th>Normal leg regenerated</th>
<th>No leg formed</th>
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Fig. 2. Despite the extirpation of most of the basal sclerites (Expt. A5) a complete leg with all basal sclerites had been regenerated (R). The anterior cut surface is indicated by the arrow.

Fig. 3. Sometimes no leg was regenerated despite the presence of basal sclerites (Expt. A4); instead, the remaining sclerites were reduplicated symmetrically. The axis of symmetry is indicated by the broken line. C, Coxa; E, epimeron; M, meron; P, praecoxa; Sp, spiracle; St, sternum; T, trochantin. In the centre of the symmetrical praecoxa there is a small piece of the trochantin.

Fig. 4. In some cases such a symmetrical praecoxal sclerite (S) was followed by a new set of basal sclerites, which themselves were followed by a complete leg.

Figs. 5, 6. Similar symmetrical structures are often observed after extirpation of the medial half of the basal sclerites (Expt. A9), but the axis of symmetry here is parallel to the anterior-posterior axis. In most cases a normal set of basal sclerites (plus leg) is adjoining medially (Fig. 5); sometimes no leg is regenerated and no sclerites are formed in addition to the symmetrical ones (Fig. 6).
Table 2. Experimental series B; types of operation and results

The figures in the second column show the area between the left mid- and hind-leg. (For comparison see Fig. 1.) Lightly dotted area: membranous parts; clear area: skeletal elements, at the left the sternites, followed (to the right) by the basal sclerites; longitudinally hatched area: wing Anlagen; cross-hatched area: amputation surface of the excised midleg; heavily dotted area: additionally excised tissues behind the leg.

<table>
<thead>
<tr>
<th>Experimental number</th>
<th>Kind of operation</th>
<th>No. of operated animals</th>
<th>Surviving</th>
<th>Normal leg regenerated</th>
<th>No leg formed</th>
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leaving the adjacent membrane as untouched as possible. Regeneration occurs in all cases. If part of the membranous area is removed together with the leg (B2), the frequency of leg regeneration is reduced to about 50%. The capacity for leg regeneration nearly disappears if the position of the posterior cut passes behind the spiracle (B3, 4). Naturally there is no regeneration if the basal sclerites and the membranous field behind the coxa are extirpated simultaneously (B5).

As was seen in the preceding series, the absence of leg regeneration does not mean the absence of all regeneration. There was a symmetrical, longitudinal reduplication of the remaining sclerites (Fig. 7). This reduplication was not
Figs. 7–10. Examples of experimental series B. Fig. 7: After extirpation of the mid-leg and the membranous area behind the leg up to the spiracle (Expt. B₃) no leg regenerates are formed, but the remaining basal sclerites are reduplicated longitudinally. P = Praecoxa of the following hind-leg. Fig. 8: Because of the more posterior position of the posterior cut surface (arrows) in Expt. B₄ the praecoxa of the regenerate had fused with that of the following hind-leg. When an experiment similar to B₃ was made with the foreleg (Expt. B₆) the duplicated sclerites mostly showed foreleg features (Fig. 9). There was only one case in which midleg sclerites had been regenerated (Fig. 10). C = coxa of the right foreleg. Broken line = position of the anterior cut surface and axis of symmetry.

complete in experiment B₄ but had stopped at the same level at which the basal sclerites of the following leg has been cut; at this level both sclerites fused (Fig. 8).

In order to find out whether the reduplicated sclerites are formed by the anterior (sclerite tissues) or posterior (membranous tissues) cut surface, an experiment similar to B₃ was performed at the base of the foreleg (Expt. B₆, not listed in Table 2). The basal sclerites of the foreleg are somewhat different from those of the mid- and hind-leg. In six out of seven animals with duplicated basal parts, the regenerated sclerites showed clear foreleg structures (Fig. 9); in only one animal had the sclerites midleg features (Fig. 10).

**Series C**

The results of the two preceding series of experiments show that there are two regions at the base of the legs which are indispensable for leg regeneration: the basal sclerites anteriorly, and the membranous field extending from the posterior margin of the coxa to the spiracle of the next segment. After
Table 3. *Experimental series C; types of operation and results*

The figures in the second column show the area between the left mid- and hind-leg. (For comparison see Fig. 1). Lightly dotted area: membranous parts; clear area: skeletal elements, at the left the sternites, followed (to the right) by the basal sclerites of the legs; longitudinally hatched area: wing *Anlagen*; heavily dotted area: excised tissues between the mid- and hind-leg. The midleg had not been amputated in this series, but it is drawn only up to the base of the coxa (broken line).

<table>
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<th>Experimental series C number</th>
<th>Kind of operation</th>
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<th>Surviving</th>
<th>Only basal sclerites regenerated</th>
<th>Extra leg regenerated</th>
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<td>Only basal sclerites regenerated</td>
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<td>2</td>
<td>Extra leg regenerated</td>
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<td>Extra leg regenerated</td>
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Figs. 11–15. Examples of experimental series C. Fig. 11: Regeneration of the excised basal sclerites (R) occurred in all cases when the anterior cut was made behind the spiracle (Expt. C2; the posterior cut was made between coxa and trochantin as indicated by the arrow, the anterior cut surface coincides more or less with the upper border of the photograph). But extra legs are formed (Fig. 12) when the coxa or trochantin comes into contact with the membranous area which extends anteriorly to the spiracle. These legs are exact mirror images of the remaining parts of the hind-leg. The axis of symmetry, indicated by the broken line, is identical with the posterior cut surface. Its position is at the anterior border of the coxa in experiment C3 (Fig. 13), in the middle of the trochantin in Expt. C4 (Fig. 14), and at the anterior border of the trochantin in Expt. C5 (Fig. 15). ML = basal sclerites of the left midleg; eL = leg; X = extra leg cut at coxa-trochanter joint for photographing.

the extirpation of the coxa these two areas come into contact, whereupon a regenerate is formed.

Now the question is: If the direct contact between these two regions is a necessary condition for regeneration, is it also a sufficient condition? In other words: Is it possible to get regenerates even when no leg parts are removed,
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only by bringing the two respective tissues into direct contact? Such a situation could be produced by the extirpation of the membranous field between the spiracle and the posteriorly following basal sclerites (zone 1 in Fig. 1). Thus the leg-promoting membrane (zone 4) of one segment is brought into contact with the basal sclerites of the following leg without damaging its distal parts.

Experiments of this kind have been made in series C in the region between the coxae of the left mid- and hind-legs (Table 3). If the anterior border of the wound area is lying behind the spiracle no additional leg is formed, but the removed sclerites of the leg base are replaced by new ones (C1, 2, Fig. 11). If the anterior wound border is lying in front of the spiracle, in many cases an extra leg is formed (Fig. 12). These extra legs are exact mirror images of the remaining parts of the hind-leg. Therefore in experiments C3 and C5 the extra leg starts proximally with the coxa (Fig. 13); in experiment C6 with the distal half of the trochantin (Fig. 14), in experiment C4 more or less with a complete trochantin (Fig. 15). On the other hand no extra legs are formed if the posterior wound margin does not touch the trochantin (C7, 8).

DISCUSSION

1. The conditions for leg regeneration

Experimental series A shows that the regeneration of a leg occurs only in the presence of at least a small part of the basal sclerites. So the anterior border of the regeneration field coincides with the anterior margin of the praecoxa (i.e. epimeron, episternum and laterosternite). In this series care was taken that the membranous parts adjoining the coxa posteriorly were not damaged. The second series, B, clearly indicates that this area also belongs to the regeneration field; for if this area is extirpated together with the leg, no regeneration takes place despite the presence of all basal sclerites. The membranous area having these features extends as far as the spiracle of the next segment (zone 4 in Fig. 1). Thus it becomes clear that the regeneration field consists of two parts: the sclerites in front of the leg and the membranous area behind. This membranous area will be referred to as the ‘leg-inducing membrane’.1 If only one of these parts is present no leg regeneration takes place. The interaction of these two types of integumental tissues seems to be necessary to allow the formation of a leg. This conclusion is further confirmed by the experiments of series C. Normally, the ‘leg-inducing membrane’ of one leg is separated from the basal sclerites of the following leg by a membranous area (zone 1 in Fig. 1), which does not possess leg inducing capacities (experiments B3, 4, 6). If this membrane is removed (series C) the formerly separated succeeding regeneration fields come into contact, whereupon an extra leg is regenerated despite the fact that all six

1 A short comment should be made about the meaning of the expressions ‘leg-inducing membrane’ and ‘sclerite-inducing membrane’ (see page 569). They are used only in a clearly descriptive sense and do not comprise any statements about the role the membranes play during regeneration.
legs of the animal are present. According to the reversed arrangement of the two parts of the regeneration field, membranous area anteriorly, sclerites posteriorly, the regenerated leg also shows reversed anterior–posterior polarity. With respect to the basal sclerites, the regenerated leg forms an exact mirror image of the leg following posteriorly. The conclusions concerning the extent of the regeneration field and the features of the different regions of the leg environment are summarized in Fig. 1.

Simple contact between the 'leg-inducing membrane' and part of the sclerites seems to be sufficient for the development of a leg irrespective of the condition at other positions, e.g. whether or not all legs are present. But in series C regeneration occurs only when the trochantin or the coxa comes into contact with the 'leg-inducing membrane'; mere contact with any part of the praecoxa is not sufficient as indicated by experiments C7,8. This seems to be in conflict with the results of experiments A3,4,5 where leg regeneration occurred at a similar level of the praecoxa. The two series of experiments are not fully comparable since the experiments of the A series were made with midlegs, while hind-legs were used for the C series. But the use of different legs for experiments A4 and C8 cannot account for the different results; for a similar experiment as C8 was made with the praecoxa of the midleg (not described in this paper) and showed similar results. Therefore the only decisive difference between experimental series A and C is the different orientation of the wound surface. The corresponding wound surfaces of the praecoxa were pointing posteriorly in the A series, but looking anteriorly in the C series. If legs were regenerated in experiments C7,8 they should have reversed anterior–posterior polarity like those regenerated in experiments C3,4. It seems that the praecoxa is not able to initiate the formation of a leg with reversed polarity.

Seeing that there are two different parts which are necessary for leg regeneration, several questions arise: In which way do these parts interact? Which kind of information is contributed by each of the counterparts? How long must they interact to allow normal regeneration? Where are the tissues of the regenerates coming from – from one or both of the two regions? The results of our experiments do not show the answers to any of these questions. At least parts of the problems could be solved by appropriate experiments. For instance, the origin of leg regenerate tissues could be elucidated by combining tissues of different segments or of different species. Experiments of this kind have been done and their results will be published in subsequent papers.

There is one experiment which deals with a similar problem, namely the origin of the tissues forming the second set of basal sclerites in experimental series B (see Discussion, II). In experiment B6 the basal sclerites of the foreleg had been combined with the anterior membranous parts of the following segment by extirpation of the connecting integumental tissues. The symmetrically reduplicated basal sclerites have mainly foreleg features (Fig. 9), more rarely, midleg features (Fig. 10). Therefore both parts of the wound area, the anterior
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part lying in the field of the first thoracic segment as well as the posterior part covering the segment of the midleg, may participate in proliferating tissues for the regenerates.

II. Sclerite regeneration

It has already been stated that regeneration occurred in series B and C even when no legs had been regenerated. In series C the extirpated parts of the sclerites had been replaced by new ones, in series B additional sclerites had been formed symmetrically to the existing ones. Leg regeneration was not possible in these cases because contact with the 'leg-inducing membrane' was lacking. But why then the regeneration of the sclerites, especially those extra sclerites of experiments B₃₋₆? Let us have a look at the structures present on the ventral surface of a thoracic segment. In Fig. 1 they are enumerated in anterior–posterior succession. The spiracle is lying at the border to the preceding segment. The following membranous area, up to the praecoxa, is indicated as zone 1. Since only sclerites are formed after contact with this tissue, the membrane is called 'sclerite-inducing membrane'. Zone 2 includes all basal sclerites. The leg is numbered as zone 3 followed by the 'leg-inducing membrane' (zone 4) which then passes directly to zone 1 of the next segment without any visible border.

It is a common feature of most animal patterns that any gaps or interruptions produced experimentally in the normal succession of structural elements are bridged over. This does not mean that all extirpated elements are replaced in all cases, as will be shown below. The main condition which must be satisfied is that all discontinuities are eliminated; that any given element – in the extreme, every cell – has its proper neighbour. This phenomenon, most frequently observed in segmented animals, is usually interpreted as the expression of some sort of gradient underlying this pattern (Lawrence, 1970; Bohn, 1970).

The results of experiments C₁₋₂ are clear: the posterior part of zone 1 and part or all of zone 2 had been removed. The extirpated 1½ zones are replaced by regenerates, thus completing the former sequence and filling up the gap. In experiments B₃₋₆ zones 3, 4, part of zone 1, and even part of zone 2 (both belonging to the next segment) had been extirpated. Instead of replacing the extirpated zones, only zone 2 (and eventually part of zone 1) is formed with reversed polarity. Thus the wide gap is closed by a relatively small amount of tissue and the discontinuity...1 2 2 1... is harmonized by the abnormal sequence...1 2 2 1... A similar situation is also produced in experiment A₆; so a similar result should be expected, i.e. formation of an extra zone 4 with reversed polarity. Unfortunately it is not possible to prove this assumption because of the lack of all morphological markers in the membranous zones 1 and 4 which could allow identification of the regenerated zones and recognition of the expected reversal of polarity.

After the extirpation of the medial half of the sclerites (experiment A₉), the remaining sclerites are doubled laterally. In addition a complete set of sclerites
is often formed (Figs. 5, 6). This lateral duplication does not necessarily conflict with the rule of an uninterrupted succession of structural units; on the contrary, structures like those shown in Fig. 5 even confirm it. But the reasons why the remaining sclerites are first doubled and only then followed by a complete set of sclerites are not clear.

III. The exceptions

There are almost no experiments in our series in which all animals show identical results. In most experiments (except $A_{1-3}$, $B_5$, $C_{1,2,7,8}$) there were some exceptions—animals which had formed regenerates under conditions which should not allow regeneration and animals that had not formed regenerates under conditions favouring regeneration—if our conclusions were right. Therefore the question arises whether our conclusions concerning the regeneration field are legitimate.

Part of the aberrant results is understandable in those cases in which at least one of the wound surfaces is lying near the border of two regions of different morphogenetic capacities. Either by slightly different cutting and/or by subsequent necrosis at the wound edges the definite wound surface in some animals may lie in one region, and in other animals of the same experiment, in the other region. Thus the results are expected to be different. Conditions of this kind may be responsible, at least to some extent, for the divergent results of experiments $A_{4,5}$ and $B_5$.

Another important factor is the mode of wound closure, especially in the cases where large areas of tissue had been extirpated. In the experimental series B and C it was not possible to remove the membranous area completely at the lateral and medial sides. The membranous areas medially are only partly separated from the corresponding membranes of the other body side by the sternal sclerites. Laterally there is also no distinct border and the membranous area extends far on to the dorsal surface of the animal. In the intersegmental area, the important region where zone 4 borders zone 1 of the next segment, the membrane even runs circularly round the whole animal. The results depend on how wound closure is made: i.e. whether the tissues of the lateral and medial wound border, or those of the anterior and posterior wound border first meet. In the first case, tissues which were expected to be eliminated by the extirpation could be transferred to the centre of the wound area and react with the tissues of the other two wound surfaces. Processes of this kind must be taken into consideration in most of the experiments, especially in numbers $A_{4,5}$, $B_{2-4}$, $C_{3-6}$, and could account for the rather high rate of ‘exceptional’ results.

There still remain experiments whose deviating results could not be explained by either of the above conjectures. Although all sclerites had been removed in experiments $A_6$ and $A_7$ some of the animals have regenerated a leg. The frequency is rather low (between 2 and 3%) but some doubt remains whether the regenerative capacity is really restricted to the basal sclerites or whether there
Regeneration field of cockroach legs

could be other tissues which after combination with zone 4 allow regeneration. The low frequency of leg regeneration argues against this possibility. More likely, part of the sclerites had been overlooked during the operation, all the more so as the anterior part of the praecoxa has no pigmentation and thus becomes very similar to a membrane. On the other hand, the basal sclerites give rise to a long apodeme (the pleural arm) which reaches far into the body cavity. It was not always possible to remove it completely. Eventually the remaining part of the apodeme could reach the surface, join the epidermis, and initiate leg regeneration.

A suitable method for removing these remaining doubts and to answer at least some of the many still open questions concerning the roles of the different parts of the regeneration field during leg regeneration would be to transplant the questionable tissues to regions of the body where legs are not formed normally. Such transplantation experiments and their results will be described in subsequent papers concerning the regeneration field of cockroach legs.

IV. Comparison with other insects

There is a slight difference in the results of authors who have studied the most proximal level from which leg regeneration is possible in cockroaches. After Bullière (1967) this level is represented by the base of the coxa; Urvoy (1963) still noticed some regeneration from a medial level of the trochantin, whereas Penzlin (1963) says that there is leg regeneration even if the praecoxal sternites are for the greatest part removed. The results of Penzlin are nearest to ours and show that leg regeneration is possible as long as fragments of the basal sclerites are present. Since the authors have used different species of cockroaches (Penzlin: Periplaneta americana; Bullière and Urvoy: Blabera craniifer; Bohn: Leucophaea maderae) the results are not really comparable. None of the authors have taken into consideration the role of the membranous area behind the leg.

The regeneration field in the Lepidoptera seems to be rather extended. After extirpation of the complete larval leg and some surrounding tissues, Bodenstein (1933) got leg regenerates. A similarly large field is found in some larvae of the Coleoptera. Even after extirpation of the trochantin and epimeron a complete imaginal leg is regenerated in Leptinotarsa (Labour, 1966). In Tenebrio large parts of the sternite region may be removed together with the leg without abolishing the capacity for regeneration (Lender & Grobocopateli, 1967). Moreover, one quarter of the regeneration field transplanted on to abdominal sternites is said to be sufficient to allow leg regeneration even in this foreign area. The results of Bourdon (1937) in Timarcha are more similar to those found in Leucophaea. He could remove some tissue proximal to the coxa and get more or less normal imaginal legs. There was a strong correlation between the absence or presence of the basal sclerites and leg regenerates in the adult animals. This may indicate that the extent of the regeneration field in Timarcha, at least in its anterior part, is similar to that of Leucophaea.

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