SOME EXPERIMENTS ON REGENERATION
IN LEGS OF BLATTIDAE

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BY
WILLIAM LORENZO DIXON

DEPARTMENT OF BIOLOGY

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INTRODUCTION

THE PROBLEM

The present paper is based upon data secured from two series of experiments suggested by the statement of Comstock (1930), page 174: "It appears to be necessary that the original coxa be not removed in order that the reproduction may take place." This conclusion was reached as a result of his own observation on spiders and studies of others on various Arthropods. A consideration of this view reveals the fact that Comstock was not absolutely certain that the coxa must always be left intact in order that the regeneration follow injury. This point seemed to warrant investigation. The experiments of the first series were begun February 23, 1932; those comprising the second series were initiated October 19th of that same year and are still under observation.

Since the first series of experiments was begun before a complete review of literature was possible, it was the main purpose of the experiments to check certain data regarding the relation of the integrity of the coxa to the extent of replacement in the appendages of insects, especially Blattidae. Also an investigation of regeneration following sections at lower levels of the appendage was planned.

It was learned very early in the course of the experiments that reproduction of the leg segments succeeding injury to the coxa is possible. Finding in the literature that this process is apparently unusual, I decided to concentrate my attention primarily upon regeneration following cuts at proximal levels of the insect's leg.
After some deliberation regarding the fitness and availability of material for investigation the cockroaches, *Periplaneta americana* and *Blatta* sp.(?) were selected. Since, according to Marlatt (1931), it is known that the European species passes through the developmental stadia more rapidly than the American species, it was hoped that the former might be secured for experimentation, but efforts to collect it were futile. Consequently the more available species was chosen. It has now been found that, although the results with this species cannot be obtained as quickly as with the European form, they are practically the same in both kinds of roaches, *Periplaneta americana* and *Blatta* sp.(?). This fact suggests that regeneration in the European species is similar.

Having obtained rather suggestive results from the first series, and having reviewed considerable literature concerning reproduction of lost appendages in certain arthropods, I resumed my experiments October 14, 1932 with the following purposes in mind:

(1) To determine the extent of regeneration following partial or total amputation of the coxa.

(2) To decide whether or not the reproduced appendage, expressing itself incompletely after the first molt, changes with additional ecdyses.

(3) To check variations, if any, in the usual regeneration of the appendage.

(4) To verify certain data obtained as a result of previous experiments on regeneration in legs of Blattidae.

(5) To check some of the results obtained by investigators who employed, as experimental material, other families of insects.

(6) To study the phenomenon of autotomy in this group of insects.
REVIEW OF LITERATURE

A general review of literature dealing with the problem of regeneration in Arthropoda, particularly Insecta, reveals the fact that comparatively few investigations have been carried out by methods and under conditions which would render the conclusions reached entirely free from question. In Arthropoda the capacity to regenerate injured appendages has been studied for some time and results obtained in this connection have been varied.

As early as 1712, Reaumur observed a lack of symmetry in the legs of Arthropoda, which legs, following injury, had been regenerated. He was probably the first to understand that this condition was caused either by injury to one appendage which was subsequently regenerated so that it approached the size of its concomitant mate, or by injury to both appendages at different periods so that one was at least partially reproduced prior to the injury of its mate. Practically all succeeding works show that growth of the injured appendage is so regulated that the reproduced appendage ultimately attains a size equal, or approximately equal, to that of its mate. Brindley (1898) observed that this capacity for accelerated growth is apparently more pronounced in Crustacea than in Insecta.

Since the times of Darwin and Weismann, scientists have attempted to link this power to regenerate lost appendages with liability to injury, pointing out the possibility of developing and perpetuating through natural selection a special mechanism for reproducing parts that are more

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exposed to environmental dangers. Moreover, there has been recognized in Arthropoda a tendency to throw off segments of an injured leg at the suture where bleeding is less liable to occur. From this point, commonly called the "breaking point," growth of another part usually takes place. This phenomenon of throwing off an appendage at the breaking point is designated "autotomy" or "self-mutilation".

Morgan (1898) concluded, as a result of experiments on the crab, *Eupagurus longicarpus*, that the ability to regenerate has not arisen through the agency of natural selection, and that there is no correlation between the frequency with which injury might occur and the power to replace these parts. He states further that followers of Darwin and Weismann have overlooked the fact that unless these animals were endowed initially with the capacity to reproduce certain parts they could not have survived under adverse circumstances.

Reed (1904) is of the opinion that the fact that the first pair of crayfish legs possesses only six podomeres while the walking legs have seven, supports the belief that the breaking joint was once a true joint which has become specially modified for self-mutilation after injury. This opinion, she believes, is fortified by the fact that the walking legs can be thrown off at the free joint between the second and third podomeres only with great effort. A close study of the segments of the walking leg shows that the line of fusion is located between the third and fourth podomeres rather than between the second and third. However, this line of fusion between the third and fourth podomeres is not present on the first pair of legs and the breaking joint is situated on the second segment. The information given above suggests that this line of fusion does not correspond to the lost joint. It was also observed that no articulation between podomeres of regenerated appendages occurred
prior to the appearance of muscles of the new leg. These muscles, according to Reed, are proliferated from the ectoderm.

Flatley and Walton (1922), in discussing regeneration in Crustacea, state that in lobsters, as in crayfish, the breaking joint is located between the second and third segments, and that in true crabs, in which autotomy reaches its height, the breaking joint is represented by a groove on the second segment corresponding to the joint between the second and third of other forms. The fact that hermit crabs, living a protected life in shells, possess autotomy to a marked degree, they believe, renders questionable the view that the phenomenon is purely adaptive.

In relation to the question of the effect of age upon the rate of regeneration following injury, Zeleny (1909 c), after experimenting upon a variety of Crustacea, offers evidence that young individuals in most species replace appendages more readily than old individuals, owing to the fact that the increase in proliferation of cells, correlated with an increase in age, is not sufficiently great to bring about completion of the limb as early as in young individuals. This means that the bulk of the appendage, which is less in young than in old animals, determines the time required for the complete replacement of the appendage.

Although experiments with Cambarus bartoni and Portunus asvi show that successive removal does not change the rate of regeneration, Zeleny's (1909a) experiments, in which Cassiopea and certain larval salamanders were used, did indicate an increase in the rate of replacement, and in none of his most accurately controlled experiments did Zeleny notice a decrease in the rate at which the new part was produced. The increase was explained either on a basis of faster multiplication of the available supply of undifferentiated cells near the cut surface of the regenerating
appendage, or on a more available form of the undifferentiated cells.

Zeleny (1905) observed in his two series of experiments with the
crayfish, *Cambarus propinquus*, that the series in which the degree of
injury sustained was greater invariably molted earlier, and also regene-
rated at a faster rate. In two species of crabs and two of crayfish,
Zeleny (1909 b) found that up to an optimum point the regenerative rate
of the appendage became greater as the amount of additional injury increased,
but that beyond this point any further injury lead to a decrease in rate.

The experiments of Cameron (1926) on regeneration in *Scutigera
forcipes* indicate a distinct relationship between temperature and rate of
growth in the developing appendage. Quoting from Cameron, p. 173: "These
observations seem to show that the rate of metabolism as measured by
molting and regeneration increases with temperature until a lethal tem-
perature is reached." He also made the significant observation that
starvation does not retard either molting or regeneration in this species,
and that the rate of growth is decidedly higher where the degree of injury
is greater. Cameron’s conclusions are slightly different from the findings
of Zeleny (1909 b) who, as we have seen, decided that the rate of regen-
eration was greater up to a certain point, beyond which further injury
lead to a decrease in rate of replacement. Autotomy in *Scutigera forcipes*
is so pronounced that appendages cut distal to the breaking joint were
not retained long enough to determine whether regeneration could take
place at levels below this joint. Since Morgan (1897), and Reed (1904)
have shown that regeneration in other *Crustacea* does take place following
cuts distal to the breaking joint, it seems not unreasonable that, if
parts beyond this point could be retained after operation, regeneration
might take place.

Loeb (1895), having convinced himself that younger animals regen-
erate more readily than older ones, expressed the belief that those younger in phylogenetic and ontogenetic series possess greater capacities for regeneration than the older individuals of the series.

Herbst (1896) noticed that in Crustacea the scape of the antenna must be left intact if post-operative regeneration is to occur. This suggested to Herbst that there is present in the basal portion some mechanism for the replacement of parts distal to the section.

Borradaile (1896), in his account of some crustaceans from the south Pacific, made frequent note of abnormalities in certain crabs. The fact that these had reproduced lost or injured members suggests again that the capacity to regenerate is an adaptation to enable the animal to cope with the dangers of nature.

Heinoken (1829), in checking the data of McCulloch, found, contrary to the conclusions of the latter, that crabs possess the power of reproducing an extreme segment, and it was upon these findings that he based his later experiments with spiders. Both investigators noted the marked occurrence of autotomy in the latter group of experimental animals.

Heinoken substituted spiders for crabs because of the difficulties experienced in keeping the latter alive. The results of his experiments indicated that spiders, and by analogy (he reasoned) crabs, could retain and regenerate amputated distal segments. The reproduced limb, although small, was perfect. He observed that "hunters" cast off crushed limbs while "web makers" usually retained them. Hence he considered this power of autotomy adaptive. He believed this plausible because of the fact that "web makers" are less liable to accidents, and consequently are not moved to action as quickly as hunters when accidents occur.

Comstock (1913) observed the phenomenon of regeneration in spiders.

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but stated that the newly formed limb is not perfect after the first molt. It appears first as a bud which grows and approaches perfection as molting continues. Heineken (1829) noticed the increase in size as molting continued, but also stated that the first appearing limb was perfect.

Turning our attention to Insecta, or Tracheata, we find that the mechanism of autotomy in connection with regeneration has been studied by many investigators. There appears to be little information concerning the process of regeneration in Coleorrhena. However, it does occur and seems to go on even after sexual maturity, which fact suggests that, as in the case with Crustacea, perhaps molting goes on after the adult stage is reached. This would distinguish them from other orders of Insecta.

In Neuroptera Watson noticed that the larva of Agrion reproduced legs following sections very close to the body, the new appendage appearing after the next ecdysis.

Such workers as Tornier (1900) and Hegusar (1907) have studied regeneration in Coleoptera, and given evidence that it takes place. According to Hegusar, reproduced legs of larvae vary in size in inverse proportion to the age of the operated larva and degree of injury. By varying the level of the cut he obtained both complete and incomplete regeneration. Tornier (1900) showed that the larvae of the meal beetle will not reproduce an amputated appendage if the operation is performed immediately before pupation, but that if the cut is made forty-five days prior to pupation the appendage is fully reproduced. This suggests

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1 H. H. Brindley, loc. cit.
2 This nomenclature is based on an old system of classification. At present the Agrionidae are classified as Odonata rather than Neuroptera.
correlation between age of larvae, time before pupation, and the actual amount of regeneration.

Conin (1894), as a result of his work on Lepidoptera, concluded that only the distal parts of the pupal legs project into those of the larvae; hence any cut at different levels of the leg would remove only fractions of the developing adult leg. Brindley (1898) reported that Rouxaur performed such experiments on the legs of Vanessa and obtained only negative results; whereas Newport (1944), by the same method, secured in the imagio either complete or partial development of previously injured appendages.

Kellogg (1904) experimented on larval legs of the silk worm, Bombyx mori. He found that these larvae possess the power to regenerate thoracic legs and abdominal pro-legs provided stumps of such legs are left intact. Each proximal part regenerates its distal part, but the body cannot produce another leg. After the first molt there was never more than wound closure. The new legs usually appeared after the second molt.

In contrast to the evidence that a complete leg is never regenerated, presented by the majority of the above investigators, and with results somewhat similar to those obtained by Cameron (1926) in his experiments on Scutigera forceri, Child and Young (1903), after extensive experimentation upon Agrionid nymphs, reached the significant conclusion that complete regeneration of an amputated part may take place at any level of the leg from the body to the most distal portion. These workers also felt, as did Reed (1904), that relation of the attachment of muscles and the subsequent appearance of joints in the new appendage is of sufficient importance to warrant investigation. Although regeneration in general was of a progressive nature, the tarsal claws appeared long before

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1 H. H. Brindley, loc. cit.
articulation was distinct.

Autotomy, although less marked than in some insects, is clearly evident in Agrionidae. Regarding the adaptive nature of regeneration Child and Young say, p. 561:

"The real criterion of the adaptive nature of the regeneration is to be found, however, not in the rapidity of the process during its earlier stages, but in the final result. If the process is adaptive, the leg regenerated from the breaking joint should be more nearly perfect, or should attain the perfect condition in less time than when regeneration takes place at other levels."

Such was not the case in their experiments.

In Orthoptera saltatoria the phenomenon of regeneration is, as we shall see, different in certain respects from regeneration in Orthoptera cursoria. Bordage (1900 a), having carried on a large group of experiments upon certain members of the Locustidae, Acrididae, and Gryllidae, decided that regeneration of the jumping legs does not occur. Bordage explains this lack of power to regenerate the posterior pair of legs in Orthoptera saltatoria by the hypothesis that individuals possessing such power would, because of natural dangers, probably not live sufficiently long to reproduce such lost legs. Furthermore, if they survived they would be unable to compete successfully for mates with uninjured animals. Owing to the fact that mutilated animals could not participate in mating, the capacity for regeneration, which may have been present in such animals, has not been propagated.

Among investigators who do not believe that regeneration of the jumping-limbs takes place, Bordage mentions Heiniken, Graber, Durieu, Frederieq, Contejeau, Werner, and Peyerasmoff. Bordage accounts for Griffini's contention concerning regeneration in these parts through the failure of the latter to recognize the fact that inequality of hind legs

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1 Bordage (1900 a).
in Pristea tuberosae and others may be due to atrophy rather than to regeneration.

Bordage (1900 b), on the basis of evidence obtained from later experiments concerned with regeneration of the two anterior pairs of limbs and of the tarsi in these insects, points to the futility of attempts to provoke self-mutilation of the first two pairs. He refers to the important observation that separation resulting from a strong mechanical pull takes place more frequently at the coxa-trochanter articulation than at the trochanter-femur line of fusion, although regeneration is more complete from the latter. The appendage re-produced from the level of the coxa-trochanter articulation is a rudimentary stump, and very often separation at that level is favorable because of the less copious loss of body fluid. Bordage (1900 b), p. 237, said:

"These facts seem in a twofold manner to invalidate the law of Lessona: 1stly, because there is regeneration at points where mutilations do not appear to be normally produced; 2ndly, because regenerations at the two places are most frequent in the one where pulling more rarely leads to the separation of the limb, and because they are without any comparison more complete in the same region."

Since, however, during molting one of the limbs may not, infrequently, be separated from the body at the trochanter-femur (very rarely at the coxa-trochanter) joint, Bordage finds no reason why the apparent double conflict with the law of Lessona should not break down. When exuvial self-mutilation does occur between the trochanter and femur a perfect limb may be formed (complete regeneration).

Regeneration of the tarsi in Orthoptera saltatoria may take place

1An exact statement of this law has not been found, but Bordage's (1900b) interpretation is that Lessona believed that there is correlation between liability to injury and capacity to regenerate. Thus parts more liable to accidental injury possess a greater capacity to replace parts.
in all three pairs of limbs after either natural injury or operation. Bordage considers this adaptive since it is likely that these parts would be torn or injured in some manner. In Phyllonotera laurifolia and Concephalus differens the regenerated part consisted of four tarsal segments (normal number) whose relative proportions differed from the normal. The regenerated anterior limbs did not reach the length of the normal, and the tibia of the anterior pair (prothoracic) had no tympanum. The essential point here is that regeneration with respect to number of tarsi is complete even though the appendage after the first molt is often so small as to render it useless.

Experiments performed on members of Orthoptera cursoria indicate that many of this group have a marked capacity for regeneration and often show pronounced autotomy.

Coquerel \(^1\) first pointed to the fact that the four jointed condition of tarsi in Phasmsids probably arose through regeneration after injury to five segmented tarsi.

Scudder \(^2\), using Diapheromera, was the first to produce experimental evidence to support this assumption.

Bordage (1897 a), while experimenting upon Monadophthorina inuca, and Raphides scabraeus (nymphs and adults), observed that a leg, when injured at various levels distal to the suture between the femur and trochanter, is often cast off at the suture. This suture is particularly well adapted for such self-mutilation since the injury to tissue and loss of body fluid are at a minimum. Bordage (1898 b) believes that the suture between the trochanter and femur is there for the purpose of autotomy. He thinks, further, that this condition of ankylosis indicated by the suture has not always existed, but that at one time there must have been a true

\(^1\) H. H. Brindley, loc. cit.
\(^2\) R. H. Brindley, loc. cit.
joint.

Godleman (1901) found that the capacity to regenerate was located more proximally, and that under certain conditions a five segmented tarsus was regenerated. Autotomy of Blatilla rossii could be induced quite readily if the necessary external stimuli were applied. This phenomenon in his specimen was regarded as a sort of adaptive arrangement for removing the insect from the molted cuticula from which molting individuals do not always free themselves. He regarded the difference in capacity for regeneration as dependent upon liability to injury.

Unaware of the fact that regeneration occurs in Blattidae so that the normal number of segments is diminished Geoffrey (1762) described the genus Blatta as possessing four tarsal segments on the metathoracic legs, and five on the meso- and prothoracic. In his plate, however, both male and female specimens were represented with five tarsal segments on all legs. His description evidently was based upon individuals which had been injured in some way.

Geoffrey's inconsistency was corrected by Serville who, however, did not discuss the four-segmented conditions. Later the four-segmented condition was noticed by Brisont de Barneville\(^1\) in several species of Blattidae.

In view of the fact that the observations of previous workers on Blattidae were based on small numbers of insects, Brindley (1897) decided to extend the experiments, and accordingly, examined over a thousand individuals bearing the abnormal tarsi. Four species of roaches were examined. His results revealed the fact that this variation was as a rule not of congenital origin, but that it was due to an injury which had been followed by

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\(^1\)H. H. Brindley, loc. cit.

\(^2\)H. H. Brindley, loc. cit.
regeneration. The frequency of the appearance of four tarsal segments was greater among old individuals than young. Quoting Brindley, p. 903: "This abnormal tarsus seemed to possess a fixity of organic stability of structure such as is held usually to be the outcome of the continued operation of selection, an influence which can hardly be called upon to account for the condition of an occasional abnormality." The tendency to drop injured appendages was also noticed in these animals. If this be considered autotomy, it is quite unusual in that dropping occurred both at the trochanter-femur suture and at the tibia-tarsal articulation.

At a later date Brindley (1898) published the result of his investigations on Arthropoda in general. Special effort was made to interpret the phenomenon of regeneration in Blattidae. His findings were, briefly, as follows:

1. Reproduction of lost limbs in Arthropoda is partial since it cannot take place in the absence of the basal portion. It does not take place after cessation of ecysis.

2. Whereas in Crustacea the reproduced portion can be seen growing from the stump (covered by a thin specially formed cuticle), in Insecta the new part is entirely concealed, and is revealed only after ecysis.

3. In some Tracheata and most Crustacea the new appendage is "an exact counterpart" of the structures it replaces; in other Tracheata the reproduced part is always unlike the normal in as much as its number of tarsal joints is lessened. The new four-segmented tarsus in Blattidae is perpetuated.

4. The reproduced portion of an appendage grows faster than the corresponding normal portion so that, although smaller than its mate, the new leg approaches the size of the normal leg and, unless prevented by the cessation of growth in the animal, attains a size almost equal to that of its mate. This suggests the operation of a special control mechanism.
(5) The peculiar structural characteristics of the new appendage remain constant.
MATERIALS AND METHOD

The usual abundance of cockroaches, especially *Periplaneta americana*, in dwelling places, warehouses, and stores rendered the problem of collecting relatively easy. Owing to the habit of roaches of concealing themselves in corners, cracks, crevices, and behind objects in daytime in an attempt to elude natural enemies, it was found advisable to use roach traps. For this purpose a commercial wire trap was selected. Meat, greasy foods, fruit, and pastries were used as bait for the roaches. The greasy foods gave most satisfactory results. The fact that the animals are largely nocturnal suggested that trapping could be done most successfully at night. Later experiences in collecting have thoroughly justified this belief. Roaches frequently lurk in vast numbers around the openings for hot water pipes and in pantries; consequently traps were always left in such places. If left in traps for long periods, many individuals found ways of escaping; therefore, it was necessary to transfer them to other containers shortly after trapping. The traps were examined at intervals of about two hours and the imprisoned roaches were removed as often as necessary. On one occasion fifty roaches varying in size from that of the newly emerged to that of the adult were captured in a single trap in less than one-half hour. At first the insects were simply shaken from the opened trap, but it was found advisable to anaesthetize them with chloroform, thereby preventing their escape. Unless this method is used they are liable to escape the most careful workers because they are surprisingly alert and move with great rapidity.

In the second series of experiments, an additional species of roach
was collected from tree stumps, beneath bark of trees, logs and rocks. The roaches so collected were Blatta sp. (?) Since the usual routine of trapping was eliminated it was less difficult to obtain outdoor specimen.

The insects were kept in large glass bowls covered with fine gauze until ready for operation. Large wire cages were used as insectaria at first, but it was found impossible to retain very young individuals in such cages because of their habit of crawling through the openings in the screen. Glass bowls were substituted with excellent results. Hereafter, I shall refer to these large glass bowls, in which my experimental animals were kept, as insectaria. Damp paper towels and other materials which would reproduce the conditions of their natural surroundings were placed in the insectaria. The food of such aggregated individuals consisted of cream, fruit, cakes, and meat. Roaches are practically omnivorous and at times prefer their own exuviae, or the damp paper towels used to provide moisture and concealment for them. In addition to the diet of the roaches collected in dwellings, those obtained in the woods were given dead leaves and other waste material. After operation each roach was removed to a small individual container (two oz. wide-mouthed bottle) which provided ample room for locomotion and at the same time afforded conditions which duplicated very nearly those under which the roaches live normally. A piece of folded paper towel which could be moistened when necessary was put on the bottom of each bottle. Food was left on the surface of the towel and allowed to remain not longer than two days, the actual time depending upon the development of mold. The paper towel was, as a rule, removed at four day intervals although it was often necessary to remove it earlier because of the accumulation of excrement of mold.

It is believed that temperature plays quite an important rôle in the regeneration of appendages in roaches; hence, a temperature of about 75° F.,
at which individuals thrived, was maintained. At one time a much overheated room caused the death of many roaches, so that the temperature was thereafter carefully regulated.

The containers for the experimental animals, having been grouped and numbered according to the type of operation performed, were examined daily and such information as was significant was carefully tabulated.

At the beginning of the investigation many operations were performed without an anaesthetic. In such cases the insect was caught and held between the thumb and fingers until the operation could be completed. Later, it was found much more advantageous to use an anaesthetic, since the cuts were more precise and accidental loss of an appendage through rough handling and struggling could be avoided. Apparently, the use of the anaesthetic had no ill effect upon the regeneration of appendages. All operations were carefully performed with sterile instruments under a Spencer binocular dissecting microscope. Very fine forceps and scissors were the only instruments absolutely needed, although for certain types of cuts a small scalpel was often very useful.

The anaesthetic was administered by soaking a small wad of cotton in chloroform and placing it in the bottle containing the roach. After the individual was reasonably quiet it was removed with the wad of cotton to a glass Petri dish which was covered until the roach could scarcely move its legs. The operation was performed quickly and the insect immediately returned to its bottle which was plugged loosely with cotton to prevent escape. After a short time the roach could be seen moving about quietly. Following the examination of an operated animal (which was always made before returning the individual to its bottle), a record was made of all significant observations. If the anaesthetic is cautiously administered, and the operation carefully performed, the chances for survival are greatly enhanced. In a very few cases
insects developed a growth of fungus on the cut surface which resulted in death, but growth of fungus was so infrequent that it was not considered a serious problem.

Along with the experimental animals, insects which were not subjected to operation were kept as controls.

In as much as the animals were examined daily it was always possible to observe the regenerating legs shortly after molting. The fact that an animal has molted is evident from the presence of the exuviae, or the changed part itself. Immediately after molting the bodies are soft and pale, but very quickly assume natural pigmentation. The exuviae are usually devoured if left in containers sufficiently long. Many exuviae were preserved in order that they might be used as evidence of the condition of the appendage before molting. Molting is more frequent in young than in older individuals. The reaches averaged about one molt per month. By varying living conditions such as food, moisture, and temperature, molting may be prolonged, or accelerated. In the experiments some individuals were kept for nearly two months without molting. This was due to a scarcity of food and low temperature. Following each molt a careful examination was made of both exuviae and regenerated appendages.

Dissection of the appendages made possible a study of the arrangement of muscles and of the form assumed by the reproduced part while beneath the old cuticula. For such dissection very small spear headed needles gave best results. Knowing the date of operation and the approximate time required to bring about another molt, it was possible to study the form of the regenerated appendage prior to ecdysis. A dissection does not always reveal the true form of the regenerated appendage, hence another method of study was adopted for comparison. By this new method examination was made easier since it was only necessary to fix the amputated appendage for a day or so in 80% alcohol which does not disturb or injure parts is not always possible.
alcohol. Very often the form of the new appendage could be studied on young
and some old individuals by transmitted light while the appendage was intact.

No effort was made to prepare histological sections of the leg, but
it is hoped that this phase of the problem may be taken up at some future
date. That such a study is desirable seems beyond question to the writer.

It is believed that some of the erroneous conclusions of previous
workers in this field originated from faulty technique; hence, the methods
used in these experiments were devised for the purpose of eliminating these
errors.

In preparing plates a normal leg was drawn by the aid of a camera
lucida and a reduced cut made from this drawing. In order that a comparison
of the regenerated appendage with normal appendage might be made possible; space
for the representation of the new part was reserved on the plate bearing the
drawing of the normal limb.
EXPERIMENTAL SECTION

THE NORMAL APPENDAGE

The Structure of the Leg

The leg of the roach which is typical of the more generalized insects is not widely different from legs of other Orthoptera. Close scrutiny of the appendage reveals the fact that it is complete as far as articular sclerites are concerned. Structurally it consists of an enormous coxa above which are located the trochantin and two antecoxal sclerites, trochanter, femur, tibia, and a characteristic tarsus.

Antecoxal Pieces.—The second antecoxal piece, usually not present as a distinct sclerite in certain specialized insects, is located between the first antecoxal piece and the episternum. It is separated from the episternum by a less marked line of articulation than that between the second and first antecoxal pieces.

The usual combination of the first antecoxal piece and trochantin is not realized; consequently it becomes necessary to speak of the trochantin as a separate part. The first antecoxa is, therefore, articulated proximally to the second antecoxa and distally to the trochantin. The first piece is distinctly larger than the second piece.

Trochantin.—The trochantin, a triangular sclerite whose tip is directed medially, is articulated with the coxa, and anteriorly joins the epimeron and episternum. This sclerite is sparsely spined and in size exceeds either of the previously described sclerites.

Coxa.—This segment, frequently considered the most important segment of the appendage from a standpoint of regeneration, is articulated to the body pleurally by a process situated at the ventral end of the suture.
between the episternum and epimeron, ventrally with the trochantin and its allied sclerites. It is the largest of the segments which constitute the appendage. Spines are numerous on the outer and inner edges of the appendage and the segment is characterized by distinct markings accompanied by a few scattered spines on its ventral surface. The coxa is articulated distally with a much smaller segment termed the trochanter. The coxa is generally considered the proximal segment of the leg.

**Trochanter.**—Joining the coxa proximally, and immovably fused to the distal end of the femur, is the small second segment, the trochanter. Its outer border is moderately armed with spines. The apex of this triangular segment is directed pleurally. The line of fusion between trochanter and femur is often spoken of as the "breaking joint" owing to the fact that separation takes place at this point.

**Femur.**—The femur or third segment of the leg approaches the coxa in size more closely than any of the other segments. It is heavily armed with rows of conspicuous spines on the inner edge. The ventral surface and outer edge is thickly set with small spines, and at the point of its articulation with the tibia large distinguishing spines may be recognized.

**Tibia.**—The fourth segment of the leg may equal or exceed the femur in length, but is always more slender. Its surface is heavily armed with both large conspicuous spines and smaller spines. The tibia of the prothoracic leg seems shorter than the femur; that of the mesothoracic leg, though closely approaching the length of the femur, distinctly shorter; while the tibia of the metathoracic leg is longer than the femur. As we proceed, therefore, from the most anterior pair of legs posteriorly, there is an increase in relative length of the tibia. This is especially evident in older specimens. The tibial spines (often called tibial spurs) are especially large and prominent at the distal end of the segment which is articulated to
the tarsus.

Tarsus.— The fifth and last division of the leg, generally known as the tarsus, consists of five tarsal segments. The most proximal of these tarsal segments, the metatarsus, is broadest and longest, being almost as long as the others combined. The segments are, beginning proximally, progressively smaller (as we proceed distally) until the fifth segment is reached. The fifth segment approaches the size of the first tarsal segment more closely than any of the others. There is located at the end of the fifth segment a pair of well articulated claws (ungues) between which one recognizes in certain Blattidae, a small pad, the empodium. This end portion or sixth segment, as many authors have chosen to call it, seems retracted or "telescoped" into the fifth.

The proximal segment of the tarsus is designated the metatarsus; the terminal part, the praetarsus. The ventral surfaces of these tarsal segments are equipped with pulvilli. The tarsal segments are densely covered with small spines which, on the ventral surfaces, are arranged in orderly rows.

A comparison of the length of the tarsi and tibiae reveals the fact that the tarsus of the prothoracic leg is longer than the tibia; the tarsus of the mesothoracic leg slightly shorter than the tibia, and even shorter with respect to tibiae on the metathoracic legs. The relative lengths of the tarsi in this instance, therefore, decreases as we proceed from the first pair of legs posteriorly.

A comparison of the lengths of the legs "in toto" indicates an increase as we proceed from the most anterior pair posteriorly.
MUSCLES

A consideration of the structure of insect legs seems to indicate that the importance of muscles and their disposition in the regenerating leg, together with their probable rôle in establishing articulations, cannot be neglected. In as much as certain authors believe that muscles and their attachments determine the presence or absence of joints, verification of this view seems desirable. In this paper observations on regenerating muscles have not been given major consideration, but it is planned that this feature of regeneration be considered in detail during an anticipated histological study. Owing to the relative compactness of the muscles in the roach appendages, an accurate study of muscle attachments, especially in distal regions of the leg is rather difficult. It is for this reason that some of the views regarding possible correlation between muscle attachment and appearance of joints (particularly in the tarsus) must be withheld until suitable information has been gathered.

It is of interest, however, to discuss briefly the general arrangement of the more prominent muscles in the normal leg as shown in Fig. 1 (Pl.I).

In the proximal segment (coxa) of the leg one recognizes the extensor of the femur, which appears to be the larger, and the flexor of the femur. The extensor of the femur originates in the thorax on one of the tubular apodemes and is inserted by a strong tendon at the proximal articulation of the coxa and trochanter. It is situated posteriorly in relation to the muscles which effect movement of the coxa. The coxa is drawn toward the median line by the large adductor muscle which originates on one of the apodemes of the thorax and is inserted on the medial articulation.
of the coxa with the trochantin. The abductor, which draws the coxa away from the median line, originates on one of the tubular apodemes and is inserted at the trochantin-coxal joint and partially along the inner wall of the coxa. None of these muscles appears to be attached to the articulations between the sclerites proximal to the coxa. The muscle which accomplishes flexion of the femur originates, as shown in Fig. 1 (Pl. I), at the point of articulation between the trochantin and coxa, being securely fastened in the pleural regions of the leg. This large muscle is inserted by a thick tendon at the coxa-trochanter articulation. The arrangement of muscles in this region is of possible significance in the reproduction of the appendage following section, since cuts through the trochanter would not interfere with muscles in the coxa whereas section at the trochantincoxal articulation removes all muscles in the coxa except a portion of the extensor femoris. After such section there would, consequently, remain only a part of one muscle which had extended into the coxa that might possibly feature in regeneration.

The flexor tibialis originates on the medial surface of the trochanter and is inserted at the proximal end of the tibia. It is of interest to note that the arrangement of muscle tissue in this insect differs from that of some other Arthropoda, such as the Agrionidae of Child and Young, and Bacillus rossii of Godellmann, in which cases the flexor tibialis does not extend into the trochanter.

The extensor tibialis, originating at the apex of the trochanter-femur articulation, is attached to about two-thirds of the upper inner wall of the tibia, and inserted by a strong tendon on the distal end of the tibia (Fig. 1, Pl. I). The muscles which occupy most of the femur are closely associated with each other, but the flexor of the tibia is distinctly larger than the extensor and extends from the trochanter through the femur to the
distal end of the tibia, where it is inserted. The muscles are attached to the inner border of the tibia, which is telescoped into the femur.

Autotomy is sometimes explained on the basis of separation through the antagonistic action of muscles located in the trochanter. This is believed to be true of the Agrionid nymphs, according to Child and Young (1903). It seems reasonable, therefore, that if autotomy takes place in any group of animals, it is less pronounced in instances where muscle arrangement does not favor it. There appear to be no muscles in the trochanter of the roach which might render the trochanter specially adapted for self-mutilation at the suture. When pulled apart at this point the muscle is usually torn, leaving fragments at its point of origin in the trochanter.

Both of the muscles which move the tarsus are attached to the ventral side of the tibia where they originate, and are so closely associated that it is difficult to separate the two, as is suggested by Fig. 1 (Pl. I), in which they have been separated just enough to enable one to distinguish them. The flexor of the tarsus narrows into a long slender tendon by which it is attached to the inner border of the empodium and claw. It is obviously much longer than the retractor of the tarsus, which is attached to the proximal end of the metatarsus. There are other muscles in the tarsal segment which have not been studied because of their small size and their relative compactness. These muscles, like all others of the leg, are largest at their points of origin and become more slender as they near the point of their insertion. This is significant, since a cut near the distal end of the muscle would leave less muscle tissue exposed and would necessitate less regeneration. A general consideration of the muscles in the leg shows that they are most massive in the portion of the leg proximal to the coxa-trochanter articulation; those situated in the femur are next
in size; those of the tibia, though smaller, are more tightly packed.
GENERAL FEATURES OF REGENERATION IN LEGS OF BLATTIDAE

Sections at any level of the insect's leg were followed by shrinkage of muscle tissue, but the amount of shrinkage, as well as the loss of body fluid (haemolymph), was determined by the level of cut made. The loss of fluid was always greatest when the cut was made between the coxa and trochanter. Bleeding was very profuse at the trochantin-coxal joint. This loss weakens the insect greatly, but excessive bleeding which might have been fatal was usually prevented by immediate coagulation of the thick oily fluid that issued from the wound. The material, at first yellowish in color, quickly changed into a brownish scab, which closed the wound securely after a short while. In a few days the wound was covered by a hard scab which was almost black. When the cut was made midway the coxa, the loss of fluid was less than when the cut was made at or above the trochantin-coxal articulation. The compactness of the muscles which occupy the coxa may in some way aid in prevention of excessive loss of blood. The waste of haemolymph following section at the coxa-trochanter joint was not copious and wound closure was not long delayed. There was frequently greater loss of body fluid following section at the suture between the trochanter and femur than from section at the junction of coxa and trochanter. If the leg was broken apart at the suture, the loss of blood was usually less than that following operation. Section at the suture left a part of the flexor tibialis in the trochanter, but if the separation was effected by pulling there often remained only small fragments of the muscle attachment. Bleeding following sections midway the femur appeared to be less than that after sections just proximal to the femoro-tibial joint. Again, the compactness of muscles
in the middle of the segment seemed to aid in prevention of blood loss. Following sections in the more proximal regions of the tibia there was almost always greater loss of fluid than in the more distal regions. In the regions below the tibia the loss of blood was never excessive, but was greater if the sections were made above the points of articulation. In general the loss of body fluid depends upon age and size of the individuals as well as on the level of the cut. Oblique cuts usually left more surface exposed, and were accompanied by a greater loss of body fluid (blood) than the transverse cuts.

The shrinkage of the muscle tissue resulting from injury was greater in the lower regions than in the more proximal regions, and greater if the section was taken midway of a segment than if taken at the joint. The tissue, after the cut had been made, was withdrawn so that the chitinous covering extended a bit beyond the surface. When the cut was made near the body there was noticeable degeneration of a small amount of tissue in immediate proximity to the cut surface. The degree of contraction of tissue, usually more obvious in older specimens, was by no means constant. Often, when the cut was made below the point of articulation, the tissue dried up to that point, and regeneration proceeded from the joint. Shrinkage and drying of muscle tissue seemed greatest in the tarsal segments in which it was very difficult to study regeneration from various levels because of the fact that such segments, though retained, were completely dried and void of live tissue. Growth of the new tissue seemed greatest where the surface exposure was most extensive. In the oblique cuts the rate of cell proliferation seemed
greatest at the more proximal end of the cut since the new part always
grew out in line with the rest of the appendage before ecdysis. On a
few occasions appendages which were crushed dried up, and were carried
around until broken off in some way, or until molting and regeneration
took place from the last sound segment.

Frequently, the leg was broken during infliction of the injury in
cutting, but this was considered mechanical injury caused by scratching or
jerking rather than typical autotomy. The experiments which were performed
to test this view will be taken up in detail later in the paper.

Replacement of the old appendage apparently begins in the proximal
regions and proceeds distally, but articulations in the tarsus (beneath
the old cuticula) were observed earlier at the point where the tibia joins
the metatarsus, and at the point between the fifth segment and the distal
claws, than at those of the other tarsal segments. Pigmentation is less dense
in the new appendage than in the old and it is possible to observe the point
where growth of new tissue began through this difference.

Externally the new appendage does not always have perfect form, being
frequently twisted and having usually fewer spines than its congenital mate.

Appendages in which the mass of material required for reproduction
was small, attained a size nearly equal to that of the mate in a short time.
In a few of the cases the regenerated appendages, following sections which
removed the entire leg, appeared to possess incomplete musculature.

A consideration of the time allowed for the regeneration of an appen-
dage showed clearly that the degree of development reached by an appendage
depended, together with other factors such as food, moisture, temperature,
etc., upon the time between operation and molting. If the operation was
performed shortly before molting, either very little new tissue could be
seen or the appendage expressed itself incompletely. Successive ampu-
tation of an appendage did not alter the power to regenerate a new leg, but did lessen the possibility of obtaining a new leg equal in size to its mate. Operations were performed upon more than one appendage at a time, and results indicated the relation between theroach's ability to replace all injured appendages in a single stadium and the extent of injury. The time allowed for growth of the new part played an equally important rôle in the completion of the new appendage. Such regenerated appendages were in no way inferior to the usual reproduced legs, except for occasional slight variations in rate of replacement.

Regeneration did not vary according to the appendage upon which the operation was made. A few special experiments were performed to determine whether regeneration after a certain section was more complete on one leg than another. The observations confirmed the view that similar results would be obtained from sections taken at the same level regardless of the location of the legs on which sections were made. Early in the course of experimentation it was found that the mesothoracic leg was better fitted for investigation, since its function seemed chiefly that of aiding in the support of the insect's body. It was, consequently, less liable to accident than the other legs which are used mostly for running, clinging, etc.
REGENERATION IN THE LEGS

For these experiments investigating the regeneration of appendages more than two hundred and fifty roaches were used. Of these about one hundred and ninety were subjected to operation in the second series, and of this group fifty eight were sectioned in the region of the coxa. The remaining individuals were sectioned at lower levels of the leg. Only three of the operations in the coxal levels of the leg performed in the first series gave desirable results, although the same operation was performed on sixteen individuals. The lack of opportunity to observe many cases of regeneration following injury to the basal portion of the leg was due to escape rather than to death resulting from injury. There were in this group only three individuals the deaths of which could be attributed to the operation. The outcome of the three successful experiments gave impetus to the investigation of the coxa as a probable factor in leg replacement. In the series of experiments which are still under observation, only four out of fifty-eight have died from the loss of blood before accomplishing a single molt. In addition to the roaches used in the above experiments, twenty-five specimens were selected to test the possibility of autotomy.

The regenerate appendage in most of the cases has been traced through three or more molts. The mortality rate was, on the whole, very low, except in the instance in which eighty roaches died from excessive heat before complete results were obtained. The greatest loss of specimens in the experiments was sustained through escape and accident. The majority of the animals operated on successfully regenerated new appendages, and
such individuals are still being watched carefully.

**Regeneration after Sections Proximal to the Coxa-Trochanter Articulation.** - When transverse cuts were made so that the legs were amputated above the articulation of the trochantin and coxa, as in roach 44 (Fig. 2 Pl. II), there was often great loss of haemolymph. The oily drop of fluid soon hardened, however, preventing further loss of blood. Shrinkage of tissue was not unusually great. Roach 44, which was injured on the right mesothoracic leg, molted for the first time after seventeen days. The time thus allowed for the development of the new appendage was short, so that there appeared only a small lateral lobe attached to the trochantin together with a larger inner portion (Fig. 3 Pl. II) which was believed to represent the coxa. The lobed portion possessed a few spines on its surface. Though expressing itself incompletely, the stump of the appendage had already begun replacing lost parts. The place of articulation was not at all well defined, and this was probably due to the fact that a portion of the articular sclerites, the trochantin and first antecoxa, had been removed. It was thought interesting to watch for further modification of the regenerated appendage since it was apparently bilobed.

After thirty-three more days roach 44 molted again, producing an appendage (Fig. 5 Pl. III) much smaller than its conuate mate, but nevertheless possessing a distinctly recognizable coxa, trochanter, and femur. The tibia was represented by a short stump. The more or less indistinct parts distal to this stump were broken off after adhering to the probe. The form of the appendage was indicative of a cramped and folded position during development beneath the cuticula, and suggested the fact that it probably hardened before it could straighten. The pleurally situated lobe, which was still present, had grown, and represented what seemed to be a sac which contained a stump of the flexor femoris muscle. The muscle was either absent or
present only as a rudiment in the regenerated coxa. The appendage was obviously incomplete, and its composition suggested that regeneration proceeds from proximal regions distally. This along with other information to be presented later in the paper, confirms the view that a reproduced appendage is not necessarily prevented from expressing itself because of its incompleteness. This is in contradiction to the view of Brindley (1898), p. 934, who said:

"The fact that non-reproduction was always total, (i.e. ecdysis left the limb in the same condition as at natulation) and that when reproduction did occur it was always complete (i.e. the several regions right down to its extremity were present and identifiable) are features generally found in Tracheataes."

The appendage (Fig. 7 Pl. IV) following the third molt, which occurred after thirty-six days, appeared complete, as far as is possible in a regenerated appendage, since it possessed the usual segments, and attained a size which compared more favorably with that of its mate. The lobe which had been traced since the first molt was still present, its attachment suggesting that it might soon grow into the coxa. The ventral surface of the regenerated coxa was slightly corrugated, and was free from the markings usually characterizing it. The trochanter was not perfectly articulated, was slightly furrowed, and like both the coxa and more distal segments, possessed few spines. The tarsus was four-segmented and possessed two well articulated claws between which was situated the small empodium. With the exception of the metatarsus, the segments of the regenerated tarsus were proportionately longer than those of the normal. The relative proportions exhibited by these segments of the reproduced appendage is typical. The regeneration of such an appendage by the part left intact suggested the possibility that the new appendage would attain a size equal, of nearly equal, to that of the unoperated, if time was allowed. At least, growth
had been more rapid in the regenerated appendage than in the normal.
Replacement of other segments after section at this level, except for
a few minor variations, gave results similar to those in the regenerating leg just described. These experiments show that regeneration can take
place in the absence of the coxa.

Sections were made on the leg of roach 45 at the joint between the
trochantin and the coxa, and regeneration from this level was studied.
The operation performed on the left mesothoracic leg was followed by a
large loss of body fluid. After the first molt, which occurred ten days
later, there appeared to be little more than wound closure (Fig. 9 Pl. V).
This was expected because of the region of injury, loss of blood, and time al-
lowed for growth of new tissue.

The second molt occurred thirty days later, and regeneration had then pro-
duced a coxa which was approximately half the size of the coxa on the right
mesothoracic leg. A furrowed trochanter which was articulated to the coxa,
and a rudiment of the femur constituted the remainder of the appendage (Fig.
11 Pl. VI). There were practically no spines and a very few of the usual
markings. The suture between the femur and trochanter was quite evident,
but the femur appeared to have been broken. It is conceivable that at the
time of the molt the femur adhered to the paper and was consequently frac-
tured, leaving the fragment of the femur.

A third molting about twenty-two days later revealed an appendage
much more complete (Fig. 13 Pl. VII). A close study of this leg showed that the
tarsus consisted of only three segments, but that the claws were present
and articulation was distinct. This condition is noteworthy since it was the only
case in which the tarsal segments were fewer than four. The coxa possessed
a few of the characteristic markings, but the number of spines on all seg-
ments was less than normal. The proportions of the major segments of the
leg, except in case of the tarsus, were not unusual. Since the individual escaped, the structure could not be traced through another molt to determine whether the tarsus would attain the four segmented form.

From a similar cut on roach 54 there appeared, following the first ecdysis, which occurred thirty-four days after operation, a new appendage consisting of a half-sized coxa to which was attached an imperfectly articulated trochanter. There was no apparent attempt to form more distal segments. After a second molt, however, there appeared a small leg (Fig. 15 Pl. VIII) which possessed segments distal to the breaking joint, and the proportions of the segments indicated the fact that effort had been made to complete the formation of the tarsus. Incidentally it may be noted that there were almost no spines present. After a few days the leg was broken at the suture between the trochanter and femur.

An appendage more than two thirds the size of its mate was produced after the third ecdysis, and this appendage (Fig. 17 Pl. IX) possessed, in addition to certain characteristic markings on the coxa, distinctly articulated segments which were well proportioned. The arrangement of spines, which were fewer than in the normal appendage, was relatively typical. The tarsus consisted of four well-articulated segments, in addition to an empodium and claws which were articulated with the fourth tarsal segment.

Other evidence definitely in support of the view that an appendage does not fail to show itself after ecdysis because of its lack of completion, was offered by the form of the regenerated appendage following section at the trochantin-coxal articulation on the right mesothoracic leg of roach 29 (Fig. 19 Pl. X). After the first molt, which occurred fourteen days following the section, the amount of regeneration was very small, as is frequently the case when there has been insufficient time for growth. After the
second molt, thirty-four days later, the leg (Fig. 19 Pl.X) was represented by a reduced coxa which bore two distinct distal lobes. These lobes were unarticulated, and were not thought of as miniature typical segments.

At the beginning of the third molt the appendage was larger and possessed, in addition to the coxa, a trochanter, a slightly furrowed and bent femur, a tibia, and a tarsus that was unsegmented (Fig. 21 Pl.XI). The appendage expressing itself in this case was obviously incomplete. The number of spines was subnormal, and distinguishing features of the typical segments were not prominent on the apparently useless leg. The animal was lost before accomplishing a fourth molt.

The left mesothoracic leg of roach 32 was removed at the coxa-trochanter joint seven days prior to ecdysis. This individual had lost all tarsal segments from all legs at some time prior to its capture and all of the right metathoracic leg below the proximal fourth of the trochanter. Although the extent of injury was relatively great, regeneration was typical in all respects. The size of the new legs varied in direct proportion to the amount removed. After the first molt which occurred ten days following the operation, the right mesothoracic leg was replaced and those legs which had lost tarsal segments regenerated four segments and claws. The lost mesothoracic leg had produced only a small amount of new tissue. More than half of the regenerated left mesothoracic coxa of roach 32 was removed after the second molt, and, following the third molt, regeneration had taken place, the segments being rather small. The regenerated leg was much smaller than the legs which had been regenerated after sections at lower levels. After the fourth molt the left mesothoracic leg (Fig. 23 Pl.XII) had been replaced to such a degree that it compared favorably with its mate, possessing many of the characteristic markings. There was, however,
a deformity, a small lobe at the point of origin of the extensor of the femur. The arrangement of spines resembled closely that of a typical appendage. The left metathoracic leg, also approaching the size of its mate, was very nearly typical, except for the four-segmented tarsus which it possessed. These results suggest that there is correlation between the extent of injury and the rate of replacement of new parts. Usually the part which has sustained greater injury does not complete its regenerative growth as early as the less injured portion.

Section through the lower third of the coxa produced results which were similar to those of the above experiments. There was, however, difference in rate of replacement. This difference depended upon the level of the cuts, surface exposure, etc. After the first molt, following such a cut taken on the right mesothoracic leg of roach 70, there appeared a very small amount of new tissue (Fig. 26 Pl.XIII). Only a few days had elapsed between time of operation and molting. The following molt, however, revealed an appendage which approached the size of its mate more closely than it did at the previous molt (Fig.28 Pl.XIV). The form of the coxa was slightly altered, but the chief divisions of the leg were easily recognized. The slightly curved tibia differed more widely from the normal than did the femur in both number of spines and size. The four-segmented tarsus possessed one incomplete joint between the third and fourth segments. Such regenerated legs as the one in question, though more or less altered in the early stages of growth, appear with the usual characteristics of the regenerated appendage if sufficient ecdyses occur before cessation of general growth.

Sections were also made at the joint between the coxa and trochanter. In one instance at the time of the section at this level, a V-shaped portion including some of the flexor femoris muscle was taken from the outer border of the coxa of roach 75 (Fig. 31 Pl.XVI). After thirty-three days
a molt occurred and the regenerated appendage was characterized by a coxa which was ventrally concave where the V-shaped portion had been removed (Fig. 32 Pl. XVI). This slightly altered the main axis of the leg, and prevented the flexor femoris from developing and assuming its usual position in the regenerated leg. All of the major divisions of the leg were restored, the regenerated tarsus being typical. The number of spines was below normal.

In many cases after similar cuts, an almost typical appendage was observed following the first molt. For example in roach 73, after section at the coxa-trochanter articulation (Fig. 30 Pl. XV) and subsequent to the first molt thirty-two days after operation, the new leg was similar to its mate. It possessed fewer spines and smaller segments, but the trochanter seemed to be the only segment possessing corrugations. The tarsus was four-segmented, but the proportions of the segments differed from those of the normal five-segmented tarsus, their greater lengths partially compensating for the decrease in number. This is usually true of the regenerated tarsus.

In addition to the study of regeneration following transverse sections at various levels of the coxa, it is of interest to note reproduction of parts following oblique sections. The left mesothoracic leg of roach 37 was cut obliquely, the cut removing a small portion of the outer edge of the coxa with the trochanter and segments distal to the trochanter (Fig. 33 Pl. XVII). After the first molt, which occurred nineteen days later, the following regenerated segments were observed: a malformed trochanter imperfectly articulated to a femur, the latter being shorter and smaller than its mate and bent ventrally, a curved tibia which was smaller and possessed fewer spines, and four imperfectly articulated tarsal segments (Fig. 34 Pl. XVII). The portion of the coxa which had been destroyed
was replaced but it lacked the characteristic markings. It had been noticed at the time of operation that the extensor of the femur was cut so that its insertion was lost. The fact that the segment could be extended showed that in the new leg the muscle was reinserted at the usual point in the leg. The five incomplete joints of the tarsal segments may indicate an attempt at the establishment of the normal tarsus. The leg was, however, not very active, and after eight days it was broken at the suture between the trochanter and femur. It was, therefore, not possible to determine whether the five-segmented form of tarsus would be perpetuated, although it seemed probable.

Other oblique cuts were made, as can be seen from Fig. 35 (Pl. XVIII) and Fig. 39 (Pl. XX). Newly regenerated parts showed that more tissue was produced from the proximal end of the cut. If the new tissue had at first grown out at right angles to the cut, the direction must have been changed rather early by a faster rate of cell proliferation at the proximal end of the cut, for an appendage in which the new growth seemed to come off at an angle from the cut surface was never observed.

An example of this type of regeneration may be studied in the appendage of roach 42 which, after the first molt, had simply reproduced the amputated portion of the coxa (Fig. 36 Pl. XVIII). After the second molt, however, the usual completely reproduced leg appeared (Fig. 38 Pl. XIX), but hardened before it had completely straightened. The articulations were perfect with the exception of that between the third and fourth tarsal segments. The leg was not heavily armed with spines.

In the case of the appendage from roach 38, selected for illustration, the portion of the coxa removed (Fig. 39 Pl. XX) was replaced after the first molt, and close examination of the less heavily pigmented area indicated that there had been greater regeneration from the proximal end of the cut. Typical markings characterized the coxa, while no attempt
to form a trochanter was made. After a second molt, however, the appendage, about two-thirds the size of its mate, possessed a furrowed trochanter, femur, tibia, and a four-segmented tarsus. A third molt revealed an increase in the size of the appendage (Fig. 40 Pl. XX), but the tarsal segments were abnormally large and the last two were imperfectly articulated. Additional ecdyses gave expression to an appendage which was more typical and which approached the size of the corresponding leg more closely.

To summarize the result of experiments so far discussed, it may be said that cuts were made at all levels of the basal portion of the leg from the antecoxal pieces to the distal end of the coxa, and regeneration was obtained in all cases in which individuals were retained for more than one molt, and, in some instances, depending on the region of the cut and the time allowed for growth, the new appendage appeared at the first molt. It seems, from the results mentioned thus far, that regeneration begins in the proximal region and proceeds distally. Also if the appendage has not completed its growth, a regenerated portion appears at the next molt even though it is incomplete in form. The appendage as it first appears is often corrugated and twisted, but many nearly typical appendages were observed, especially when the cut was made in the more distal regions of the coxa sufficiently long before molting. A comparison of the rates of replacement of parts following section through various levels of the coxa indicates earlier completion of the appendage in which the cuts are more distal. It is readily seen that the loss of blood, together with the amount of new tissue to be replaced, is greatest as the level of cut approaches the body. These experiments suggest that there is no localization of power to reproduce lost segments in any particular region of the coxa. Furthermore, regeneration from the joints themselves is not necessarily more complete than
that from regions between joints.

Regeneration from Sections through the Trochanter.—Regeneration of appendages following cuts through the trochanter are also of special interest, since some authors have suggested that certain Arthropoda do not regenerate appendages if section is made above the suture. In some cases sections through the trochanter were followed by formation of a thick scar, which extended back to the joint between this segment and the coxa, from which regeneration then took place. However, in many instances regeneration took place from a point just proximal to the cut surface, and the appendages produced were typical regenerated appendages except for the corrugations found midway of the trochanter. The loss of fluid following such sections was not particularly great, and regeneration from such cuts was not less complete than that from the breaking joint. It occurred readily, and the part replaced was not obviously smaller than the appendage produced as a result of regeneration from the so-called point of adaptation. This was shown by removing two mesothoracic legs at the same time, one at the breaking joint, and the other midway of the trochanter.

The completeness of regeneration from this point can be observed in Fig. 42 (Pl.XXI). The appendage, taken from reach 80, differed from the normal only in tarsal segments, number of spines, and corrugations, or furrowed regions, marking the place from which regeneration began. Articulations of segments were complete. This well formed appendage appeared following the first molt which occurred twenty-nine days after operation. An additional molt revealed an appendage nearer the size of its conurate mate and free from the corrugations. The proportions of the tarsal segments in the regenerated appendage differ from those of the normal in that the middle segments are longer than the normal. They are heavily
armed with spines, but not so heavily as those of the normal appendage.

Regeneration following a transverse cut in the distal half of the trochanter of reach 32 (Fig. 43 Pl.XII) revealed at the first molt, eleven days after operation, a portion of the leg divided into four distinct parts (Fig. 44 Pl.XII) which were unarticulated except at the coxa-trochanter joint. The two distal divisions suggest an attempt to form portions below the trochanter. This condition again offers evidence opposed to the view that regenerated appendages express themselves only in a complete form. The appendage became more complete as growth proceeded until, after the fourth molt, the individual had produced a new leg (Fig. 46 Pl.XIII) typical in every respect. Expressing itself at first as an in-complete part, it gradually became more perfect as molting continued and is at present closely approaching the size of its mate.

Regeneration following Sections through the Suture between the Trochanter and Femur.— It was expected, in view of the various theories concerning autotomy and regeneration in Arthropoda, that the appendage reproduced from this point would exhibit a more typical and complete form both in size and in articulation of segments. This was found, however, not to be true in this insect. The appendage (Fig. 48 Pl.XIV) produced from this point was in the end not more complete than one regenerated from the joint between the coxa and trochanter. The complete reproduced appendage appeared earlier than those regenerated from the coxal cuts, probably because of the fact that less injury to tissue was sustained and loss of blood was reduced.

When operations were performed on different legs at a given time and at the same level, as for example, from the breaking joint, the regenerated legs were about the same, both in structure and in size. From identical cuts at higher levels variation in amount of regenerated
tissue was more common.

Reconnaissance after Section at various levels of the Femur.—Section of the femur at any level distal to the breaking joint and proximal to the femoro-tibial articulation injured both muscles which affect movement of the tibia. After all such cuts partial or complete replacement of the muscles located in the tibia was necessary. Regeneration following such sections depends upon the level at which the injury was sustained. Usually, if less than one-fourth of the femur remained, it happened that the tissue dried back to the breaking point from which reproduction proceeded. In some instances, however, as little as one-fifth of the femur was left after amputation, and the operations were followed by regeneration of new appendages which were not different because they had been reproduced from different levels. Appendages replaced following sections through the femur were in no respects less complete than those replaced from the breaking joint. If more than one-half of the appendage was left after the operation, the new appendage, in certain cases, was found to be larger than the one produced from the breaking joint. The difference in size was, however, slight. Such differences in size of the said appendages would be expected, unless it happened that accelerated growth in the more injured leg was of such nature as would accommodate the need for more tissue in the same length of time required for the replacement of the less highly injured leg. It seems that the rate of growth of new tissue may be greater at sections midway of the femur because of the fact that transverse cuts here expose more tissue. If so, this accounts for the slight difference in size of the regenerated leg resulting from cuts taken midway and beyond the middle of the femur. Such cuts were made and a comparison of the two regenerated legs revealed small differences in size. Fig. 50 (Pl. XXV) illustrates the form of a new leg after section through one-half of the femur.
Regeneration from Section through the Tibia. — The new parts reproduced following cuts through the tibia were, after the first molt, more complete with reference to size than those appearing after the first molt following section at the breaking joint. Fig. 52 (Pl. XXVI) shows the appendage of reach 39 which was amputated midway the tibia twenty-five days prior to ecdysis. The leg, though a typical regenerated appendage, approaching its fellow in size, was distinctly smaller. If less than one-third of the tibia was left after operation, regeneration usually occurred from the next proximal joint.

It appears, therefore, that regeneration takes place at different levels of the tibia as at levels above, that the appendage produced is a typical new part, and that there is no definite point of localization of the regenerative capacity. It is rather surprising that abnormal appendages were not produced, since there is relatively little space for the developing appendage in the tibia.

Regeneration after Section through the Tarsus. — It has been noticed that reproduction of tarsal segments takes place if the injury is sustained at the tibio-metatarsal articulation. Although cuts were not made below this point in the first series of experiments, a checking of Brindley's conclusion concerning the possibility of replacement after injury to various segments of the tarsus seemed desirable. Brindley (1898) said, p. 929:

"Regeneration may commence at this point (meaning femoro-trochanteric suture) or from the stump of either femur or tibia, but not from any part of the tarsus. The stump of this latter region may be retained for some time after mutilation, but it is invariably dry and shrivelled."

In the second series a dozen individuals were selected for these experiments, half of which were injured on more than one leg. It is
difficult to study regeneration following section through the tarsus because these segments are easily broken off after injury. Sections were made at every joint between the metatarsus and the claws. After a cut at the first tarsal joint, the metatarsus on one roach was carried about in the dried condition until the next molt, regeneration occurring from the tibial. In four cases the remaining tarsal segments were lost at different times, but in five cases very interesting results were obtained. The tarsus in three instances was reproduced after section just beyond the articulation between the fourth and fifth segments, but the new tarsus possessed only four segments. Fig. 54 (Pl.XXVII) shows regeneration following section through the distal end of the fifth tarsal segment, in which instance, that portion of the end segment which is drawn into the fifth remained. The experiments, performed on Periplaneta americana because of the well-formed empodium between the claws, showed that reproduction could follow such cuts. In this insect there appeared a five segmented tarsus equipped with a terminal empodium and claws, but the new claws were smaller than the normal, and were abnormally pressed together. The claws seemed to project ventrally from the distal surface of the empodium. It is probable that this may have been caused mechanically, owing to the very small space in the tarsus which could accommodate growth of a new part, or the cause may have been physiological, since regenerating appendages develop under conditions that are not normal. Regeneration was complete despite the modification in form of the new part. Reproduction following section at the joint between the fifth segment and terminal portion was complete, and in three instances gave rise to a normal empodium with claws.

These experiments show that regeneration follows section through the tarsus, if the remaining tarsal segments are retained and do not become
dried. Evidence has been obtained from these experiments which shows that there are instances in which the new leg appears complete after injury. These experiments are to be continued as further interesting results are anticipated.

**Disposition of the Regenerated Appendage before Ecdysis.**—The form of the replaced parts prior to their appearance at ecdysis was studied frequently, and several satisfactory demonstrations were obtained. By studying the amputated coxa containing the regenerating appendage at various periods before molting, it was observed that the reproduced portion assumed a more or less contorted position beneath the cuticula.

It was observed that as growth of the new appendage proceeded beneath the cuticula, there was lack of room for expansion; hence the new appendage assumed a rather cramped position, gradually folding back on itself. The muscles in the coxa are apparently rather closely packed, so that there must be either more available space in the coxa than is evident, or reorganization of old tissue so as to render available more space for the developing segments. This increase in space may be made possible by a loss of fluid from the old muscles.

The regenerating appendage of roach 90, shown in Fig. 56 (Pl. XXVIII), was taken from an individual which, prior to its capture, had lost the portions of the leg distal to the breaking joint. Its position in the coxa indicated that the new tissue had, as a result of pressure exerted in the region of the scar, begun to fold back on itself into the space available in the coxa. It was evident that the material already occupying the coxa was being crowded as growth proceeded. The age of the regenerated appendage was unknown since the animal was captured in this condition.

A study of an individual (Fig. 55 Pl. XXVIII) which had produced a new appendage following section at the breaking joint of the right meso-
thoracic leg after approximately thirty days shows that the new appendage, which is obviously older than that shown in Fig. 56, has, after folding back, grown so that it now occupies about one third of the coxa. The trochanter, femur, and tibia can be distinguished readily, but the segments of the tarsus are not well defined, although discernible. The femur is partly hidden by the coxa and small spines can be seen on its ventral surface. The spines of the tibia are quite evident. It is obvious from the segments seen that the new appendage is rapidly nearing completion. The relative position of these segments in the coxa has not been altered by dissection or fixation since the study was made immediately after its removal from the animal's body.

Another such study was made on the left mesothoracic leg (Figs. 57 and 58 Pl. XXIX) of the same individual. The appendage, before removal for examination, had been almost completely following previous amputation at the breaking joint. The time elapsing between injury and removal for examination was thirty-six days. This leg might serve as a good specimen for comparison since the amputation was made about one week after the observation of the right mesothoracic leg which had been cut at this level on the same date. The scar on this leg was rougher and larger. The tarsus was more fully formed and the empodium and claws were quite distinct. Segmentation was more complete and clearer. The appendage was drawn in position from both a dorsal view (Fig. 57 Pl. XXIX), and a ventral view (Fig. 58 Pl. XXIX) in order to demonstrate the relationship between segments. The tarsus has folded back upon itself and extends partially into the coxa. The new appendage obviously occupies a portion of the space previously filled by the muscles of the coxa, so that unless there is considerable expansion just after ecdysis one may expect the new coxa to be much smaller than a normal one. However, if there is a difference,
it is certainly so slight that it is not detectable.

Many specimens were examined to determine the form of the regenerategenerated
appendage beneath the tibia after removal of the tarsus. Fig.59 (Pl.XXX)
shows the form of the new part in roach 94 after separation at the tibio-
tarsal joint and before a dissection of the tibia had been made. No fixing
reagent was used. The part coiled about itself could be seen clearly be-
neath the old cuticula, but it was difficult to study segmentation and to
ascertain whether the claws and exoskeleton had been completed. The diagram
shows the natural arrangement of the new segments after they have grown
for forty-three days. A dissection of the tibia (Fig.60 Pl.XXX) disclosed
a tarsus which was four-segmented, though the most distinct points of articu-
lation were the joints between the tibia and metatarsus, and between the
claw and fourth tarsal segment. The other joints, though faint, could be
recognized. It is believed that development was still in progress at the
time of observation.

A study of an appendage five days older than that shown in Fig.59
(Pl.XXX) was made on roach 95 and the parts were diagramed Fig.61 (XXXI).
The essential difference to be noted is that articulations are more distinct.
The appendage must be viewed from both sides in order to study all seg-
ments effectively. Those segments which are covered by others are repre-
sented by the dotted lines in the figure.
AUTOTOMY

The phenomenon of autotomy has been mentioned frequently in the present paper in connection with regeneration, not because the writer is convinced that such self-mutilation follows injury of the roach, but because of its rôle in connection with reproduction of legs of Arthropoda generally. Since it was found possible to effect separation relatively easily by pulling the segments, it was thought necessary to examine the insect for possibilities of autotomy. It had been observed during the course of experimentation that whenever separation occurred, it either took place at the suture between the trochanter and femur or at the tibiotarsal articulation. Separation apparently resulted from mechanical pulls exerted at certain points along the leg. In order to examine the insect with reference to the breaking off of its appendage at the suture (commonly called the breaking joint) certain of the experiments which have been performed on other Orthoptera now believed to throw off limbs were tried on the roach, Periplaneta americana.

Bordage (1897) performed experiments on Phasmids relative to their ability to throw off limbs. During the course of his experiments, he noticed that ants attacked certain of his experimental animals and caused them to throw off the limbs at the breaking joint. This autotomy was attributed to stimulation caused by formic acid which he believed given off by the ants, and not to biting or other injuries. Autotomy was evoked in the insects by stimulation of the legs with hot needles. In certain instances the legs were burned to mere stumps without being thrown off which fact suggests that autotomy is not entirely dependent upon the intensity of the stimulus.
In order to test the effect of formic acid on roaches, fifteen animals of varied ages and sizes were selected and subjected to the treatment. The acid was applied to various regions of the leg, and roaches always responded violently, but not to the extent that the leg was thrown off. Individuals thus treated were placed in a smooth glass bowl so that separation because of contact with a rough surface might be avoided. Even when the animals were cut and subsequently treated with acid, they did not respond to the treatment by throwing off their legs. On one occasion ants found their way into some of the containers and interfered with the animals, causing death in some cases, but in no instance did the observer find that appendages were cast off. Individuals which had been given the acid treatment were watched carefully for some time following the treatment and the appendages were retained even though they were repeatedly stimulated with formic acid.

If the roach is held by one of the appendages and pinched it frequently breaks away leaving the appendage in the hands of the experimenter. The appendage, though usually separating at the suture, sometimes divides at the tibio-tarsal joint. If, however, the animal's leg is pinched and released instantly, breaking does not take place, which suggests that the appendage must be held in some way in order to insure separation. The point of major interest here is that division may be affected at two points under the necessary conditions, but such separation is not usually considered autotomy.

Ten animals were stimulated by a hot needle in order to determine whether or not they could throw off appendages under very powerful stimuli. The sizes and ages of the individuals varied as in the above roaches. Results indicated that individuals would not throw off legs even when the irritation was exaggerated. Many legs were burned to mere stumps without being thrown off.
In some cases portions of the leg were dried under the influence of the hot needle and in the course of a few days the leg had lost certain of the burned portions. Since the appendage was very infrequently lost at the breaking joint it was thought by the author that the separation was due to cracking of a useless dried part.

The fact that very few appendages became separated during the experiments on regeneration in the legs of the roach, although many of the operations were performed without the aid of an anaesthetic, indicates that separation must be aided by contact so that the animal is able to pull away from the leg. The possibility of a physiological separation at the breaking joint, the uninjured part retaining its morphological connection with the uninjured, has not been overlooked, but it seems that if such a separation occurred one should expect regeneration to take place from the point of physiological separation. In most cases, however, as was shown both by the experiments on regeneration and those on autotomy, regeneration took place from the section at the level of the cut.

In addition to the above we are confronted with the problem of where the separation in autotomy should take place since there are two possible points of separation. Following injury to the tarsal segments it is rather difficult to study regeneration because of the fact that all segments are frequently lost within a few days. Typical self-mutilation was not observed in these segments so that the loss of segments may have been caused by drying, or healing, following physiological separation. The former seems more likely since in cases in which the appendage was retained and did not become dried, regeneration took place from the level of cut, rather than at the point where a physiological separation might have taken place. It may be argued that the phenomenon, while not occurring frequently, may have taken place in instances where the appendages
were not retained. The possibility of both physiological and morphological separations is not denied but the results of these experiments do not seem to support such a view. It can be said with definite assurance that there are two points in the log of a roach where the log may very easily be separated by a mechanical pull, and it seems that if the insect possesses the power of autotomy, it might take place at one or both of these levels. If it were possible for autotomy to take place at both points, it would certainly be unusual.

If the roach possesses the power of autotomy, that power seems not pronounced, and cases which could be recognized as self-mutilation did not occur in these experiments.
DISCUSSION

The first point of significance here is that regeneration of portions of the leg of a roach may take place from different levels between the body and the tip of the leg.

Comstock (1931) as has already been noted, suggested a correlation between the integrity of the coxa and the ability to replace injured appendages, stating that there had been no cases observed in which reproduction of the entire leg had taken place. As a result of his observations on spiders, he concluded that the original coxa must be left intact in order to insure reproduction of lost parts. Brindley (1898) considered the power of regeneration in most Arthropoda limited, since he was unable to observe regeneration in the absence of the basal portion, the coxa.

Regarding the question of regeneration of lost parts, Brindley says, p. 925:

"If an appendage is wholly removed from the body it seems that reproduction never occurs. Thus in a Crustacean the coxopodite of an appendage, in an Insect the scape of an antenna or the coxa of a leg, must be left intact if there is to be reproduction of the extremity. An appendage is therefore never reproduced as a whole by the trunk, but the event is really the formation of a new extremity by a larger or smaller basal portion."

The larger or smaller basal portion referred to may mean the coxa alone, or the coxa and various distal portions. If he meant larger or smaller portions of the coxa itself, his statement would be self-contradictory.

In opposition to the statements of Comstock and Brindley, Child and Young (1903), using Agrionid nymphs, have shown that regeneration may take place at any level between the body and the tip of the leg, and that in most cases, depending upon the level of cut and time allowed for growth,
the appendage is perfectly completed. The contrast in these cases is
twofold: the appendage may be reproduced completely; and it regenerates with-
out the coxa. Child and Young are consequently of the opinion that there is
lack of localization of power to regenerate lost parts at any level of the
leg in Agrionida. Mégueur (1907) found that replacement in the absence of
the coxa was possible in beetles, the degree of success depending upon the
conditions of the operation.

This investigation of certain species of Blattidae offers evidence
in support of the view that regeneration may take place in the absence of
the coxa. It is obvious that injury in the upper levels of the coxa and
above is more extensive, and that the loss of body fluid which is very
necessary in nourishing the regenerating tissue is greater. It is probable
also that disintegration of tissue is more extensive; consequently one may
expect retardation in development. The appearance of new parts in the prox-
imal regions of the leg may be delayed by variations in the level of cut and
time allowed for regeneration so that differentiation and appearance of new
segments becomes progressive. The regenerated legs illustrated in Fig. 3
(Pl.III) and Fig. 7 (Pl.IV) give support to this view. After the first molt,
as indicated in Fig. 3, there was little more than healing of the wound.
There appeared, however, a small portion of new tissue, directly proportional
in size to the time allowed for readjustment and growth prior to the molt.
Additional ecdises revealed new parts in progressive sequence. The fact
that these parts appeared after molts despite their obvious incompleteness
is contradictory to the view of Brindley (1898b), who believed that the new
part would not appear unless regionally complete; that is, the principal
regions from the basal portions to the tip of the leg. It is essentially
in agreement with the conclusions of Child and Young (1902) who pointed
out the fact that very often more than one molt is necessary before the
the limb will be completed. The outstanding difference in regenerated parts between their Agrionids and the specimens used in this investi-
gation is that the legs of Agrionids were completely reproduced, whereas in the roaches the tarsus had four segments in contrast to the five seg-
ments of the normal tarsus.

Regeneration in roaches occurred from various levels of the proximal portion of the leg between the cut through the antecoxal pieces and the distal part of the coxa. There seems to be no reason why regeneration could not take place in the complete absence of the antecoxal pieces, un-
less either excessive bleeding results in death, or there is localization of the capacity to replace parts in the proximal regions of the antecoxa.

In general the appearance of the leg or regenerated part after molting depends upon the degree of development reached prior to the molt. Re-
generation of the new part continues after molting until a leg possessing a four-segmented tarsus has been completely developed, provided there has not been cessation of general growth. The peculiar structural characters of the first part to appear are not usually perpetuated throughout the growth period.

The condition of legs regenerating from the coxal region depend upon the level at which the section is made, the rate of individual growth, and length of time between the operation and ecdysis.

In agreement with the phenomenon of regeneration in other insects, the degree of replacement of lost parts is also influenced by such factors as shrinkage, disintegration of tissue, loss of fluid, age and physical condition of the individual, temperature, food and moisture.

Plates II - XXIII show parts regenerated from various levels above the suture which has been considered by some investigators as the point proximal to which regeneration of injured appendages can not take place.
It is seen from these figures that reproduction is possible in roaches, not only from sections through the trochanter at a level slightly above the suture, but also at levels far above the suture, as for example the antecoxa. Although the injury sustained in a cut through the antecoxal pieces was great, a new leg possessing all major divisions was produced after the third molt (Fig. 7 Pl. IV). Legs regenerated from cuts midway of the trochanter (Fig. 42 Pl. XXI) were as complete as new legs appearing after section at the suture (Fig. 48 Pl. XXIV).

In regard to the question of localization of the power to reproduce legs, Weismann (1904) states that it is quite evident in Insecta and Crustacea, and assumes that the "regeneration primordium" is located in the suture between the trochanter and femur of certain forms, because regeneration followed autotomy at the suture rather than reproduction from a distally torn or cut part. He suggests that the phenomenon is similar in Phasmids, with the difference that in the latter there are three primordia, one in the suture, one in the tibia, and one in the tarsus at the tibio-tarsal joint. Each of these primordia, according to Weismann, should possess the power of regenerating only parts distal to it. This means that the cuts would have to stimulate individual primordia, and that if a primordium located at the suture were stimulated, the determinants of the segments distal to the suture would effect the activation of growth. Assuming that there is localization of the primary constituents of the "regeneration-idioplasm", he raised the question as to how these constituents were able to reconstruct segments in such exact proportion and detail. He suggested that certain determinants may have been aroused to activity by the cut and in turn stimulated the determinants of the succeeding parts in an orderly sequence. This explanation was not satisfactory to Weismann, and it is certainly not an adequate explanation of regeneration in Blattidae because there is one segment missing
in the reproduced tarsus, and it is not the distal segment. Weismann dismissed the matter with the statement that there are certain internal directive forces which we are unable to define.

Since reproduction did not occur from other levels, Bordage (1899) concluded that the regenerative power in Phasemis is located in the tarsus, in the distal third of the tibia, and at the suture, but Godelmann (1901) obtained regeneration in the legs of Bacillus rossii, both in the proximal region of the tibia and at the joint between the femur and tibia.

Regeneration was obtained by Child and Young (1903) from all levels of the legs of Agrionida, and by Megusar (1907) from various levels of the legs of beetles.

In this investigation regeneration has been observed at many levels between the episternal sclerite and the claws. The majority of the appendages produced have attained a size practically equal to that of the normal mate, and for the most part, the regenerated tarsi have possessed only four segments.

Since it has been noted that reproduction occurs after injury to the various regions of the leg, it seems that, if this capacity be considered as dependent upon certain regenerative primordia, it must also be assumed that such tissue is located in all regions rather than at particular points in the leg. It appears more reasonable to assume that the hypodermis at any point below the most proximal cut (in these experiments) is endowed with capacity to replace parts distal to the cut surface, being stimulated to proliferate either by such factors as the cut and brief exposure, or by something produced at the level of the cut which might initiate development. The possibility of localization cannot be dismissed, however, because it is possible that such a primordium is located above the most proximal cut that has been made in these experiments. If this is true, it would seem that
cuts below the point of localization would fail to stimulate the primor-
dium as appeared to be true in the experiments of Bordage. Bordage failed
to get regeneration at certain other levels both above and below the non-repro-
ductive points. In the present experiments, however, tissue was always
stimulated to growth by the cut, regardless of the level at which section
was made. It appears, therefore, that there is no localization in the leg
of a roach, since there was no region in which the cut failed to stimulate
growth. The difficulty of establishing definitely localized points from
which reproduction may be induced is readily realized when one considers
such instances as these in the present investigation.

In the posterior limbs of Orthoptera saltatoria Bordage has noticed
a total absence of the power to reproduce parts. His explanation, which has
been considered in the review of literature, does not present a satisfactory
reason to reproduce limbs. It seems unreasonable to assume that environ-
mental difficulties which might eradicate certain animals would eliminate
the need for the regenerative capacity. An instance in which animals often
beset with great difficulties have retained the power to replace lost members
may be recalled in the experiments of Cameron. Cameron's:
experimental animals, *Scutigera forcaea*, survived when every leg was missing.
Such great injury has probably occurred frequently in these animals while in
their natural surroundings, since injured legs are always thrown off by these
animals. The improbability of survival that confronted Bordage's animals,
(Orthoptera) which had lost only the jumping legs, is certainly no greater
than that where animals have been rendered unable to move through a loss of all legs. Concerning Bordage's view that animals possessing the ability to regenerate jumping legs have been eliminated through natural selection, the question is raised as to why animals possessing a characteristic which is not a liability were eliminated, whereas those developing under the same difficulties without the capacity to regenerate survived.

Regeneration from oblique sections exhibits a faster rate of growth in the more proximal regions, which fact suggests the operation of a tropic control. The phenomenon of difference in rate of growth from oblique sections in other animals has been studied by Morgan (1902, 1906). He points out the possible existence of certain formative factors which may be considered "pressure" or "tension" relations. These relations which inaugurate growth and determine the rate at which the new part is regenerated account for the difference in rate of replacement along the oblique cut. Mabrit (1929), considering the question of oblique cuts, suggests that in Fundulus and the goldfish this faster growth in the proximal region is due to greater surface exposure of fin ray material.

In the leg of the roach there is greater surface exposure of hypodermis in oblique sections, but whether the difference in rate of replacement is governed by the difference in the size and number of hypodermal cells exposed along the level of cut, or whether there is a definite retardation ("holding back") of development in the distal region of the cut due to pressure or tension relations, nothing definite can be said. The former interpretation seems more likely. Whenever a section is made through the leg, there is an upset in the normal equilibrium. It seems possible that either this disturbance, or the action of something produced at the injured surface by the tissue in that region, may initiate growth of new parts. If the disturbance is physiologically more intense at the proximal
end of the cut, the degree of disturbance which would stimulate growth from the cut surface might cause more rapid proliferation of cells in the proximal regions. Furthermore, if a certain primordium which possesses the power of influencing replacement of lost parts is localized at some point above the regions which have been injured in these experiments, it is conceivable that stimulation of cell proliferation will be greatest in those regions of the cut nearest the source of the influence.

There is also the possibility that the operation of a special control mechanism causes acceleration of growth in the more proximal region, or retardation of growth in the distal region, or both. Inasmuch as the regenerating part is not developing under normal conditions, it is obvious that many factors must be considered in trying to explain abnormalities, such as the bilobed appendage in Fig. 3 (Pl.II), and variations in the reproduction of parts from sections at the same level. In some instances, as in Fig. 19 (Pl.I) and Fig. 44 (Pl.XXII), the part reproduced possessed incomplete and unarticulated joints so that almost none of the segments could be definitely identified. A study of the disposition of the regenerating appendage before ecdysis reveals the conditions under which new parts are produced; consequently it is not difficult to see how such abnormalities may result.

Autotomy in the sense of immediate self-mutilation through a special reflex mechanism depending upon the integrity of the nervous system has not been observed in these specimens, but regeneration from the suture has been studied, both after tearing, and after cutting the limb apart at this point. The reproduced parts have been studied in Fig. 48 (Pl.XXIV), where it is seen that the only part missing is one of the tarsal segments.

This suture has been considered an adaptation for autotomy and subsequent regeneration by such investigators as Geismann, Godelmann, and
Bordage. In opposition to their view, Child and Young (1903) admit no such adaptation for autotomy in Agrionida, and doubt the existence of such in other cases on a basis of insufficient evidence to warrant the conclusions reached.

In the specimens used in these experiments the appendages produced from the suture are not usually more complete and larger than parts produced from other sections, and in some instances, are smaller and even less complete. If a portion of the last segment is removed, it may be replaced so that the leg appearing at the next molt is a complete leg, but if the cut is made at the suture, the regenerated tarsus is apparently always incomplete in number of segments. The appendage reproduced from the suture in a given period may be smaller than that produced from a cut at a lower level in a similar period. The essential difference between cuts taken midway of the trochanter and at the suture seems to be in markings which characterize the regenerated trochanter, the degree of development of the new leg being about the same in a given period. In the light of regeneration from this point as compared with that from other levels, it seems unwise to regard this point as specially adapted for regeneration.

Child and Young (1903) have suggested a correlation between the development and attachment of muscles, and the appearance of joints in Agrionida, especially in regards to the appearance of articulations in the tarsus. They point out that muscles are usually differentiated after the regeneration of the leg is well advanced, and that articulations appearing first in the upper regions of the developing appendage are seen only if muscles become inserted at the various points. The appearance of joints except in the tarsus was progressive, the direction depending upon the "order of differentiation and attachment of muscles". In the tarsus the distal joint appeared first and was followed by the proximal, but if muscles
were not inserted, articulations failed to appear.

Brindley (1898) stated that regeneration from the tarsus was not possible in roaches, adding that, though it may be retained for a few days after operation, it always becomes dry and shriveled. This contradicts certain data obtained in the present investigation. Fig. 54 (Pl. XXVII) shows the reproduced portion after section through the distal part of the fifth tarsal segment. The empodium and claws were reproduced, but the claws seemed to project from beneath the empodium rather than at angles as in the normal leg, and they were smaller than the normal claws. This condition was obtained in two instances. In one instance the reproduced claw appeared normal. Although it is possible that other factors were involved in the production of the abnormal claw, it seems probable that the lack of space for the developing part may have been the chief cause. In instances where the section was made through the joint between the fifth and terminal portions of the tarsus, the normal empodium and claws were reproduced without reducing the number of tarsal segments. There may be variations in the regenerated tarsus such as incomplete articulations, lack of spines, etc., but usually the tarsus produced appears normal except for the absence of one segment. Careful study of the regenerated tarsus shows that when it is compared with the segments of the normal tarsus there is a difference in the proportional size of the segments; the reproduced segments being relatively larger.

Giard, while collecting information concerning "hypotypic regeneration", attempted to explain the four-segmented condition of the tarsus as "atavistic". His view must have been based on observation of certain forms such as Locustidae, which normally have four tarsal segments. Brindley (1898) feels, as does the writer, that there is insufficient information to justify a belief

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in reversion to ancestral forms. If we accepted this view, we should have to explain the fact that the regenerated tarsus of the Agrionida of Child and Young, and that of Bacillus rossii of Gudelmann often possessed five segments (normal). It is not absolutely clear whether the tissue appearing first forms the base or the tip of the regenerated leg, but a morphological study of various stages in the development of the new leg after extensive injury suggests regeneration of the proximal regions first. However, the segment missing in the tarsus is neither the distal nor the proximal, but one of the intermediate segments. A more thorough understanding of the appearance of articulations may throw valuable light upon the matter.
SUMMARY

(1) Following section of the legs of the roaches, *P. americana* and *Blatta sp.* (?), regeneration takes place from the cut surface.

(2) Regenerated structures may be expected to replace injured appendages as long as general growth is possible. With cessation of general growth, the power of reproduction is no longer evident and the appearance of the regenerated leg in the imago depends upon the extent to which development has proceeded prior to the final ecdysis.

(3) For regeneration to succeed injury it is not necessary that the coxa be left intact, replacement of parts being possible from all levels of the coxa.

(4) The new leg reproduced from the basal portions is not always complete with respect to regions, and, depending on the degree of injury, may require one or more ecdysis for completion. The leg produced after sections through proximal regions of the coxa is usually small and useless at first, but becomes functional after additional ecdyses.

(5) There is a direct relation between such factors as degree of injury to tissues, loss of blood, level of cut, time of operation prior to ecdysis, and completion of the leg. The leg regenerated from a proximal section, where the tissue is greatly injured, loss of blood is heavy, and the time of operation before ecdysis is short, is likely to be less complete than one reproduced from an appendage less seriously injured immediately after molting has occurred.

(6) The new part becomes revealed at ecdysis whether complete or incomplete. The morphological features of the regenerated part first appearing
are not perpetuated throughout all stadia because of the fact that there is further regeneration in the later stadia. Indistinct articulations appearing at the first molt may become distinct (complete) with additional ecdysis.

(7) Young individuals are more likely to produce an entire leg than older individuals because of the time available for growth and development.

(8) If the operation is made sufficiently early in the developmental stadia, a full-sized leg may be produced, differing from the normal only in the segmentation of the tarsus and the number of spines. The number of spines of the reproduced leg is usually less than normal, the degree depending upon the injury sustained. There is accelerated growth in the injured leg and this continues until the regenerated appendage attains a size approximately equal, or equal, to that of its con specific mate.

(9) Successive amputation of an appendage does not alter the capacity to reproduce, but may hinder or prevent the production of a full-sized leg.

(10) The four-segmented condition of the tarsus as it first appears is perpetuated throughout the stadia without increase or decrease in number of tarsal segments, although such a tarsus may attain a size either approximately equal or equal to that of its five-segmented mate, and in this respect the stability of this division is comparable to that of the corresponding normal tarsus. In completely regenerated legs unarticulated empodia and claws were never noticed.

(11) There are two points from which loss of segments through tearing takes place rather easily; namely, the suture between the trochanter and femur, and the joint between the tibia and the metatarsus. Autotomy in the sense of immediate self-mutilation resulting from stimuli applied to various portions of the leg has not been observed in these specimens. When breakage
did occur, it apparently resulted in some way from a mechanical pull on the leg, and never occurred unless contact had been made with some object which would make possible a certain amount of strain. There is no evidence of autotomy and special adaptation for regenerating segments at the suture.

(12) Regeneration from an oblique section is faster in the proximal region of the cut.

(13) The new parts developing beneath the old cuticula gradually became folded or coiled, and tightly packed because of a lack of available space. These parts can be studied while in normal position or after dissection.
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BOOKS


MISCELLANEOUS MATERIAL

GENERAL DESCRIPTION OF THE PLATES

The normal leg appearing on the plates has been drawn by the aid of a camera lucida so that all of the essential characteristics are shown accurately. From the camera lucida drawing which was magnified 70.8 times, a reduced cut of 9.8 magnification was made, and all figures of the normal legs were printed from the cut. On Pl.II-XXVII the figures of the basal sclerites, similarly represented, have been printed at the right of the normal leg in order that the regenerated part be drawn in position.

Since the cut represents the left mesothoracic leg, regenerated left legs have been selected for illustration when possible, but in certain instances right appendages offered better illustrative material. When used, these have been drawn as their mirror images in order that they may be readily compared with the normal. The regenerated parts have been drawn to scale as nearly as possible.

The leg on Pl.I has been magnified approximately 9.8 times; the figures on Pl.XXVII have been enlarged 100 times; the figures on Pl. XXVIII-XXIX, 26.1 times; the figures on Pl.XXX, 30 times; and the figure on Pl.XXI, 37.5 times.

Shaded portions on the regenerated leg indicate irregularities of some sort. The empodium is not highly developed on the normal legs, but is well illustrated in Fig. 53 (Pl.XXVII) where it is seen as a pad between the claws. In practically all instances the spines have been omitted from the regenerated parts since they were not of major significance in this paper.
The levels at which the cuts were made are indicated by red dotted lines.
PLATE I

Fig. 1 The normal left mesothoracic appendage of a roach nymph showing the arrangement of the major muscles in the leg which had been preserved in alcohol. The cuticula is represented in red ink. The muscles of the leg are tightly packed normally, but in the figure a small space has been left between so that they may be distinguished. For contrast the tendons are unshaded.
Fig. 1
PLATE II

Fig. 2 The normal left mesothoracic leg. The dotted line represents a section made above the trochantin-coxal articulation.

Fig. 3 Early stage in the regeneration of the right mesothoracic leg of roach 44, showing only a small portion of the coxa bearing a small lateral lobe. The new portion is quite irregular.
Fig. 4 The normal left mesothoracic appendage. The dotted line indicates the level of the cut.

Fig. 5 Regenerating right mesothoracic appendage of roach 44 after the second molt. The following segments are represented: a minature coxa which possesses a small lateral lobe and which is free from the characteristic markings, an imperfectly articulated trochanter, a distorted femur, and a portion of a distal segment, presumably the tibia.
PLATE IV

Fig. 6 The normal left mesothoracic leg. The dotted line represents the level at which the cut was made.

Fig. 7 The regenerated right mesothoracic leg of roach 44 as it appeared after the third molt. The lateral lobe has grown slightly larger, but is being drawn into the coxa proper. Slight corrugation on the ventral surface of the coxa are indicated by shading.
PLATE V

Fig. 8 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 9 Regenerating portion of the left mesothoracic appendage of roach 45 as it appeared after the first molt.
PLATE VI

Fig. 10 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 11 A reduced coxa, imperfectly articulated trocanter, and fragment of the femur which appeared on roach 45 following the second molt.
PLATE VII

Fig. 12 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 13 Regenerating left mesothoracic leg of roach 45 after the third molt, showing the coxa which has increased in size, a trochanter, a femur, a tibia and a three-segmented tarsus bearing an empodium and claws which are well articulated.
PLATE VIII

Fig. 14 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 15 Regenerating left mesothoracic appendage ofroach 54 appearing at the second molt, showing a reduced coxa, imperfect trochanter, much reduced femur and tibia, and a four-segmented tarsus. The joints of the tarsus with the exception of the tibio-tarsal are incomplete and the fourth segment possesses a small unarticulated lobe which could not be definitely identified as the empodium.
Fig. 16 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 17 Regenerated left mesothoracic leg of reach 54 which appeared after the third molt. The coxa has taken on some of the characteristic markings and all of the segments are well articulated. The segments are fairly normally proportioned except in the region of the tarsus where the new segments are fewer in number and proportionately larger than those of the normal leg.
Fig. 18 The normal left mesothoracic leg. The dotted line represents a section taken through the trochanter-coxal articulation.

Fig. 19 Incomplete stage of regeneration of the right mesothoracic appendage from reach 29 after the second molt. The reduced coxa bears two unarticulated divisions at its distal end.
Fig. 20 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 21 The incompletely regenerated right mesothoracic leg of roach 29, appearing after the third molt. The coxa, possessing a very small lateral lobe, is articulated to a reduced trochanter, femur, and tibia to which is articulated a single tarsal segment.
PLATE XII

Fig. 22 The normal left mesothoracic leg. The dotted line represents a section taken through the trochantin-coxal articulation.

Fig. 23 Almost completely regenerated left mesothoracic leg of roach 32 as it appeared after the fourth molt. The leg was reproduced by an individual which had also received injury to all other appendages. Many of the characteristic markings have been restored on the coxa, but a small lateral lobe persists.
PLATE XIII

Fig. 25 The normal left mesothoracic leg. The dotted line represents a section taken through the distal third of the coxa.

Fig. 26 Early stage of the regeneration of the right mesothoracic leg of roach 70 as it appeared following the first molt. Only a small amount of regenerated material could be recognized on the distal end.
Fig. 25

Fig. 26
PLATE XIV

Fig. 27 The normal left mesothoracic leg. The dotted line represents a section taken through the distal third of the coxa.

Fig. 28 Later stage in the regeneration of the right mesothoracic leg of roach 70. The appendage is slightly twisted and has not entirely straightened. There are four tarsal segments, but the joint between the third and fourth segments is incomplete.
Fig. 29 The normal left mesothoracic leg. The dotted line represents a section taken between the coxa and trochanter.

Fig. 30 Regenerated left mesothoracic leg of roach 73 after the first molt, showing a corrugated trochanter, a femur, a tibia, a four-segmented tarsus, empodium and claw. The leg is smaller than the normal, but well articulated and proportioned.
Fig. 31 The normal left mesothoracic leg. The first dotted lines represent a V-shaped section taken from the coxa; the second dotted line indicates a section taken through the coxa-trochanter articulation.

Fig. 32 Regenerated left mesothoracic leg of roach 75, showing the slightly reduced coxa which is somewhat corrugated and indented in the region of the V-shaped cut, the trochanter, femur, bent tibia, and the tarsus which is four-segmented and irregular. The segments are distinctly jointed. The appendage appeared after the first molt.
PLATE XVII

Fig. 33 The normal left mesothoracic leg. The dotted line represents an oblique section taken through the coxa leaving a very small fraction of the trochanter.

Fig. 34 Regenerating left mesothoracic leg of roach 37 after the first molt, showing the coxa, an abnormal trochanter, a femur still bent because of its folded position beneath the cuticula, a slightly twisted and bent tibia, and an abnormal tarsus. There are marks which point toward an attempt to form five segments. The reduced claws project abnormally from beneath the epipodium and the joints are incomplete.
PLATE XVIII

Fig. 35 The normal left mesothoracic leg. The dotted line represents an oblique section taken through the lower region of the coxa.

Fig. 36 Regenerating left mesothoracic leg of roach 42 after the first molt. Regeneration of tissue has been more rapid in the proximal region of the cut, the amputated portion of the coxa being almost restored.
PLATE XIX

Fig. 37 The normal left mesothoracic leg. The dotted line represents an oblique section taken through the lower region of the coxa.

Fig. 38 Later stage of regeneration of the left mesothoracic leg of roach 42 which appeared after the second molt. The reproduction of the leg has been almost completed, but the coxa lacks certain markings and the other segments are abnormal, being either twisted or bent or both. The joint between the third and fourth tarsal segments is not well established.
Fig. 39 The normal left mesothoracic leg. The dotted line represents an oblique section taken through the distal part of the coxa.

Fig. 40 Regenerated left mesothoracic leg of roach 38 appearing after the third molt, and consisting of the major divisions including a four-segmented tarsus, the segments of which are abnormally large. The tibia and femur approach normality more closely than the tarsus, and the trochanter, the latter being slightly furrowed. The coxal portion has been completely replaced, growth having been more rapid in the proximal region of the cut.
PLATE XXI

Fig. 41 The normal left mesothoracic leg. The dotted line represents a section taken through the trochanter.

Fig. 42 The regenerated left mesothoracic leg of roach 80 which appeared complete after the first molt. Aside from the four-segmented tarsus, and the trochanter which bears a furrow indicating the place of section. The leg is whole and in size closely approaches that of its mate.
PLATE XXII

Fig. 43 The normal left mesothoracic leg. The dotted line represents a section taken through the distal part of the trochanter.

Fig. 44 Early stage in the regeneration of the right mesothoracic leg of roach 32 after the second molt, showing what appears to be a trochanter and three additional divisions. The first molt occurred shortly after operation and the regenerated portion at that time was very small.
PLATE XXIII

Fig. 45 The normal left mesothoracic leg. The dotted line represents a section taken through the distal part of the trochanter.

Fig. 46 Regenerated right mesothoracic leg of roach 33, which after the fourth molt appears normal except for the four-segmented tarsus.
PLATE XXIV

Fig. 47 The normal left mesothoracic leg. The dotted line represents a section taken at the suture between the trochanter and the femur.

Fig. 48 Regenerated left mesothoracic leg of roach 35 as it appeared after the first molt. It is complete with respect to principal divisions, but smaller than the normal.
PLATE XXV

Fig. 49 The normal left mesothoracic leg. The dotted line represents a section taken about midway the femur.

Fig. 50 A complete regenerated left mesothoracic appendage (except for the four-segmented tarsus) which appeared after the first molt, being nearly normal in size and larger than one regenerated from the trochanter in the same length of time.
PLATE XXVI

Fig. 51 The normal left mesothoracic leg. The dotted line represents a section taken through the middle of the tibia.

Fig. 52 Regenerated left mesothoracic tarsus and portion of the tibia of roach 39 after the first molt. The regenerated part is nearly normal in size. Only the regenerated portion shown.
Fig. 53 The fifth tarsal segment showing the empodium and claws of the normal leg. The spines have been omitted. The dotted lines represent the portion that is "telescoped" into the fifth and also a portion of the tendon which is inserted there. The dotted red line represents the level of section.

Fig. 54 Regenerated portion of the tarsus ofroach 91 the regenerated claws of which are smaller than normal and project from a position beneath the end of the empodium, being closely grown together. This may be contrasted with the normal position in which the claws seem to come off at an angle with respect to the empodium.
PLATE XXVIII

Fig. 55 Regenerating leg appearing beneath the coxa thirty days after operation. It is obviously older than the leg in Fig. 56. The appendage, also taken from roach 90, is well developed, consisting of a trochanter, femur, tibia and an incomplete tarsus. Although the joints are not distinct, the distal part of the tarsus can be recognized.

Fig. 56 Disposition of an early stage in the regeneration of segments distal to the breaking point between the trochanter and femur. The exact age of the appendage seen beneath the coxa was not known, since the appendage was taken from roach 90, which was already injured at the suture when captured. Segmentation is indistinct, but it can be seen that regeneration is advanced and the parts have begun to fold into the coxa as a result of the pressure exerted on the scar and the lack of space for development. Probably the clearest indication of jointing is seen between the coxa and trochanter, and between the femur and lower region.
Fig. 57 The position assumed by the regenerating appendage of roach after thirty-six days. Viewed from the dorsal side, a well formed trochanter, femur, tibia, and indistinctly segmented tarsus can be recognized. The appendage is older and more complete than that shown in Fig. 56. The tarsus has folded back on itself and the distal part extends into the coxa. The femur partly overlaps the tibia, so that in this figure only the spines of the femur and those on the inner border of the tibia may be seen. The shaded portion represents the scar.

Fig. 58 A ventral view of the appendage described above, showing the tibia which now overlaps the femur. The segments of the tarsus are clearer in this view, the claws being quite distinct. The articulations of the segments of the regenerating leg are easily recognized.
Fig. 59 Regenerating tarsus shown beneath the cuticula of the old tibia of roach 94 as it appeared 43 days after removal of the old tarsus. The appendage appears coiled about and twisted so that, while in position, it is difficult to distinguish the segments and especially, the distal claws. The dotted lines represent segments which are not in view. The spined segment is a part of the new tibia.

Fig. 60 A dissection of the tibia showing the tarsus and a part of the tibia described above. A part of the tibial cuticula has been removed and the normal position of the regenerating leg disturbed so that the segments are easily seen and studied. The tarsus is apparently well articulated except between the second and third segments and the fourth is slightly bent.
Fig. 61 Disposition of the regenerated tarsus beneath the cuticula of the old tibia of reach 95,48 days after operation. The segments of the tarsus which could not be seen from this view because of its coiled condition are represented by the dotted lines. The spined portion is the new tibia. The tarsus is distinctly articulated and consists of four segments and empodium with claws.