

# BIOLOGICAL BULLETIN

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## THE ORIGIN OF THE MYCETOCYTES IN PSEUDOCOCCUS.

FRANZ SCHRADER,

BRYN MAWR COLLEGE, BRYN MAWR, PA.

### INTRODUCTION.

Symbiotic fungi or mycetozoa which are to be found in many species of insects, have lately been made the subject of some interesting work chiefly in Germany and Italy. This peculiar association between fungus and insect body seems especially well developed in the Homoptera and there a great variety of special features is to be found in its development. In some cases, among which are the mealy bugs of the genus *Pseudococcus*, the symbionts are lodged in or associated with cells that originate in the insect body—the mycetocytes. These cells have a peculiar interest in that they are evidently very much specialized and are restricted to a very definite locality in the body of the insect. In all of the Homoptera, the symbionts are transmitted from one generation to the next through the eggs; each of which receives a certain number of the fungi from the mycetome or symbiont mass of the mother. In *Pseudococcus*, such a transfer to the ova involves a dissociation of the symbionts from the mycetocytes, for the latter do not pass into the eggs. When the infection of the egg is complete, the fungi are therefore found “naked” near the anterior pole and always in the form of a number of spherical clumps or packets, each of which contains a large number of the symbionts. During the development of the embryo, these clumps once more become associated with mycetocytes which arise in some way in the embryo.

The exact nature of the mycetocytes has received a variety of interpretations. Breest ('14), working on *Aspidiotus*, a coccid



not distantly related to *Pseudococcus*, suggested that they arise from the yolk cells—that is, cells that remain behind in the yolk at the time of blastoderm formation.

Strindberg ('19), who worked on *Lecanium*, reported that in that coccid also, the mycetocytes took their origin in the yolk cells.

Pierantoni, who was the first worker to make a detailed investigation of *Pseudococcus* gives a different account of the mycetocytes ('10, '11, and '13). According to him, some of the cleavage cells in traveling to the periphery to establish the blastoderm, encounter the symbionts. This association, more or less accidentally initiated, becomes permanent, and the cleavage cells assume the characters that stamp them as mycetocytes. Exactly the same process has been described by Pierantoni also in *Icerya*.

Buchner ('21) in reviewing previous investigations, seems inclined to agree with Pierantoni.

Finally, Shinji ('19), also working on *Pseudococcus*, describes a migration of cells to the symbionts, shortly before the germ band commences its growth. These migrating cells he interprets as potential germ cells. Some of them become permanently associated with the symbionts and constitute the mycetocytes, but others migrate once more to form the definitive gonads.

In criticizing these varying results, I am not in a position to pass judgment on the conclusions of Breest and Strindberg. Certainly my own results in regard to the point in question, *i.e.*, the origin of mycetocytes, have led me to an entirely different interpretation.<sup>1</sup> But this difference may very well be due to an actual difference in the development of mycetocytes in the three genera under consideration.

The work of the other investigators mentioned has already been considered in a recent paper ('23). Although a considerable part of that paper was devoted to showing that Shinji's conclusions are untenable, I also took up briefly the statements of Pierantoni. My own position on this question of the nature of the mycetocytes can best be presented by giving a short resumé of those of my findings that are involved in the present discussion.

In both *Pseudococcus citri* and *P. maritimus*, the somatic num-

<sup>1</sup> Regarding other points in the early development, I am in essential agreement with Strindberg.



ber of chromosomes is ten, all being alike in size and shape (Figs. 1-3). Five tetrads are formed in the maturing egg, and the egg nucleus undergoes a reduction and an equation division. The order in which these two divisions occur cannot be ascertained. The first division results in two daughter groups each consisting of five dyads. One of these groups of course represents the first polar body. It is not extruded but remains in an inactive state at the periphery. The other group of dyads undergoes the second maturation division, the results of which are two groups, each containing five unit chromosomes or monads. One of these last two groups remains at the periphery, constituting the second polar body. The other sinks into the egg and there combines with the male pronucleus.

The five dyads of the first polar body now break up into their unit elements, ten in number, and then enter the resting condition. The five monads of the second polar body also become diffuse. The two polar bodies then approach until in touch with each other. Fusion may then actually occur and I have described such a case in my previous paper ('23). In other cases however fusion is delayed until the chromosomes of each have been almost completely reformed and the nuclear walls begin to break down. The chromosomes then intermingle to form a single group, and this of course contains 15 chromosomes. With this act of combination the polar nucleus becomes established (Fig. 4).

The polar nucleus undergoes two or three divisions which appear normal in every respect (Fig. 4 to 7). Following the last of these divisions, irregularities occur, and in the course of these the chromosomes are greatly increased in number and the size of the cells is enlarged. These phenomena occur in every egg and the resultant cells I have called giant cells. The giant cells once established, undergo some apparently normal divisions and then become separated from the periphery and migrate a considerable distance through the egg to the symbionts. With these they enter into association and thus form the mycetocytes, which in this manner are formed anew in every embryo.

Although this account portrays the general course of events, it leaves unexplained the exact nature of the irregularities which



convert the derivatives of the polar nucleus to giant cells. It is this point with which the present paper is concerned.

The work was done on two species, *Pseudococcus citri* and *P. maritimus*.

#### CHROMOSOME COUNTS.

The short statement regarding the origin of giant cells was given as follows in my previous paper ('23): "Later divisions of the polar nucleus derivatives are subject to irregularities. Apparently nuclear division is then very often or even generally not accompanied by cytoplasmic division, so that the two resulting nuclei may lie side by side in a single protoplasmic area. At the ensuing division, there may be an intermingling of the chromosomes evolved, or else a multiplicity of spindles. Possibly also, cleavage cells nearing the edge may at times fuse with the derivatives."

This statement covers the problem only in a very general way and is hardly definite enough to be regarded as a solution of the complexities that are to be observed in the behavior of these peculiar cells. The one point established is that the polar nucleus derivatives are involved in some way in giving rise to the giant cells.

Without making any reference to this early period in the embryology of *Pseudococcus*, Buchner ('21) in his work on symbiosis comments on the fact that the mycetocytes in the adult contain a multiple number of chromosomes. This condition I had also observed, but it was not until recently, when the embryology was worked out, that the relationship between the giant cells of the embryo and mycetocytes in the adult became clear to me. Buchner apparently did not study the younger stages of *Pseudococcus* and therefore did not observe the giant cells at all.

The giant cells when first making their appearance in the egg are marked by a peculiarity that was observed very early in my investigations. This is, that although the number of chromosomes they contain is clearly variable and always greater than the true somatic number, it nevertheless varies only within very definite limits. This led to a more careful examination of the chromosome numbers in these cells.

A large number of chromosome plates was investigated. Un-



fortunately, the large size of the cells and plates often results in their being cut in the process of sectioning. Even if the chromosome plate studied is entire and flat, the chance of an overlapping of several chromosomes is very great, considering the large number involved. The latter defect may often make a count unreliable, but the cutting of a plate must throw it out of consideration at once. Discarding therefore all counts in which any doubt as to the number can possibly be entertained, the following data were obtained:

There are 6 plates containing 25 chromosomes; 3 plates containing 30 chromosomes; and 5 plates containing 35 chromosomes. These numbers become more impressive in view of the fact that during this period not a single perfect plate carrying any other but these three numbers was encountered (Figs. 12-17).

Among the plates discarded because overlapping of chromosomes made their counting uncertain, there were several in which doubt existed only with respect to a single chromosome, *i.e.*, whether to count it as one or two. Accordingly as these two possibilities were taken, these doubtful plates were found to fall under one of the three types given, or differ from it by one chromosome. In this uncertain group of plates there are 5 plates containing 25, or 1 more or less chromosomes; 3 plates containing 30, or 1 more or less chromosomes; 1 plate containing 35 or 36 chromosomes. Again, no plate in which doubt as to one chromosome exists was found to approximate any number but 25, 30 and 35.

How are these numbers to be explained?

In every perfectly clear plate, it was evident that the chromosomes resemble each other very closely as to size and shape. This fact coupled with the observation that the multiple numbers found are all multiples of five makes it improbable that an irregular process of fractionation of the chromosomes is to be held accountable. Again, although a definite regularity obtains in these chromosome numbers, it has also been observed that one egg may carry cells of more than one type. Thus a plate with 25 may be found close to another plate with 35 chromosomes. This too would be difficult to explain on the basis of a fractionation, especially as the character of the numbers would make



inevitable the hypothesis that only certain definite chromosomes break up into smaller units.

If effective polyspermy were common, the possibility that the supernumary sperms might combine with each other or any of the embryonic cells should receive some consideration. It would then remain to be explained why such numbers as 20 or 40 should not be encountered, since the 5 chromosomes of each sperm should make almost any combination possible. But aside from this consideration, I may point out as I have previously done, that supernumary sperms even if present rarely evolve chromosomes and certainly not as part of a regulated process, whereas giant cells with their multiple numbers of chromosomes are to be found in every developing egg at the right stage.

Finally, as the last of these possible but unlikely hypotheses remains an irregular behavior of the polar bodies. It is perfectly possible that the first and second polar bodies may not combine and that one or both may continue to divide independently. In combination with embryonic cells, the second polar body might thus bring about such numbers as 25 and 35. But against this I need repeat only my former observations that in all eggs at the crucial stage under observation, the polar nucleus is always formed and its 15 chromosomes go through several normal mitoses. None at all show an independent development of the two polar bodies. This of course still leaves the possibility that the first polar body may at times divide before fusion with the second. It may even be admitted that independent divisions or development of the polar bodies may sometimes occur. But such cases, and I have not seen any, are not normal.

These considerations leave only two types of cells as factors in the origin of the giant cells. They are the polar nucleus derivatives carrying 15 chromosomes and the true cleavage cells with 10 chromosomes.

Taking up the three types of giant cells in order, it is plain that the 25 chromosome type can arise only by a combination of a polar nucleus derivative (15 chromosomes) with one cleavage cell (10 chromosomes).

The 30 chromosome type must arise from a fusion, or recombination after mitotic division, of 2 polar nucleus derivatives.



Numerically, the same number could be attained by the combination of 3 cleavage cells. The latter possibility is more than doubtful if it is considered that giant cells are formed only in a certain and limited area at the periphery, whereas if a fusion of cleavage cells alone is a possibility such cells with 30 chromosomes should then be formed at any part of the periphery where cleavage cells are forming the blastoderm. Weighty although negative evidence against this hypothesis is to be adduced from the seeming nonexistence of giant cells with 20 chromosomes. The failure to find such cells is all the more significant if it is considered that the chances of two cleavage cells coming together for fusion are greater than the chances of three combining in that way.

The 35 chromosome type can originate only from a combination of a polar nucleus derivative with two cleavage cells. Other chances of combination to bring about this type have been ruled out by the preceding considerations.

Why a 40 chromosome cell should not be found at this stage it is not possible to say. Possibly there are such cells but their less frequent occurrence has prevented their discovery. More probably the three types already mentioned represent all the combinations possible.

#### THE GIANT CELL CHROMOSOMES AT LATER STAGES.

As already mentioned, the giant cells originate always at the time that the cleavage cells are migrating to the periphery of the egg to establish the blastoderm. With the completion of the latter and the first stages of germ band formation, another period in the history of the giant cells is initiated. In the course of this, giant cells with still greater numbers of chromosomes than those already described, are encountered. Beside them, the three older numerical types may continue to exist. The increase in the number of chromosomes, together with the fact that divisions in the giant cells tend to decrease their size, make certain counts in these later cells a much more difficult matter. Apparently most of these greater chromosome numbers hover in the neighborhood of 60 or 70. Only one certain count could be made, that being of a plate containing 60 chromosomes (Fig. 18).

Cytological evidence to be considered later, makes it probable



that the period of fusion has now been passed. It is indeed possible that there is an occasional combination of a giant cell with one of the yolk cells, the latter representing nothing but cleavage cells that failed to migrate to the periphery to establish the blastoderm. Fusion with what were formerly cleavage cells but must now be termed blastoderm cells, can of course take place only at the edge of the giant cell area, where the two types of cells are in contact. But aside from the fact that the giant cells in that location are not larger as a rule than those more centrally placed, it must be considered that the blastoderm cells in their expansion seem to exert actual pressure on the giant cells. The latter are heaped up and finally actually leave the periphery altogether. If such a pressure really exists, it would be constantly cancelled by fusion of adjoining giant and blastoderm cells.

Nor can there be a continued tendency of giant cells to fuse with each other. Such occurrences are at least not general, for the number of giant cells is at this time slowly but steadily increasing, while their individual size is decreasing. Nevertheless the size is evidently variable, so that an occasional division of the chromosomes unaccompanied by cytoplasmic division remains as the most plausible explanation.

Once the giant cells have migrated to the symbionts and entered into association with them, divisions become rarer. At the same time it must be observed that in the adult *Pseudococcus*, the former giant cells, then called mycetocytes, contain relatively enormous numbers of chromosomes. The association with the symbionts must therefore have a disturbing effect on the few divisions that still occur, and most probably it is the failure of cytoplasmic division following a normal division of the chromosomes that thus causes a multiplication of the chromosomes. This idea has already been expressed by Buchner ('21).

#### CYTOLOGICAL EVIDENCE.

A complete cytological consideration of the problem should begin with a study of the maturation phenomena in the egg. Since these primary steps have already been considered at some length in my previous paper ('23), it is sufficient to begin the present account with the polar nucleus, *i.e.*, the combined first



and second polar bodies. The first difficulty arises in determining the number of divisions to which the polar nucleus is subjected. It seems certain that at least two divisions take place regularly and that they are always normal. In some cases, the 4 nuclei or polar nucleus derivatives resulting from these two divisions certainly undergo a third division. But whether this last division occurs in every egg is not so certain. If so, there will then be 8 polar nucleus derivatives at the periphery of the egg (Fig. 4-7a).

Almost the same difficulties are encountered in determining the number of divisions that the fertilization or zygote nucleus undergoes, before the resultant cleavage cells take up their migration to the periphery to establish the blastoderm. Typically there appear to be about 32 cells in the interior of the egg when the migration begins.

It will be apparent that there is a distinct variation in the rate of division of polar nucleus derivatives and cleavage cells respectively. While the polar nucleus is undergoing at most 3 divisions, the fertilization nucleus undergoes approximately 5. The result of this is that while all the nuclei of each type taken by itself are at about the same stage of division, they may not be at all synchronous with the division stages of the other type. It is this condition that lies at the bottom of the difficulty in determining the number of divisions that the nuclei in question undergo before the process of fusion is begun. Thus in one egg, there are 8 polar nucleus derivatives, all in slightly varying stages of telophase, and still connected in pairs by spindle fibers (Fig. 7). The cleavage cells of this egg have begun the peripheral migration, but none have yet reached the edge. It can be assumed that here 8 polar nucleus derivatives will be involved in the processes of fusion to follow. In contrast with this is another egg in which there are only 4 polar nucleus derivatives. The chromosomes are in the final stage of condensation but the nuclear walls have not yet been broken down. The cleavage cells, present in about the same number as in the previously mentioned egg (32), are again in the stage of migration, and one has actually come in touch with one of the polar nucleus derivatives at the periphery. This cleavage cell like its sister cells is in the resting phase. The question therefore arises whether the polar nucleus derivative will complete its



impending division regardless of the proximity of the cleavage cell, or whether the presence of the latter will make that division abortive. In the first case, 8 nuclei will commence the fusion process as before; in the last named eventuality only 4 will be at hand (Fig. 7a).

On the whole it may be assumed that the polar nucleus as well as the fertilization nucleus undergoes a definite number of divisions. As has been noted previously, the chromosomes of any single giant cell are from their first appearance alike in size and shape. The chromosomes of the polar nucleus derivatives however decrease in size with each succeeding division (Fig. 4 to 6) and the same is true of the cleavage cells. If fusion or combination of these two types of cells could occur after a varying number of divisions, it would be expected that the chromosomes of the combination nucleus would often be of two sizes. But this, as has been said, is not the case. It is of course possible that there is some regulative mechanism capable of equalizing differing sizes of chromosomes, but for this assumption there is little or no basis.

Regarding the phenomena of fusion which now occur, the conclusions based on the numerical data receive the full support of the purely cytological evidence. In giving this last named proof I am fully aware of the ease with which in a case like the present, a number of isolated figures can be seriated to fit a preconceived hypothesis. Standing alone, the cytological proof would therefore be advanced with considerable caution. Nevertheless one or two of the figures found are of considerable value in themselves.

Every step in the migration of the cleavage cells to the periphery, their approach to the polar nucleus derivatives, the flowing together of the protoplasmic areas which surround each nucleus and the final apposition of the nuclei within the single protoplasmic area resulting, can be traced through closely seriated stages. Similarly, what appears to be a pair of polar nucleus derivatives may at times be seen in close proximity. However unless the chromosomes of the apposing nuclei are close to full condensation, no definite conclusion can be reached as to the nature of the nuclei involved in either case. All of the figures show that the process takes place in either two or three cells, and a greater number has not been observed (Figs. 8 to 10).



It might be supposed that even during the resting phases the sizes of the fusing nuclei would suffice to identify them. And indeed it seems well established that when the two types of nuclei are at precisely the same stage, that of the polar nucleus derivative with its 15 chromosomes is slightly larger than a cleavage nucleus with 10. But it is practically impossible to exactly identify the phase of the nucleus during its preparatory phases. At the same time it has already been noticed that variations in the size of any one type of nucleus are extreme. The changes in size seem directly related to the condition of the contained chromatinic material and are such that the nuclear volume is smallest just after the formation of the nuclear wall at telophase, and largest immediately before the dissolution of the nuclear wall prior to the following division. Thus the increase in size of the female pronucleus between the telophase of the last maturation division and the time when the chromosomes are again almost fully condensed before the first segmentation division, are very considerable (Figs. 8*a* and 11, '23). Changes of size almost as great can be observed in the polar bodies prior to the formation of the polar nucleus and the polar nucleus derivatives. It is therefore manifestly impossible to arrive at any conclusion regarding the nature of fusing nuclei by simply comparing their size when it is considered that a further complication arises from the fact that apposing nuclei may be at entirely different phases (Fig. 7*a* and 10).

In spite of the very different phases that two or even three apposed nuclei may be in, it is apparent that a normal plate of chromosomes, which represents the summation of the numbers contained in each of the fusing nuclei, is finally attained. This may happen only when all of the nuclei are in a very definite generation of cells as has been pointed out in regard to the question of the number of divisions undergone by the polar nucleus. It is also a consequence of these observed facts regarding the varying phases of apposed nuclei, that the chromosomes of one or two of the nuclei will reach their full condensation prior to those of the other nuclei involved. Those first evolved must therefore be subjected to a suspension of further activity until those lagging behind have caught up. All of my figures make it



plain that condensation of chromosomes progresses regardless of the phase of an apposed nucleus, and that therefore the period of suspension of activities occurs when the chromosomes have been fully evolved.

Whether complete fusion of such nuclei is ever brought about before the condensation of chromosomes cannot be answered with certainty. A cytological demonstration would be next to impossible if the act is a very short one—say like the fusion of two soap bubbles to make a single larger one. That I have no stages showing such an act is therefore not complete proof that it does not occur. Nevertheless the normal course consists of a condensation of the chromosomes entirely independent of any other nucleus, and the fusion occurs only when the nuclear walls break down and permit an intermingling of all the chromosomes.

It is owing to the conditions brought out in the preceding paragraphs that a very good cytological demonstration of the act of fusing can be given. In Fig. 10 are shown three nuclei in apposition, and in the light of the numerical data they may safely be assumed to represent one polar nucleus derivative and two cleavage nuclei. Without the numerical data however, no such assumption would be justified. Fig. 11 on the other hand furnishes strikingly independent proof. Here there are 20 chromosomes almost fully condensed, and these show a slight trace of being arranged in two groups of 10 each. But in addition there are 15 chromosomes still in a more threadlike stage, and evidently at an earlier phase of condensation. The figure evidently represents a case in which the nuclei when coming into apposition were at different phases. The conclusion seems inescapable that here is represented the fusion of a polar nucleus derivative with two cleavage cells.

Spindles formed in the first division of these combination or fusion nuclei are apparently perfectly normal. Multipolar spindles are indeed encountered but little if any more frequently at this time than they are in the normal tissue of many animals. I am entirely at a loss to explain how the mitotic mechanism of two or even three combining nuclei is adjusted to the process of fusion. Certainly all the involved nuclei are capable of dividing perfectly independently. Bowen ('22) has recently pointed out a



similar case in *Loxa florida* where a fusion of cells is likewise unaccompanied by any irregularity in the mitotic spindle formation.

#### CONDITIONS IN OLDER EMBRYOS AND IN ADULTS.

As explained previously, the fusion process is limited chiefly to the period in which the blastoderm is laid down. When the latter is fully established, figures showing apposed nuclei in a single cytoplasmic area become very rare. Most of such figures arise from what is probably an accidental migration of yolk nuclei to the periphery, for a few have been found in the blastoderm as well as in the giant cell area. It is even doubtful whether in these isolated cases a fusion of nuclei is finally consummated, for no multiple cells have been found in the blastoderm cell region. The increase in the numbers of chromosomes in the giant cells, which is certainly still occurring at this time as well as later, is therefore due principally to a division of chromosomes unaccompanied by a cytoplasmic division of the cell concerned (Fig. 18).

When after leaving the periphery the giant cells have become associated with the symbionts, mitotic figures are not so often found in them. Nevertheless they do occur, and successfully as far as the chromosomes are concerned. Cytoplasmic division which before the migration, was undoubtedly completed successfully in some of the mitoses, is now completed less often. Only in this way can the relatively enormous numbers of chromosomes in the mycetocytes of the adult be explained. Further fusion is in these later stages practically eliminated, since most of the mycetocytes are almost completely hedged in by the symbiont spheres which they harbor.

Buchner ('21) estimates the chromosome number in some of the mycetocytes as over 200. In this estimate I can only concur with him. There is in addition to this a decided increase in the individual size of the chromosomes, although this seems to be a variable feature in different mycetocytes (Fig. 19 and 20).

My material is not favorable for a detailed study of the spindle formation in these later mycetocytes. The centrosomes seem extremely small under even the most favorable circumstances, and special staining methods are difficult to apply to these as to other insect eggs. Apparently mitotic figures are normal now as



well as in embryonic stages and I am induced to regard Buchner's figure of a multipolar spindle as an exceptional case. That such may occur I have no reason for denying, and it is indeed strange that abnormalities are not the rule rather than the exception in all of the mycetocytes.

The size of the cells is not proportionate to the increased amount of chromatinic material. It is augmented considerably when compared with that of the giant cells in the time of first association with the symbionts, but never reaches the dimensions that one would expect from an examination of the contained chromosomes.

#### GENERAL CONSIDERATIONS.

Breest's and Strindberg's conclusions that mycetocytes arise from the yolk cells may safely be discarded as far as *Pseudococcus* is concerned. By Strindberg's own definition, yolk cells are cleavage cells which have been left behind after the general migration to the periphery to establish the blastoderm. The giant cells however, which are the direct progenitors of the mycetocytes, arise even before the migration of cleavage cells is complete, and therefore before the yolk cells have been established as such.

Pierantoni's conclusions have already been taken up in my previous paper ('23). Undoubtedly he is correct in his explanation that the cleavage cells wander in among the symbionts, but in *Pseudococcus* I consider this association, if such it can be called, as one of the most temporary nature. It is the natural consequence of the general peripheral migration of cleavage cells.

The whole series of developments, as here described, seems extraordinary. And yet, most of the stages regarded individually are not unprecedented; the processes involved have been described before in other forms.

A fusion of polar bodies and the persistence of the polar nucleus thus formed has been described in several polyembryonic Hymenoptera. The formation of a polar nucleus is therefore not the only case among insects. It is indeed only rarely that such instances are met, but the old assumption that polar bodies never develop in a normal case of embryology certainly does not hold. That the polar nucleus does not behave like the female pronu-



cleus is of course quite evident. In *Pseudococcus* for instance, its derivatives tend to stay at the periphery and do not sink into the egg as does the pronucleus. Nevertheless those inherent qualities in the latter which cause it to fuse with the male pronucleus, may be present to a certain extent also in the polar nucleus derivatives and cause them to combine with any cell that happens to come in contact with them. Certainly this tendency is not to be observed in the cleavage cells, for these are never found to fuse with each other under ordinary circumstances. It is found only in the polar nucleus derivatives, which can fuse both with each other and with the cleavage cells.

In much of their further behavior they are not anomalous at all. It is almost unnecessary to mention that in case of a great many animals and plants, complete fusion of the pronuclei may be delayed for some time. In such extreme cases as *Cyclops* (Rückert, '95; Haecker, '95) and *Cryptobranchus* (Smith, '19) the individuality of the two pronuclei may be traced even through the early cleavage stages. The failure of immediate fusion of two apposed nuclei in *Pseudococcus* is therefore not peculiar. As a matter of fact, it seems to be a rule in insects that the two pronuclei lie in apposition and the chromosomes of each are evolved independently of those of the other. It is only when nearly fully condensed that the nuclear walls break down and the chromosomes intermingle. Such seems to be the case in *Archimerus* (Morrill, '10), "goumi aphid" (Stevens, '06), *Trialeurodes* (Schrader, '20) and finally in the pronuclei of *Pseudococcus* itself.

Another aspect in the process of fusion of polar nucleus derivatives and cleavage cells is to be observed in the fact that two apposed nuclei may be in different phases. It has been explained that at such times the chromosomes of the nucleus in a more advanced phase are fully condensed but then enter on a period of suspended activity. During this period the chromosomes of the apposed nucleus or nuclei also become condensed and only then the common spindle is formed and the different sets of chromosomes are arranged in a single plate. The mechanism involved in this regulative process is not clear to me. But it may be stated that this aspect also is paralleled by the behavior of pronuclei



in several forms. Here may be mentioned *Lilium* (Weniger, '18), the "goumi aphid" (Stevens, '06), and once more the pronuclei of *Pseudococcus* itself. In the latter case I have mentioned ('23) the possibility that delay in the condensation of chromosomes in one of the two pronuclei may be connected with the peculiar chromosome conditions of the male. This is at best only a working hypothesis.

The present account makes it evident that, generally speaking, the fusion of the polar nucleus derivatives with migrating cleavage cells is a more frequent occurrence than I had previously supposed. It therefore seems best to apply the name "polar nucleus derivative" only to the cells carrying 15 chromosomes, which are products of the division of the original, single polar nucleus. In my previous paper ('23) this term was applied somewhat indiscriminately to cells arising from division of the polar nucleus as well to some of those that had already undergone fusion with other cells and therefore contained a multiple number of chromosomes. The latter type of cell has been called "giant cell" throughout the present paper and of course includes cells arising from the fusion of polar nucleus derivatives among themselves, as well as with cleavage cells. The distinction between the giant cells and the polar nucleus derivatives is thus made a very definite one. Giant cells that have entered into association with the symbionts therewith become mycetocytes.

No attempt has been made here to discuss the exact relations between the insect body and the symbionts harbored by it. It should be pointed out however that the mycetocytes are insect cells. But they are in a measure extraneous to the organization of the body of the insect and even their actual connections with the latter are confined to branches of the trachea. Even during development they do not stand in a more direct relation to the various organs of the embryo than do the symbionts themselves. Their physiological importance nevertheless may be considerable; but this is a problem in itself.

I realize that the difficulties of the case are not removed by listing parallel instances of various stages—as I have done in this discussion. Such a proceeding however does serve to emphasize that many of the questions brought up by the investigation are



identical with problems that have troubled the cytologist for years.

#### SUMMARY.

1. The first and second polar bodies of *Pseudococcus* undergo fusion and form a polar nucleus. This contains 15 chromosomes.
2. The polar nucleus divides several times (probably a definite number of times) giving rise to the polar derivatives.
3. The polar derivatives may fuse with either migrating cleavage cells or with each other to form the giant cells. The numerical data furnished by chromosome counts as well as the purely cytological evidence support each other in arriving at this conclusion.
4. The giant cells migrate from the periphery to the symbionts to enter into association with these. When this process has been completed, the giant cells are known as mycetocytes.
5. Discussion regarding the nomenclature of the cells involved in these phenomena. Statement of the problems presented during the various stages of the investigation.

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## EXPLANATION OF PLATES

All figures have been drawn at table level, using a camera-lucida. Tube length = 160 mm., 2 mm. Zeiss oil immersion objective, and No. 12 X compensating ocular. No reduction has been made in the reproductions.

PLATE I. (all figures of *Pseudococcus citri*).

FIG. 1. Chromosomes of the fertilization nucleus.

FIG. 2. Blastoderm cell during early stage in the blastoderm formation.

FIG. 3. Blastoderm cell at time when blastoderm has become complete and giant cells have begun to migrate to the symbionts.

FIG. 4. The polar nucleus.

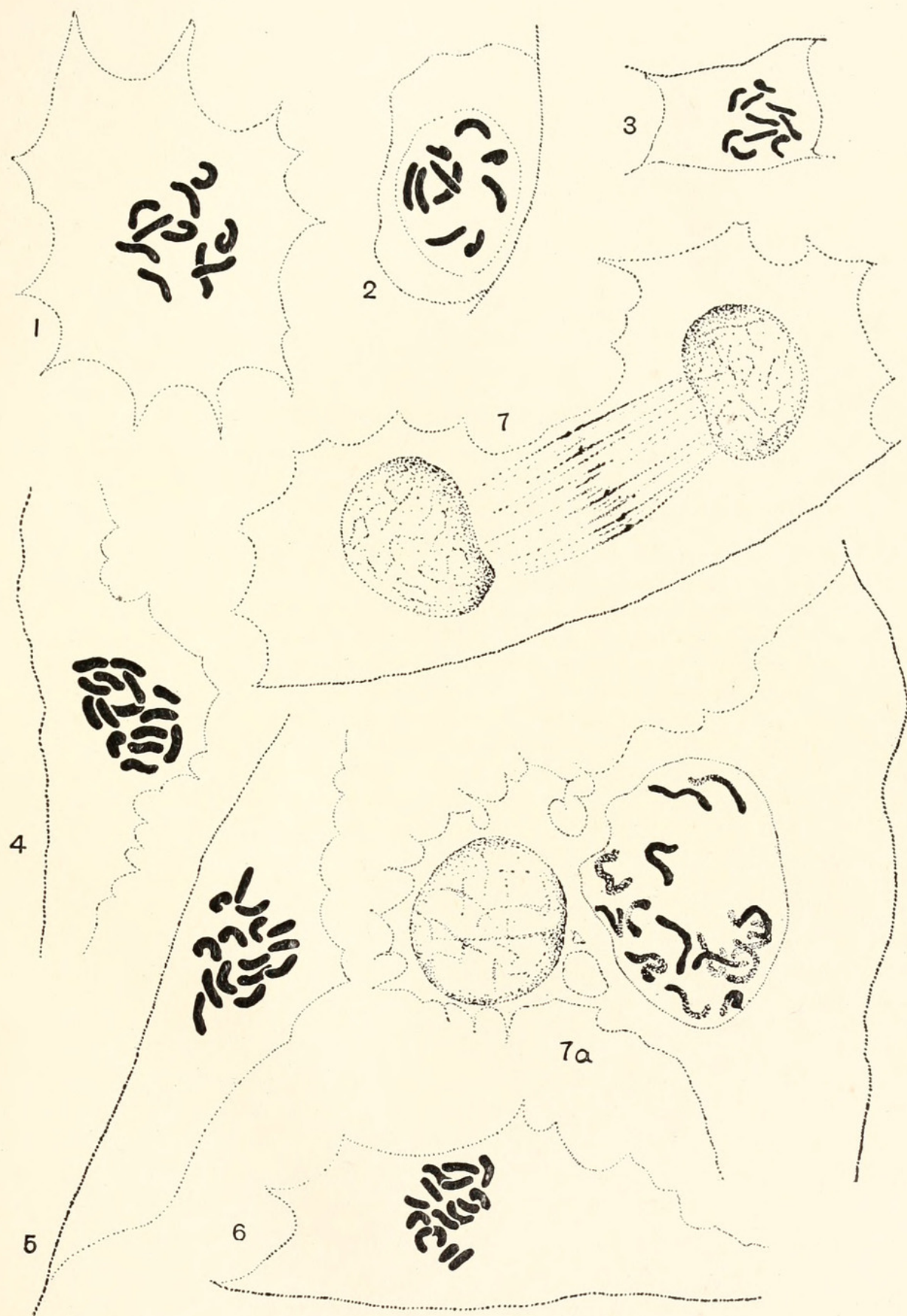
FIG. 5. One of two daughter cells derived from the first division of the polar nucleus, *i.e.*, one of first polar nucleus derivatives.

FIG. 6. One of two daughter cells derived from the division of one of the first polar nucleus derivatives. Four derivatives present in the egg at this time.

FIG. 7. Telophase of the third division of the polar nucleus. Eight derivatives in the egg when this division is complete.

FIG. 7a. One of four polar nucleus derivatives with fifteen chromosomes almost condensed for the next division. Cleavage cell in resting stage coming into apposition.















## PLATE II.

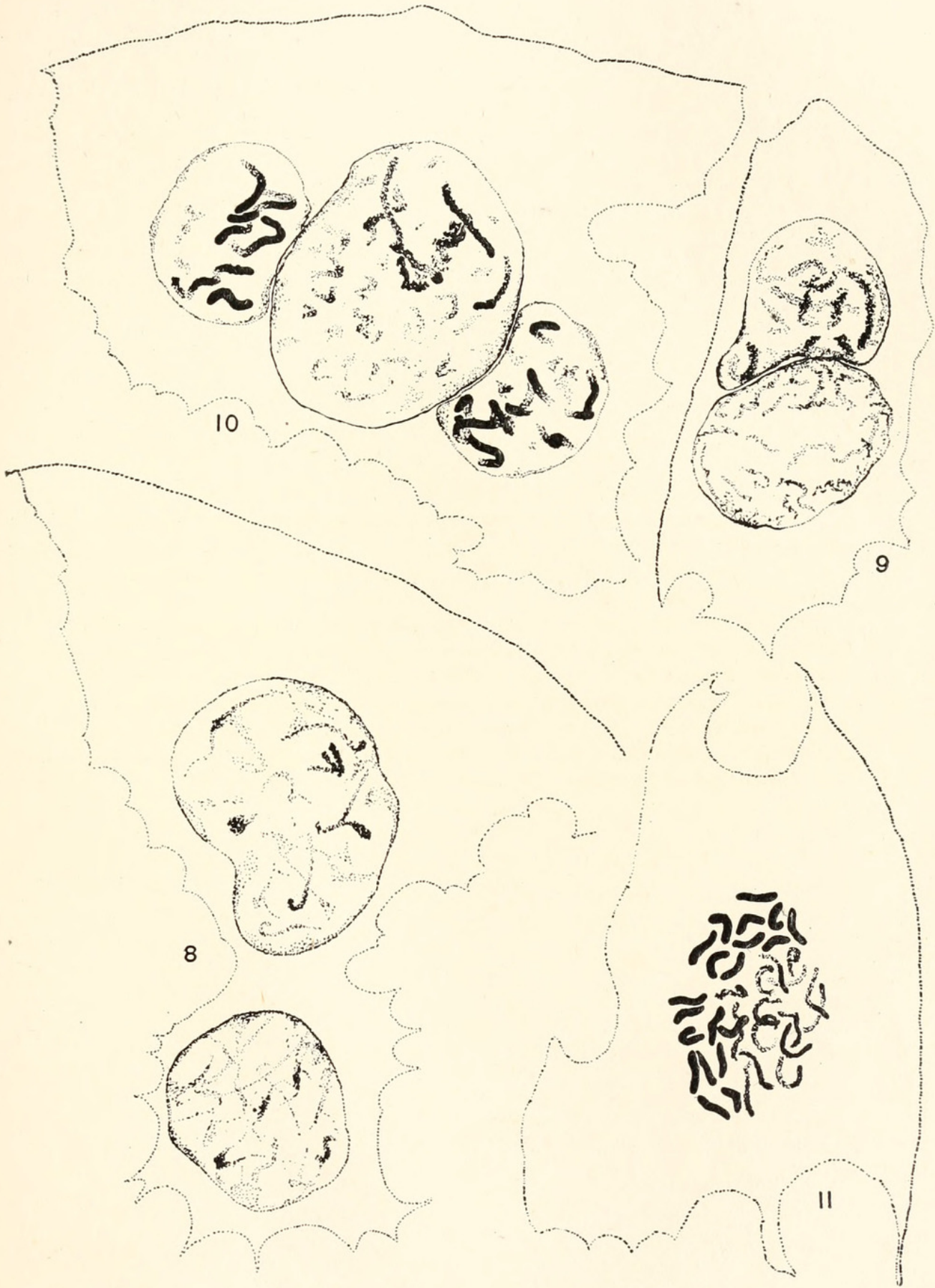
FIG. 8. Cleavage nucleus approaching a polar nucleus derivative. Cytoplasmic area fusing. (*P. maritimus*.)

FIG. 9. Two nuclei in apposition. Not certain whether both are polar nucleus derivatives. (*P. citri*.)

FIG. 10. Three nuclei in apposition. The two smaller with chromatin slightly more condensed than that of the larger nucleus. Probably two cleavage nuclei and a polar nucleus derivative. (*P. maritimus*.)

FIG. 11. Nucleus showing 20 chromosomes almost fully condensed and 15 at a slightly earlier phase. The 20 condensed chromosomes seem to be arranged in two groups of 10 chromosomes each. Probably originated from two cleavage nuclei and a polar nucleus derivative. (*P. maritimus*.)











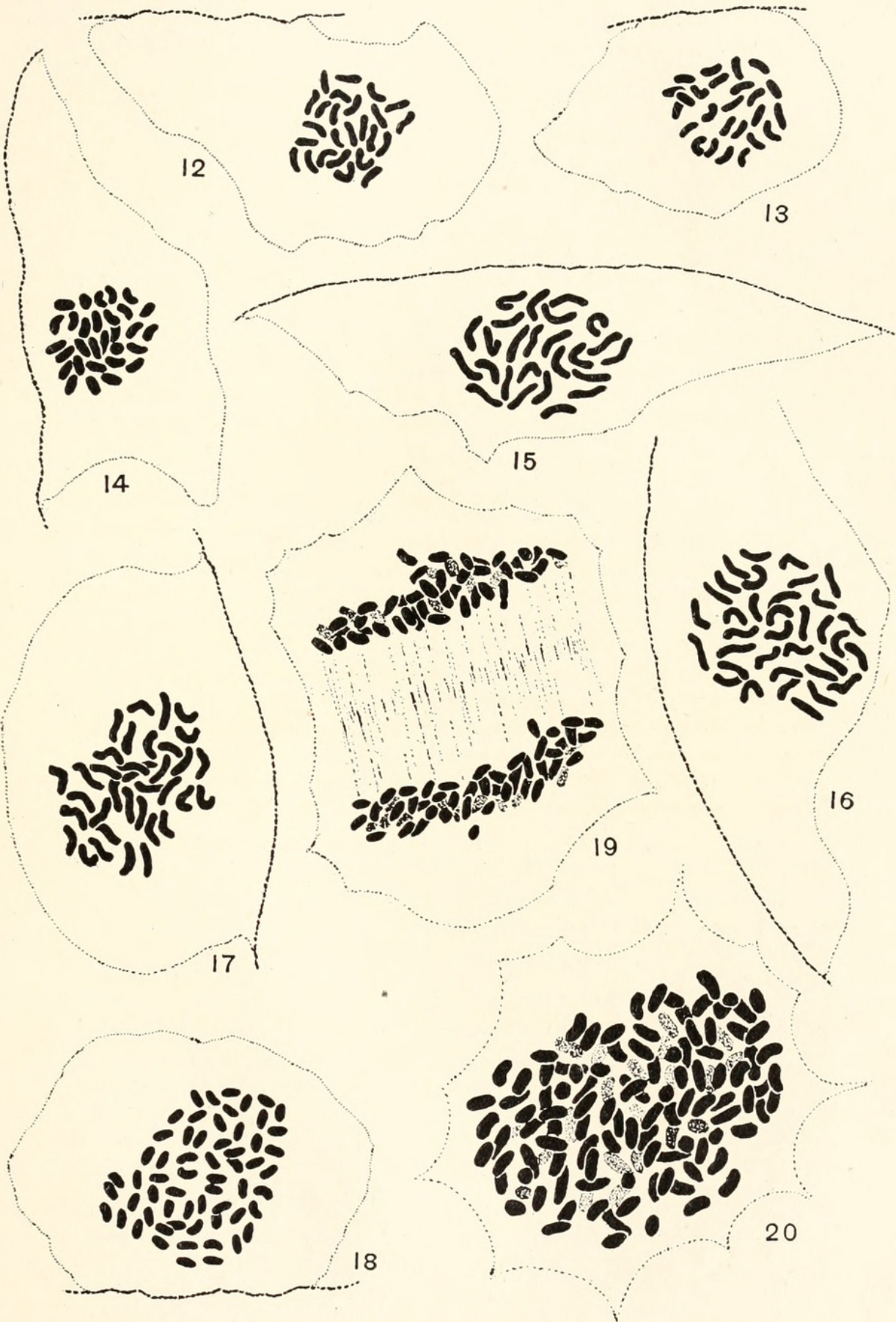




## PLATE III.

- FIG. 12. Giant cell containing 25 chromosomes. (*P. citri*.)  
FIG. 13. Giant cell containing 25 chromosomes. (*P. citri*.)  
FIG. 14. Giant cell containing 30 chromosomes. (*P. citri*.)  
FIG. 15. Giant cell containing 25 chromosomes. (*P. maritimus*.)  
FIG. 16. Giant cell containing 35 chromosomes. (*P. maritimus*.)  
FIG. 17. Giant cell containing 35 chromosomes. (*P. maritimus*.)  
FIG. 18. Giant cell containing 60 chromosomes. (*P. citri*.)  
FIG. 19. Mitotic division in a mycetocyte of an adult female. (Two other sections not shown.) (*P. maritimus*.)  
FIG. 20. Metaphase plate of chromosomes in a mycetocyte of an adult female. (Two other sections not shown.) (*P. maritimus*.)











## THE ENDOCRINE SYSTEM OF *TYPHLOMOLGE* *RATHBUNI*.

EDUARD UHLENHUTH, PH.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, N. Y.)

The endocrine system of *Typhlomolge rathbuni*, the blind Texan cave salamander, has been a matter of controversy for some time. In order to clarify some of the points under discussion, I have made serial sections of the region of the lower jaw, throat, and heart of 5 specimens, and the entire head of 2 specimens of this animal. The specimens were captured in Texas, as described in a previous article.<sup>1</sup> Six of them died during the trip from Texas to New York, one after 14 months of captivity in the laboratory. Since they are preserved after death, only the anatomical features of the various organs can be studied. A histological study must be postponed until suitable material can be secured.

### THE THYROID.

Emerson<sup>2</sup> was the first one to call attention to the possible absence of the thyroid gland in *Typhlomolge*. In 1905 she examined sections through the head of one specimen and was unable to find a thyroid. At the time Emerson published her paper the interest in the endocrine system of amphibians was very slight and her paper remained unknown to most biologists. In several of my papers on the thyroid function of salamanders I have called attention to Miss Emerson's interesting findings, which I had recognized to be correct. Soon after my return from Texas in 1916, I sectioned one of the *Typhlomolge* captured there and found the thyroid absent.

But at the 1921 Christmas meeting of the Anatomists, Swingle, apparently unacquainted with the literature on these facts spoke of the thyroid of *Typhlomolge* as a matter of fact and claimed to have isolated and observed this organ under the microscope.

<sup>1</sup> Uhlenhuth, E., *Biol. Bull.*, 1921. XL., 73.

<sup>2</sup> Emerson, E. T., *Proc. Soc. Nat. History, Boston*, 1905, XXXII., 43.



Although I mentioned my own findings, Swingle's very definite claims made the correctness of my observations doubtful, and even Doctor Wilder, from whose laboratory Emerson's paper was published, was ready to admit the possibility of an oversight on the part of Miss Emerson.

Immediately after my return from this meeting, I made sections of the 7 specimens discussed in this paper, and upon examination of the first one I was convinced that I was correct. In response to a letter in which Swingle admitted that the organ which he had claimed to be a thyroid was another vesicular organ, I communicated my new observations to Mr. Swingle. Neither this communication nor the incident at the Anatomists' meeting has been mentioned in an account recently published by Mr. Swingle in which he<sup>1</sup> states that 3 specimens examined by him possessed no thyroid.

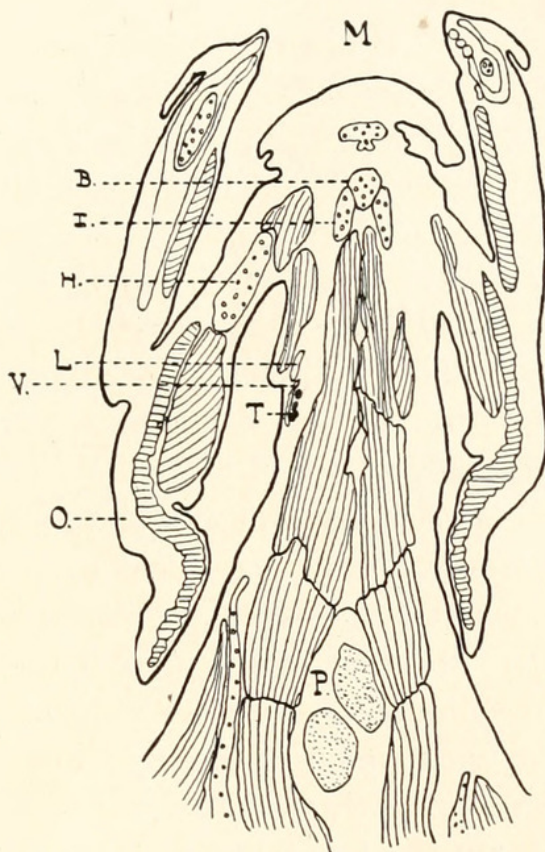
The examination of the 7 specimens, together with previous findings, shows that while some specimens of *Typhlomolge* are without even vestiges of the thyroid, others possess epithelial structures, evidently undifferentiated thyroid rudiments whose development was inhibited by an unknown factor.

Before describing these rudiments, the location of a normal thyroid may be briefly referred to. For comparison I shall use the thyroid of the Ambystomidæ, which may be called representative of a normal salamander thyroid. In the Ambystomidæ, the median thyroid rudiment splits up into two epithelial cell masses, which migrate in a posterior, lateral and largely ventral direction until, in *Ambystoma opacum*, they are closely attached to a large lymphatic space (Fig. 1). This space is located in the interstitial space formed by the muscles which surround the first gill arch, ventral and median to the epibranchial of the first gill arch. In other species the thyroids may be located slightly further posterior, but always the lymphatic space where it is in touch with the thyroid is adjacent also to the anterior cardinal veins. At the site where the ventral end of the thyroid is attached to the lymph space, the anterior cardinal vein comes into close contact with this space, leaving there the thyroid, at its ventral and posterior end, as a large vessel into which collects the blood from the interfollicular rete of the thyroid.

<sup>1</sup> Swingle, W. W., *Jour. Exp. Zool.*, 1922, XXXVI., 397.



In addition to the main portions of the thyroid, most specimens possess accessory thyroids. These develop from small cell groups which during migration become detached from the main portions and thus mark the path along which the main portions



In reproduction figures 1 to 8 have been reduced by one third, figures 9 and 10 by slightly more than one third.

FIG. 1. Location of main portion of right thyroid in an advanced larva (59.2 mm. total length) of *Ambystoma opacum*; I, first gill arch; B, first basibranchial; H, hyoid; L, lymph space; M, cavity of mouth; O, operculum; P, pericard; T, thyroid; V, anterior cardinal vein.

migrate. Their location varies greatly. They are located usually anterior and may be either ventral or dorsal or, in case of several accessories, both ventral and dorsal to the main portions. Or they may be at one level with the main portions. They consist either of one or several median rudiments located in the median interstitium of the muscles ventral to the basibranchials of the visceral skeleton (genio-hyoideus) or of two lateral portions, one on each side, which either are attached to the sides of the basibranchials or are located in the interstitia of the muscles lateral and ventral to the basibranchials. In some *Ambystomidae*



(*A. tigrinum*) the lateral portions may develop into normal thyroids of considerable size.

The thyroid rudiments of *Typhlomolge* occupy a position closely resembling the location of the various thyroid portions of the Ambystomidae.

*Typhlomolge* 1, a sex-mature animal of 111 mm. total length and 58.2 mm. body length, does not possess even vestiges of a thyroid. The region of the lower jaw, throat, and heart was sectioned into a complete series; no section is missing. The anterior cardinal vein was followed in its entire course, the visceral cartilages and muscles were carefully searched through, but no traces of a thyroid could be found.

*Typhlomolge* 3, a small, apparently young, animal of 57.7 mm. total length and 32.6 mm. body length possesses a median thyroid rudiment. It is entirely detached from the pharyngeal epithelium and partly imbedded into the muscles just ventral to the basibranchial (genio-hyoideus). It is located between the attachments, to the basibranchial, of the hyoids and first gill arches and just anterior to the latter ones. The tissues are not well preserved, but the rudiment is seen to consist of several vesicles possessing epithelial walls and containing no colloid. No other epithelial structures were found, although the anterior cardinal vein was searched in its entire course down to the *Ductus cuvieri*, and the lymphatic space, the muscles, and cartilages of the visceral skeleton were carefully inspected.

In *Typhlomolge* 7, an animal of 75.6 mm. total length and 43.1 mm. body length, a median rudiment is attached to the ventral surface of the muscles just ventral to the basibranchial, about in the middle between the attachments of the hyoid and first gill arches (Fig. 2). It consists of a single solid cell mass of epithelial structure (Fig. 3). In the center a network-like structure is noticed, produced very likely by the cell walls of the clear inner cell ends; the same structure is frequently found in tangential sections through the walls of the follicles of normal thyroids. No colloid is contained in this rudiment. In addition to the median rudiment, two lateral rudiments are present, one on each side. The anterior ends of these rudiments are located near the connection between ceratobranchial and epibranchial of the first



gill arch and median to this arch (Fig. 4). They extend in a posterior direction; the posterior end approaches closely the wall of the lymph space, but does not come in contact with it. It is

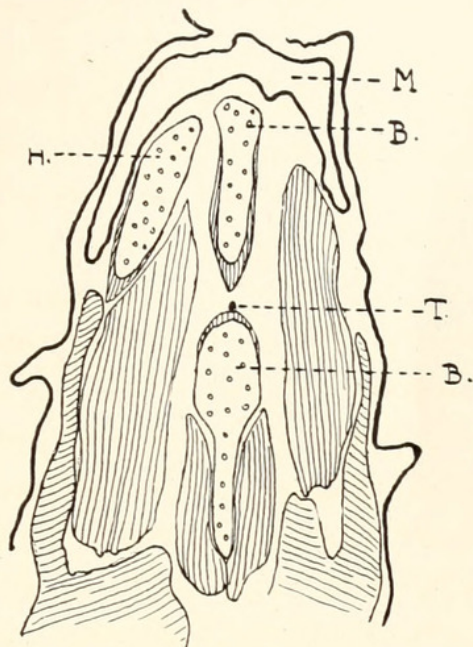


FIG. 2. Location of median thyroid rudiment in *Typhlomolge* 7. B, basibranchial; H, hyoid; M, cavity of mouth; T, thyroid rudiment.

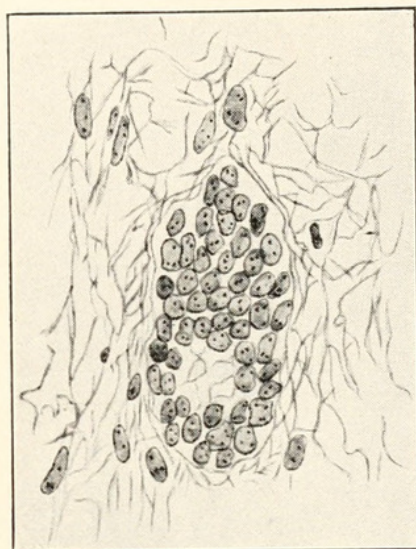


FIG. 3. Median thyroid rudiment of *Typhlomolge* 7.  $\times 340$ .

evident from this account that the lateral rudiments of *Typhlomolge* occupy a location similar to that of the main portions of the thyroid in *Ambystoma opacum*. They are, however, located further anterior, as if they had stopped migrating before attaining the definite position. Moreover, the place where the anterior cardinal vein passes the lymph space is located considerably more ventral; therefore, the lateral rudiments are nowhere in contact with this vein. It is indeed impossible to see any vessels supplying the thyroid rudiment;

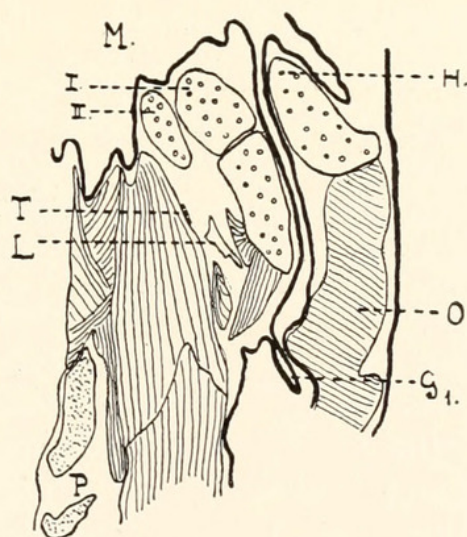


FIG. 4. Location of lateral thyroid rudiment of *Typhlomolge* 7. I., first gill arch; II., second gill arch;  $G_1$ , plate of first gill; H, hyoid; L, lymph space; M, cavity of mouth; O, operculum; T, thyroid rudiment.



if there are any they must be very small. The lateral rudiments consist of a series of tiny epithelial cell masses; some of them are solid, others are hollow, but none of them contain colloid.

In *Typhlomolge* 6, the smallest and probably youngest animal (56.0 mm. total length and 32.0 mm. body length), no median but one lateral rudiment on each side is present. They consist of a series of cell masses (Fig. 5), some of which are solid while

others contain a lumen. Colloid is absent in all of them. The location is similar to that of the lateral rudiments of the previous specimen. In particular they do not touch the lymph space and are situated dorsal to the place where the anterior cardinal vein comes into contact with this space. They are without a blood supply resembling that of a thyroid.

In *Typhlomolge* 2, an animal of 77.5 mm. total length and 43.6 mm. body length, only the lateral rudiments are present. Their location is the same as that of the lateral rudiments described above. Instead of being broken up into several separate cell masses, each of them has the shape of one continuous epithelial cell tube possessing a narrow lumen (Fig. 6).

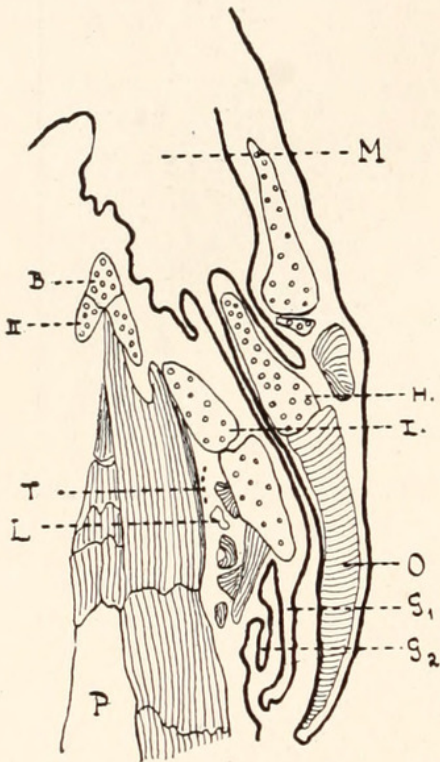


FIG. 5. Location of lateral thyroid rudiment of *Typhlomolge* 6. I., first gill arch; II., second gill arch; B, basi-branchial; G<sub>1</sub>, blade of first gill; G<sub>2</sub>, blade of second gill; H, hyoid; L, lymph space; M, cavity of mouth; O, operculum; P, pericardium; T, thyroid.

In *Typhlomolge* 5, an animal of 66.0 mm. total length and 36.5 mm. body length, only one lateral rudiment, the left one, is present. It is located near the connection between the ceratobranchial and epibranchial of the first gill arch, median to it and anterior to the location of a normal thyroid of *Ambystoma opacum*. It is composed of a small number of vesicles (Fig. 7) which, instead of being arranged in an antero-posterior row as in the other specimens, are crowded together in one place. The



vesicles have a distinct epithelial lining and are hollow; they do not contain colloid. In addition to this rudiment, *Typhlomolge* 5 possesses another one of similar structure, located on the same side but more median. It is attached to the left side of the muscle just ventral to the basi-branchial and just anterior to where the first gill arch connects with the basibranchial. Thus it has very nearly the same location as the median rudiment of other animals, but is displaced slightly to the left side. Apparently the primary median rudiment of this animal split into two rudiments; one of them, the left one, moved into its normal position. The right one not only failed to do so, but was dragged along a short distance by the left rudiment before complete separation was

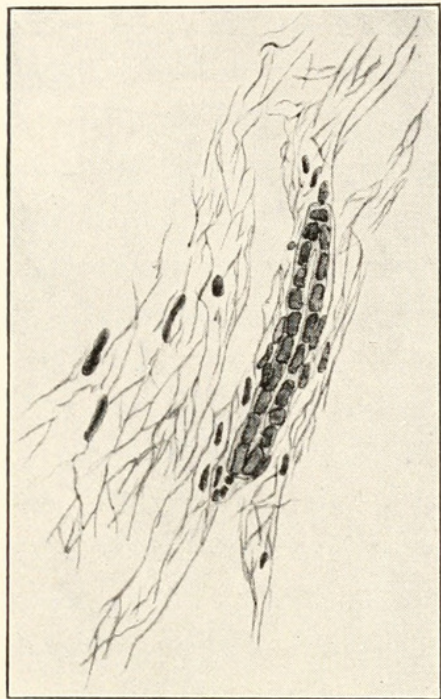


FIG. 6. Left lateral thyroid rudiment of *Typhlomolge* 2.  $\times 340$ .



FIG. 7. Left thyroid rudiment of *Typhlomolge* 5.  $\times 340$ .



accomplished, and thus was dislocated from its primary median position to wards the left side.

The 6 specimens described so far died on the way from Texas to New York, shortly after they had been removed from the caves. The seventh animal, *Typhlomolge* I, a specimen of 97.5 mm., died after it had been kept alive in the laboratory for 14 months. The largest part of this time (12 months) it lived at a temperature of 15° C. and in darkness; for the last two months it was kept in an aquarium stocked with plants, small crustaceans and young tadpoles, in daylight and at a temperature of approximately 22 to 25° C. The sections of this specimen are greatly torn and I am not sure that our inability to find a median and left lateral rudiment is due to the absence of these organs and not to the poor condition of the sections. On the right side, however, a lateral rudiment is present. It is located near the lymph space, anterior and dorsal to the location of the main portion of the thyroid of *Ambystoma opacum* and resembles more closely a thyroid structure than the rudiments of the specimens described previously. It consists of a small number of hollow vesicles which compose an elongate, egg-shaped organ possessing a connective tissue capsule and hence impressing one as a distinct and individual organ. The walls of the vesicles are of epithelial character; but no colloid is contained in the lumen of the vesicles (Fig. 8).

Summary: Although *Typhlomolge*, in its advanced stages, does not possess an organ resembling the normal thyroid of a salamander, epithelial structures are found which indicate that in the young embryo of this animal the thyroid rudiment forms in a similar manner as in other amphibians. This rudiment, however, for some reason, fails to develop into a thyroid. In some animals, development ceases after the median epithelial outgrowth has separated from the pharyngeal epithelium and the rudiment remains a single vesicle. In other cases it may partly split up into lateral portions which, as in other salamanders, move in a posterior direction and, in some instances, may approach closely the lymph space; but they never reach the anterior cardinal vein. The unsplit part of the median rudiment may retain its median position and primitive vesicular structure; the lateral portions



may either develop into a continuous epithelial tube or may break up into a series of solid or hollow cell masses. In some animals no median rudiment is found; if this condition develops in consequence of a complete splitting-up of the median rudiment or of later degeneration of the median rudiment, can be decided only by studying the embryology of this animal. In one specimen no vestiges of the thyroid are present at all. If Emerson's and Swingle's statements are not due to an oversight, on their part, of the inconspicuous epithelial structures, there are now 6 specimens of *Typhlomolge* known which did not possess any vestiges of the thyroid. One was described by Emerson, 3 by Swingle, one was found previously by me and the sixth animal is the one described in this paper. Either no thyroid rudiments developed in these six animals, or they were reabsorbed shortly after they had developed.

It is certain that the thyroid rudiments of *Typhlomolge* which do persist retain permanently a primitive epithelial structure and fail to develop, among many other structures of a normal thyroid, a venous rete and the colloid.

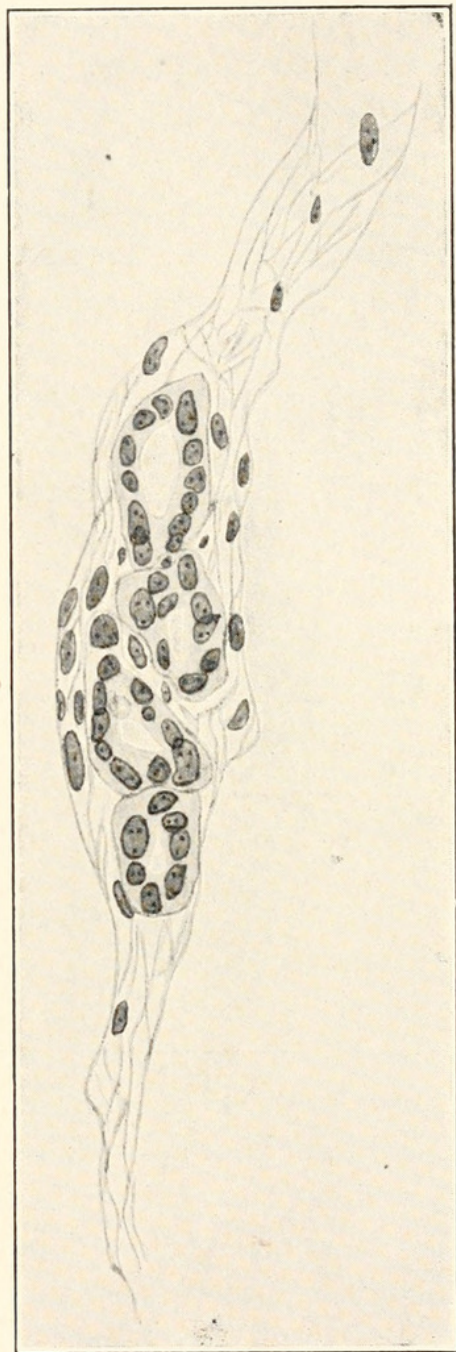


FIG. 8. Right thyroid rudiment of *Typhlomolge* I.  $\times 340$ .

#### OTHER ENDOCRINE ORGANS.

It may be briefly mentioned that the thymus glands, the hypophysis, and the postbranchial body were found to be present in



every specimen. Like other salamanders, *Typhlomolge* possesses 3 pairs of thymus glands; in one animal they were found fused into two large glands, one on each side. This condition is frequently met with in adult salamanders.

The postbranchial body, although, on a whole, it resembles this organ in other salamanders, shows certain peculiarities (see Baldwin's paper <sup>1</sup> for a description of this organ). Its structure is very similar to that found in *A. opacum*; in particular, it is found only on the left side. It is an epithelial structure of the shape of a tube possessing, in places, epithelial diverticula. A lumen is frequently absent, while in *A. opacum* and other Ambystomidæ this organ possesses often a very considerable lumen. The cephalic end of the organ is located in the pharyngeal epithelium with which it connects near the place where in Ambystomidæ the *Aditus laryngeus* is situated. In the Ambystomidæ the posterior end of the organ is often very large as compared to the thin duct-like anterior end and is located on the left side of the pericardium, posterior to the fourth aortic arch. Frequently it is closely attached to the pericardium and posterior wall of the fourth aortic arch. In *Typhlomolge* the fourth aortic arch is missing; the postbranchial body attaches itself to the third aortic arch. In some animals it reaches back to the heart and is found attached to the pericardium. Its posterior end, however, does not attain the size which this part is found to attain in *Ambystoma*. Moreover, in some specimens the organ remains short, extending backward only to the middle between pharynx and pericardium. In these cases its posterior end becomes attached to the wall of the third gill arch approximately half-way between the pericardium and the entrance of the arch into the gill blade of the third gill. It seems that the postbranchial body of *Typhlomolge*, although it possesses, on the whole, the structure of the normal organ of the Ambystomidæ, shows sometimes signs of developmental inhibition.

The hypophysis was studied only in two animals and only in transverse sections. Like the hypophysis of the Ambystomidæ <sup>2</sup> it is composed of 4 parts, the pars anterior proper, the partes tuberales, the pars intermedia and the pars nervosa. In the pars

<sup>1</sup> Baldwin, F. M., *Jour. Morph.*, 1918, XXX., 605.

<sup>2</sup> Atwell, W. J., *Anat. Record*, 1921, XXII., 373.



anterior, the largest part of the entire organ, the individual tubes are discernible more distinctly than in the *Ambystomidae*. They take an antero-posterior course and are arranged parallel to each other (Fig. 9). In the spaces separating the individual tubes,

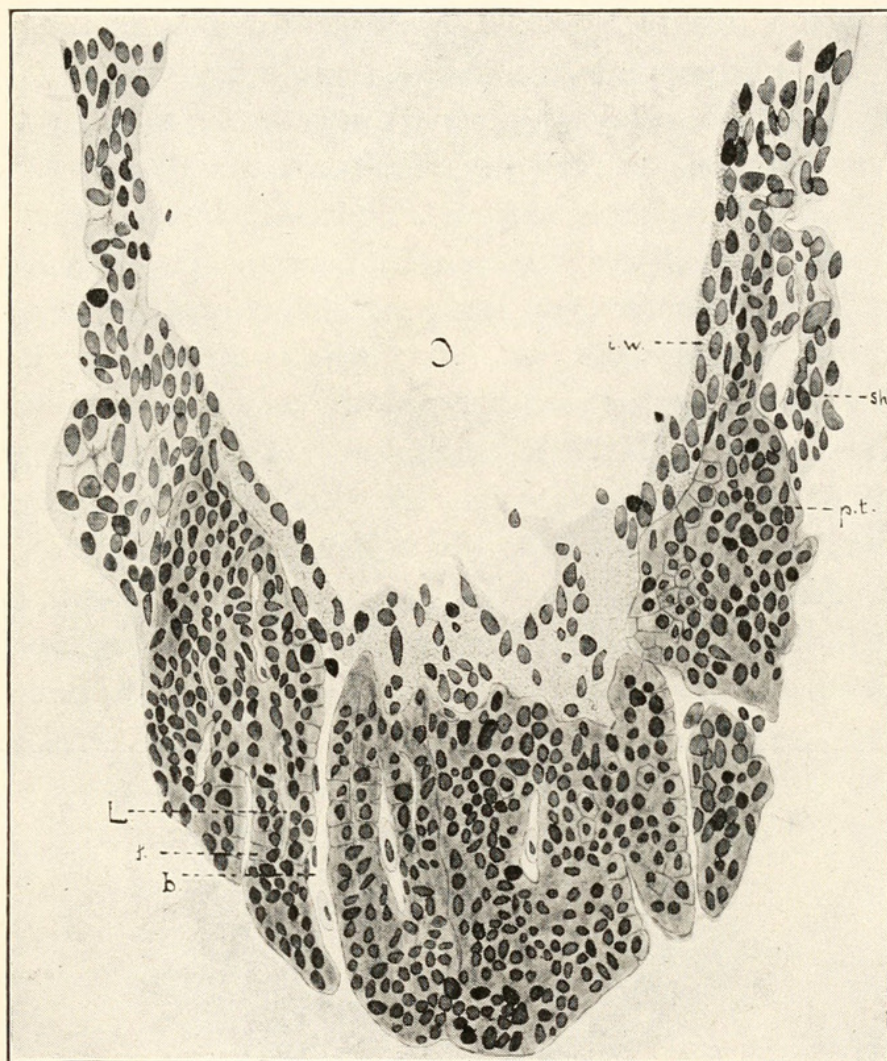


FIG. 9. Transverse section through the hypophysis of *Typhlomolge* 7. *b*, blood vessel; *i*, infundibulum; *i.w.*, infundibular wall; *L*, lumen of individual tube; *p.t.*, left pars tuberalis (of the right pars tuberalis this section contains only the connective tissue sheet of the ventral surface). *Sh*, connective tissue sheet. *t*, individual tube of pars anterior.  $\times 340$ .

large blood vessels are located. Frequently the individual tubes possess a distinct lumen; the nuclei of the cells are located at the distal end of the cell, towards the lumen of the tube. The cell walls are sometimes very distinct. The anterior end of the pars anterior continues into two lateral processes, the partes tuberales,



which, like in other salamanders<sup>5, 6, 7</sup>, are continuous with the pars anterior and attach themselves to the ventral wall of the infundibulum (Fig. 9). The partes tuberales of *Typhlomolge* are apparently smaller than in the adult *A. opacum* and resemble in size the partes tuberales of a larvæ of *Ambystoma opacum* of about 55 mm. total length and showing no signs of metamorphosis as yet. The pars intermedia of the amphibians cannot well be discriminated from the anterior part in transverse sections. But from such sections as shown in Fig. 10, it would seem that the dorsal part of the pars intermedia is bilobed, the two lobes being separated by a median antero-posterior space. The pars nervosa, as in other salamanders, consists in a thickening of the wall of the infundibulum where the pars intermedia is attached to it (Fig. 10). In comparing the pars nervosa of *Typhlomolge* with that of other salamanders, Haller's description<sup>7</sup> of the pars nervosa of *Proteus anguineus* is of interest. According to this author, the pars nervosa of *Proteus* is hardly differentiated from the rest of the infundibular wall. In *Typhlomolge* the pars nervosa is not only well-differentiated, but seems to be larger and more sacculated than is the case in *Ambystoma opacum* (Fig. 10). Summarizing

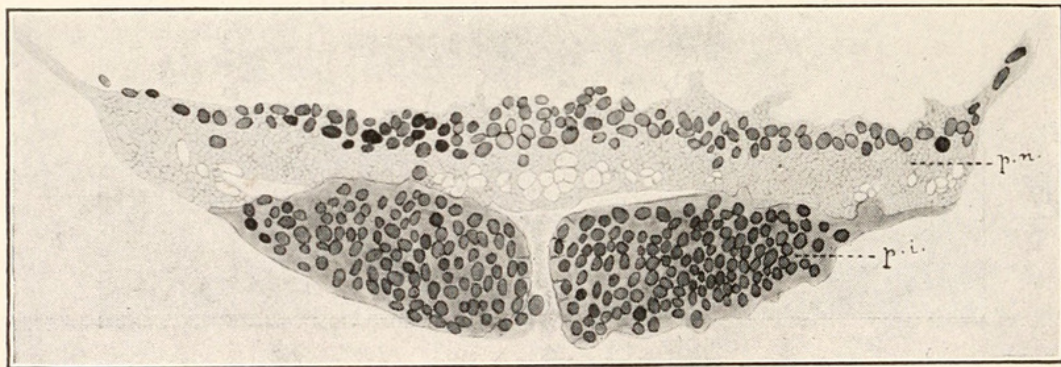


FIG. 10. Transverse section through dorsal regions of hypophysis of *Typhlomolge* 7. *p.i.*, pars intermedia; *p.n.*, pars nervosa.  $\times 340$ .

the description of the hypophysis of *Typhlomolge*, one may say that it resembles closely the hypophysis of other salamanders. In particular, it does not seem that the hypophysis of this species presents indications of an atrophic state, although, with a larger amount of material at our disposal we might find that the partes tuberales of *Typhlomolge* are of the size of a larval organ.

<sup>6</sup> Haller, B., *Morph. Jahrb.*, 1898, XXV., 30.

<sup>7</sup> Haller, B., *Arch. Mikr. Anat. und Entwicklsg.*, 1909, LXXIV., 812.



## DISCUSSION.

The factor which led to the inhibition of the thyroid development is unknown. The researches of Leo Adler<sup>8</sup> and Bennett M. Allen<sup>9</sup> showed that extirpation of the hypophysis in amphibians inhibits the development of the thyroid. One could imagine that defective development of the hypophysis might have been the immediate cause of the inhibition of the thyroid development in *Typhlomolge*, but so far, no abnormalities in the structure or mutual relations of the various parts of the hypophysis have been discovered, which could account for the thyroid atrophy of *Typhlomolge*—a phenomenon singular in the vertebrates.

It is not known whether the atrophy of the thyroid of *Typhlomolge* is only one of the results caused by certain factors which lead to the inhibition of the general development of this animal, or whether the inhibition of the thyroid was primary to other developmental inhibitions. As pointed out in previous papers<sup>10</sup> the athyroidism of this species possesses special interest, because in the same species metamorphosis also is suppressed. I have assumed, in a purely tentative manner and in order to obtain a basis for further experiments, that the latter phenomenon is the direct result of the lack of the thyroid. In the absence of adequate experiments and in the light of the well known fact that extirpation of the thyroid inhibits the amphibian metamorphosis<sup>11, 12</sup>, this explanation still seems to be the most feasible one.

Swingle,<sup>13</sup> in a recently published article, takes occasion to criticise my attitude, outlined above, towards the problem of neoteny in *Typhlomolge*. He has made certain observations confirming the existence of a releasing mechanism in salamanders. Regarding the facts communicated in this article, these surely should be welcome to the writer of the present article, in so far as they led Swingle to exactly the same conclusion as that at which I arrived as early as 1919. But certain statements made in this paper are apt to give rise to misunderstandings. To prevent these a discussion of Swingle's paper seems desirable.

<sup>8</sup> Adler, L., *Arch. Entwicklgsmech. d. Org.*, 1914, XXXIX., 21.

<sup>9</sup> Allen, B. M., *Biol. Bull.*, 1917, XXXII., 117.

<sup>10</sup> Uhlenhuth, E., *Endocrinology*, 1922, VI., 102.

<sup>11</sup> Allen, B. M., *Science*, 1916, N. S. XLIV., 755.

<sup>12</sup> Hoskins, E. R., and Hoskins, M. M., *Jour. Exp. Zool.*, 1919, XXIX., 1.

<sup>13</sup> Swingle, W. W., *Jour. Exp. Zool.*, 1922, XXXVI., 397.



Throughout his paper Swingle attempts to create in the reader's mind the impression that in my previous work I have laid too much one-sided emphasis on the thyroid gland as the only organ potent in the amphibian metamorphosis. This attitude is perplexing in view of the circumstance that I have been able to disclose facts demonstrating the existence of a releasing mechanism outside of the thyroid and in view of the other circumstance that Swingle received the discovery of this "releasing mechanism" when communicated by the writer in 1919<sup>14</sup> in the following way<sup>15</sup> (p. 600): "Uhlenhuth, while accepting the conclusions stated regarding the relation of iodine to amphibian metamorphosis, thinks that still another substance is needed to cause the thyroid gland to excrete the iodine necessary for metamorphosis. This hypothetical factor he terms excretor substance and thinks that it is evolved during the growth processes of the organism. The assumption of an excretor substance obscures rather than clarifies the already sufficiently complicated problem of amphibian metamorphosis."

As to Swingle's criticism regarding the omission, on my part, of the possible defectiveness of the releasing mechanism in the explanation of the neoteny of *Typhlomolge*, it should be pointed out that the writer of this article has, reluctantly, refrained from suggesting this possibility, because no experiments suggesting it have been—or are today—available. As to the actual interpretation of the neoteny of *Typhlomolge* and as to my attitude<sup>16</sup> towards Jensen's<sup>17</sup> experiments which showed that adult *Proteus* and *Necturus* do not metamorphose upon thyroid administration, the following statements may be made: (1) the interpretation of this phenomenon as given in my previous papers<sup>16</sup> does not form an integral part of the theory of a releasing mechanism; (2) the most pertinent problem in regard to the neoteny of *Typhlomolge* was the question whether or not this animal possesses a thyroid gland proper, a question to the solution of which Swingle has contributed nothing, as will become evident from a perusal

<sup>14</sup>Uhlenhuth, E., *Jour. Gen. Phys.*, 1919, I., 473.

<sup>15</sup>Swingle, W. W., *Jour. Gen. Phys.*, 1919, I., 593.

<sup>16</sup>Uhlenhuth, E., *American Naturalist*, 1921, LV., 193.

<sup>17</sup>Jensen, C. O. *Oversigt klg. Danske Vidensk. Forhandl.*, Copenhagen, 1916, No. 3, 251.



of the introduction to this article and of the facts described above; (3) the lack of an effective releasing mechanism may have been the primary cause of the neoteny of *Typhlomolge*, but so far nothing as to this effect can be quoted; (4) the question of whether or not *Typhlomolge* and physiologically similar species, such as *Proteus* and *Necturus*, possess, in their present state, the ability to metamorphose upon thyroid administration is a problem entirely aside from the rôle of the releasing mechanism and has been considered so in the writer's previous papers. (It is possible that permanent suppression of the thyroid function over long periods may cause complete loss of the reactivity of the organism. The demonstration of such complete loss, however, does not decide the question as to whether primarily the thyroid ceased to function, in *Typhlomolge*, on account of a defective releasing mechanism); (5) it has not been proved as yet that *Typhlomolge*, *Proteus*, and *Necturus* have completely lost their ability to react to the thyroid hormone. It was merely this point which gave occasion for the criticism of Jensen's work. Jensen<sup>17</sup> exposed only the adult specimens of *Proteus* and *Necturus* to the action of the thyroid hormone. That he did not succeed in enforcing metamorphosis does not necessarily mean that the responsiveness of these animals has been completely lost. In order to show that *Necturus*, *Proteus*, and *Typhlomolge* could not metamorphose even if they were in the possession of a complete and normal thyroid mechanism, the young larvæ or even the parents at the time of development or ripening of the ova and spermatozoa may have to be subjected to thyroid administration. Swingle has merely repeated Jensen's experiments on *Necturus*, without modifying Jensen's technique. Like Jensen, he did not use the young larvæ, but the adult animals. Nowhere in Swingle's paper, however, can there be found any reference to Jensen's experiments on *Necturus*.

The same attitude is met with in Swingle's paper regarding the releasing mechanism. Although no progress beyond the present state of the problem has been accomplished, there is, in Swingle's article, no mention made anywhere of previous work on the same problem.

That the thyroid mechanism of salamanders consists of two physiologically distinct parts was found by the writer of this



paper as early as 1919.<sup>14</sup> The discovery of the factor necessary to release the thyroid hormone was summed up in the following statement,<sup>14</sup> (p. 476): "Hence, besides iodine, still another substance is needed in the amphibian metamorphosis; namely the 'excretor substance' which causes the thyroid to excrete the stored-up iodine." Swingle's statement<sup>15</sup> (p. 600), made in 1919 in reference to this work and quoted above, shows that he did not recognize the existence of such a releasing factor.

Since organs of internal secretion or any other organs would manifest themselves physiologically in a manner essentially similar to a substance and since it seemed undesirable to reflect in the term applied to the releasing factor upon any preconceived theory, the term "excretor substance" was replaced later on by the term "releasing factor"<sup>16</sup> (p. 207) and "releasing mechanism"<sup>10</sup> (p. 112), both of them implying merely the function by which this factor manifests itself and which was actually observed.

Since the first communication was made my experiments were continued and it was shown, for 3 different species of salamanders, that in low temperature metamorphosis is greatly retarded in proportion to general growth, while the development of the thyroid gland shows no such retardation. It was concluded from this fact that since the thyroid developed at a normal rate in proportion to general growth the development of the releasing mechanism was retarded. Several papers were published in regard to this problem and the difference between the retardation of the thyroid and the releasing mechanism in response to the same degree of lowered temperature was explained by assuming a lower temperature coefficient for the thyroid than for the releasing mechanism. In 1921 the results of this work were summarized in the following statement<sup>16</sup> (p. 206): "The most conspicuous character in the salamander metamorphosis is the fact that although it certainly is dependent on the thyroid hormone, it does not necessarily take place in larvæ whose thyroid is mature. This can only mean that two factors are required in order to bring about the metamorphosis of salamander larvæ, namely a mature gland and a factor which releases the thyroid hormone from the follicles of the gland."

Further confirmation of the existence of a releasing mechanism



has been found in the iodine experiments.<sup>10, 18, 19, 20</sup> Administration of an excess of inorganic iodine does not enforce the metamorphosis of salamander larvæ,<sup>10, 20</sup> yet the elaboration of the colloid is accelerated by iodine feeding. This result was to be expected if the release of the hormone does not depend on the quantity of hormone developed in the follicles of the thyroid but is controlled by a particular releasing mechanism.

The results outlined above were checked also by histological sections of large numbers of thyroids of normal and experimental animals. Although the publication in full of this work has been postponed in order to assure greater completeness, single results have been referred to in various papers and have been demonstrated to colleagues and before meetings. In every case it was found that the elaboration of the colloid and the excretion of it are two distinct and independent processes, physiologically as well as structurally. Elaboration of normal colloid is frequently met with in cases of inhibition of metamorphosis and in normal larvæ long before metamorphosis, and, in this case, is combined with complete absence of the structures characteristic of the excreting stage of the thyroid. This relation has been interpreted as further testimony in favor of the existence of a releasing mechanism.

I must also refer here to Swingle's criticism of my iodine experiments, since, if correct, it would question the value of these experiments as supporting the theory of the releasing mechanism. My experiments<sup>10, 20</sup> showed that, contrary to anuran larvæ, in the larvæ of salamanders metamorphosis cannot be enforced by the administration of inorganic iodine. The bearing of this fact upon a general theory of the rôle of iodine in the specific effect of the thyroid hormone has been outlined in detail in two previous papers.<sup>10, 20</sup> Swingle's general attitude in his paper tends to create the impression (1) that I have claimed "iodine has nothing to do with the axolotl metamorphosis"<sup>13</sup> (p. 417) and (2) that somewhere in my papers are to be found statements to the effect that organic iodine compounds cannot enforce the metamorphosis of the axolotl and other salamanders.

<sup>18</sup> Uhlenhuth, E., *Jour. Gen. Phys.*, 1922, IV., 319.

<sup>19</sup> Uhlenhuth, E., *BIOL. BULL.*, 1921, XLI., 307.

<sup>20</sup> Uhlenhuth, E., *BIOL. BULL.*, 1922, XLII., 143.



As to the first point I should like to refer the reader to the following statement,<sup>10</sup> (p. 114) into which my results on the rôle of iodine were summarized: "That iodine if supplied in excess does not produce metamorphosis of salamander larvæ does not mean, according to what has been said above, that it is not necessary in the metamorphosis of salamanders. Very likely if larvæ of salamanders would be raised on an iodine-free diet and kept in iodine-free water, metamorphosis could not take place."

Regarding the second point Swingle quotes against me his own experiments<sup>13, 21</sup> in which he thinks he has shown that 3-5 di-iodo-tyrosine can enforce metamorphosis of thyroidectomized axolotls, and Jensen's experiments (22) with iodized proteins. Neither Swingle's own experiments nor Jensen's experiments referred to have proved that inorganic iodine can be utilized directly by the axolotl tissues to elaborate the thyroid hormone. The facts regarding the influence of inorganic iodine on the axolotl metamorphosis are, however, widely different from what Swingle would like them to be.

In the first place, Jensen has not only not shown that inorganic iodine does enforce metamorphosis of the axolotl, but on the contrary has shown that inorganic iodine as such is ineffective in the axolotl metamorphosis. In one of his papers, Jensen<sup>23</sup> points out that the effectiveness of thyroid preparations in enforcing the axolotl metamorphosis does not correspond to the iodine-content of these preparations. In a personal conversation, Professor C. O. Jensen told me that he had tested the action of inorganic iodine, but found it ineffective in enforcing the axolotl metamorphosis. Jensen's experiments are therefore entirely in accord with my own experiments. Moreover, Professor Jensen's experiences which are well in accord with my own observations may serve as a warning against the reliability of those experiments which resulted in "enforced metamorphosis" of the axolotl. Among Professor Jensen's strains of the European race of the axolotl there were, in the beginning, animals which gave rise to offspring 50 per cent. of which would metamorphose

<sup>21</sup> Swingle, W. W., *Science*, 1922, N. S., LVI., 720.

<sup>22</sup> Jensen, C. O., *Compt. rend. Soc. Biol.*, 1920, LXXXIII., 315; 1921, LXXXIV. 423; 1921, LXXXV., 391.

<sup>23</sup> Jensen, C. O., *Hospitalstidende*, 1920, LXIII., 505.



spontaneously. Early in his work he began to select carefully individuals which produced 100 per cent. neotenus larvæ.

Swingle also quotes the experiments of Huxley and Hogben,<sup>24</sup> and of Hirschler<sup>25</sup> against me. What Huxley and Hogben really found, however, is that inorganic iodine does not enforce the metamorphosis of axolotls. There are still Hirschler's experiments; these are represented by "one" successful experiment. The total number of Hirschler's experiments on inorganic iodine in relation to axolotl metamorphosis is "two." One animal was given an intraperitoneal injection of iodoform; it died before a conclusive result was obtained. The other animal received an injection of iodine dissolved in potassium iodid; it metamorphosed completely. But the animal illustrated, as a control alongside this experimental animal, shows, contrary to the authors claim, distinct signs of metamorphosis, a reduction of the tail fin and instead of the larval gills mere stubs. It seems to me the number of Hirschler's positive experiments will have to be increased before they can be held against the negative experiments of Jensen, Huxley and Hogben, and myself.

As to Huxley's and Hogben's positive results<sup>24</sup> on the larvæ of *Salamander maculosa* and *triton*, quoted by Swingle against me, it should be stated that the method employed in these experiments is such as to permit of no conclusions whatsoever. In the first place, they did not use the first moulting, but the sizes of the gills as an indicator of metamorphosis. The gills may become reduced in size by the action of many factors different from metamorphosis, particularly by starvation. Since strong iodine solutions were used, it is almost certain that contrary to the authors' impression (quantitative measurements of the food intake were not made) the experimental larvæ fed less well than the controls. Secondly, nowhere in Huxley's and Hogben's paper can I find any statement indicating the size and stage of the larvæ at the beginning of the experiment. Yet if the larvæ were in an advanced larval stage any irritation as serious as that caused by iodine solutions would be sure to bring about precocious metamorphosis.

<sup>24</sup> Huxley, J. S., and Hogben, L. T., *Proc. Royal Soc.*, 1922, XCIII., 36.

<sup>25</sup> Hirschler, J., *Arch. Entwicklgsmech. d. Orgn.*, 1922, LI., 482.



It is evident that none of the observations according to which inorganic iodine does enforce the metamorphosis of salamanders can be accepted as correct at the present time.

That organic iodine compounds may enforce the metamorphosis of neotenuous forms of salamanders has been claimed repeatedly and may be true, although the axolotl used generally in these experiments appears, for reasons stated above, to be an unreliable material. Jensen was the first one who studied, in an extensive manner, the influence of organic iodine-compounds upon the metamorphosis of axolotls. Where he left the problem it is still at the present time. In particular, Jensen deserves the credit for having recognized that the experiments with iodine could not advance the problem unless thyroidectomized larvæ are used. He was the first one who administered organic iodine compounds to thyroidectomized axolotls and stated <sup>26</sup> that thyroxine can be used directly by the organism without the intermediation of the thyroid. Swingle repeated these experiments <sup>13, 21</sup> using 3-5 di-iodo-tyrosine, a substance which Jensen <sup>27</sup> had found ineffective in the normal axolotl. Swingle reports that 3-5 di-iodo-tyrosine does enforce the metamorphosis of thyroidectomized axolotls. Both Jensen's and Swingle's experiments, however, should be taken with caution as far as the successful thyroidectomy is concerned. I am not certain at all that Swingle realizes that an axolotl possesses 4 thyroid glands, two main portions and two accessory ones. He mentions it nowhere and it is likely that only the main portions were extirpated. The accessory thyroid glands of *A. tigrinum* have a tendency to become very large and, after removal of the main portions, may enlarge considerably, so as to cause finally metamorphosis, as I observed in many larvæ of *A. tigrinum*. It is likely that Swingle's "thyroidless" axolotls were in the possession of two developing accessory glands; that an axolotl does not possess accessory glands I would be willing to believe only if sections through the entire region of the lower jaws, throat, and heart could be presented, since dissection, because of the hidden position of these accessory glands, may fail to demonstrate them. If the main thyroids are removed, it takes a long time before the accessories, in the event that they

<sup>26</sup> Jensen, C. O., *Compt. rend. Soc. Biol.*, 1921, LXXXV., 391.

<sup>27</sup> Jensen, C. O., *Compt. rend. Soc. Biol.*, 1920, LXXXIII., 315.



have been small, attain a size and structure capable of producing metamorphosis. But as shown in my iodine experiments, the feeding of iodine would greatly accelerate the elaboration of the hormone and, if the releasing mechanism is set active (which it was in Swingle's specimens, according to his own statements), metamorphosis may occur months before it takes place in the untreated controls. Swingle has observed his animals apparently only for 6 months; it would be important to know whether the untreated "thyroidectomized" animals did not finally metamorphose.

Swingle<sup>13, 21</sup> mentions also that 3-5 di-brom-tyrosine, when fed to thyroidectomized axolotl larvæ, is incapable of enforcing metamorphosis and thinks that this result is contrary to my own views on the rôle of iodine in the amphibian metamorphosis and in the thyroid hormone. Apparently he did not see the following statement, in which my views were summarized<sup>10</sup> (p. 114): "The views elaborated above are in no way contradictory to the fact that nevertheless, in a biological sense, iodine is an important and essential part of the thyroid hormone; if it were possible to substitute the iodine by any other substance without changing the reactivity of the hormone, biologically this would not make iodine less important, for it is the only substance which, by the mechanism actually available to the organisms, can be used in the manufacture of the thyroid hormone. Although chemically bromine or any other halogen may be able to substitute iodine without changing the chemical or even the physiological reactivity of the thyroid hormone, the organism is unable to use bromine, as shown by Swingle, and presumably the other halogens to make thyroid hormone." I have never claimed that the thyroid or any other organ can manufacture the thyroid hormone from bromine. Swingle has not touched, by his experiments, the real problem. This centers around the question whether the finished thyroid hormone could enforce metamorphosis if it contained bromine instead of iodine; Swingle did not employ such a product in his experiments.

As Swingle correctly states, the crux of the problem of thyroid function is now to find the organ or tissue or substance which plays the rôle of a releasing mechanism to the thyroid gland. I have intentionally refrained, in my previous papers, from forming



any theories, aside from those directly suggested by the results of my experiments, as to the nature of the releasing mechanism; devoting pages to discussing assumptions and hypotheses does not materially advance the problem. We know, of course, that the hypophysis has something to do with the development and, possibly, with the function of the thyroid. I have made some experiments, to be published shortly, which seem to indicate that some unknown factor is located in the gills, in the absence of which the thyroid, although it develops in a normal manner, remains incapable of releasing the hormone. Swingle mentions one experiment which was intended to test the activity of the hypophysis of a neotenus axolotl by the grafting method. Although ultimately it may turn out that the hypophysis controls, in some way, the releasing mechanism, Swingle has so far contributed nothing to the solution of this problem.

Hence it is very evident that Swingle has not advanced, by a single step, the problem of neoteny and thyroid function beyond the stage at which my own researches left it.

#### SUMMARY.

1. Only in one, a sex-mature specimen, among 7 specimens of *Typhlomolge rathbuni*, is the thyroid completely absent; in the other 6 specimens rudiments of the thyroids are present.
2. The thyroid rudiments are undifferentiated epithelial cell masses located along the path of migration of the thyroid, typical for salamanders. They may contain a lumen, but never contain colloid and blood vessels.
3. *Typhlomolge* possesses 3 pairs of thymus glands.
4. The hypophysis is similar to that of other salamanders. But the partes tuberales are perhaps smaller than in the adult *A. opacum* and the pars nervosa is larger.
5. The postbranchial body resembles much that of other salamanders, but sometimes is shorter and lacking a lumen.



## BREEDING EXPERIMENTS WITH CONFINED BREMUS (BOMBUS) QUEENS.<sup>1</sup>

O. E. PLATH,

COLLEGE OF LIBERAL ARTS, BOSTON UNIVERSITY.

The biologist who attempts to give a complete account of the life-history and habits of the bumblebees of any part of the world, is generally confronted with the following difficulties: (1) He rarely, if ever, has the opportunity to study the beginning and early stages of a bumblebee colony, and (2) it is usually impossible for him to ascertain the biology of the less common species, because he is unable to secure their nests. The first attempts to overcome these difficulties were made by the Austrian zoölogist Hoffer ('82). This eminent bumblebee student confined a large number of *Bremus* queens in a museum at Graz, and thus was able to observe how the queen of *Bremus lapidarius* constructs her nest and the first egg-cell. However, none of these breeding experiments of Hoffer ('82, p. 413) produced a colony.

Better results along this line were obtained by the late F. W. L. Sladen ('12), who succeeded in rearing several colonies of *Bremus terrestris*, a species which is very common in most parts of Europe. About the same time, similar experiments were carried out by the Danish biologist Lindhard ('12) with queens of *Bremus agrorum*, *distinguendus*, *hortorum*, *lapidarius*, *subterraneus*, *sylvarum*, and *terrestris*. With the exception of *Bremus hortorum* and *subterraneus*, at least one queen of each of these species started a nest, some of the resulting colonies later becoming self-supporting. In this country, Mr. Theodore H. Frison ('18) was equally successful in artificially rearing a colony of *Bremus auricomus*, a species concerning whose biology little was known up to that time.

I became interested in this subject during the summer of 1921 and decided to try similar artificial breeding experiments with our

<sup>1</sup> Contribution from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 222.



New England species of *Bremus*. During the following spring and summer, about fifty queens belonging to eleven <sup>1</sup> of the thirteen New England species listed by Franklin ('12/13, pp. 190, 191) were captured in the Arnold Arboretum, within the city limits of Boston. After being confined for various periods of time, at least one queen of each of these species became broody and oviposited, but self-supporting colonies were only produced by six.<sup>2</sup> The names of these six species, and the number of colonies obtained from each, are listed in the accompanying table:

TABLE I.

Species.	Number of Colonies.
1. <i>Bremus bimaculatus</i> Cresson.....	2
2. <i>Bremus impatiens</i> Cresson.....	1
3. <i>Bremus perplexus</i> Cresson.....	1
4. <i>Bremus separatus</i> Cresson.....	2
5. <i>Bremus ternarius</i> Say.....	1
6. <i>Bremus vagans</i> Smith.....	2

Before discussing the methods which were used in these breeding experiments, it seems desirable to describe briefly those employed by the earlier workers. Hoffer ('82) supplied his queens with nesting material and plenty of fresh flowers. Each queen was probably confined in a separate box. At first, Sladen ('12) also confined each queen separately, giving her an artificial nest and a liberal supply of honey and pollen, but he was unable to get a colony started in this way. He then placed two queens (of the same species) in each box, and this method yielded better results. However, Sladen (p. 131) found that one of the queens always killed her companion about the time the eggs were laid, and that the victorious queen invariably deserted the nest, unless she was supplied with one or more workers. In order to avoid this killing of queens, Sladen (p. 132) modified the experiment by confining one queen with one or more workers in each box. This also proved successful, even when the workers were of a different species.

<sup>1</sup> *Bremus affinis*, *bimaculatus*, *borealis*, *fervidus*, *impatiens*, *pennsylvanicus*, *perplexus*, *separatus*, *ternarius*, *terricola*, and *vagans*.

<sup>2</sup> On June 13, several of these incipient bumblebee colonies were exhibited at a meeting of the Cambridge Entomological Club.



The methods employed by Lindhard ('12), differ in several respects from those of Hoffer ('82) and Sladen ('12). Lindhard (pp. 337, 338) used nest-boxes which were constructed as follows: Each nest-box consisted of two compartments of about 20x20x20 cm. each, one of which may be called the front compartment, or No. 1, and the other the rear compartment, or No. 2. On one side, compartment No. 1 had a glass pane to admit light. At the base, this compartment was provided with two flight-holes, *a* and *b*; *a*, communicating with the outside world, and *b*, with compartment No. 2. Both flight-holes could be opened and closed.

After lining compartment No. 2 with a layer of sod, about 5 cm. thick, Lindhard (p. 337) filled the interior with dry grass and the like. In some cases (cf. p. 341), a small paste-board box, filled with moss, and provided with a glass cover, was placed in the compartment instead of sod, and was surrounded with loose earth. With this arrangement, it was possible to place food in compartment No. 1 without disturbing the queen while she was engaged with her nest in the other compartment, and to keep the nest at a more uniform temperature.

As food, Lindhard (p. 337) provided a 50 per cent sugar solution and flowers, preferably those from which bumblebees were then obtaining pollen out in the open. After the queen had begun nest-building, the flight-hole was opened so that she could gather pollen from large bouquets which were put in the room in which the box was kept. As soon as the first worker emerged, the colony was placed out of doors where further development proceeded under normal conditions. In subsequent experiments, the queens were permitted to forage in the open shortly after they had begun nest-building. This method promised equally satisfactory results at the time Lindhard ('12) reported his work.

In his breeding experiments with *Bremus auricomus*, Frison ('18) confined two queens in one box, but, unlike Sladen (p. 131), found that they did not kill each other. As nesting material, Frison ('18, p. 44) supplied an old field-mouse nest in which he placed a honey-moistened lump of pollen. In addition to this, the queens were given a mixture of honey, rye-flour, and water.

The methods which were used in my own experiments, may be



described briefly as follows: Each nest-box was provided with a double cover, the lower being made of glass and the upper of wood or tar-paper. At one end of the box, a round hole,  $\frac{1}{2}$  inch in diameter, which could be closed by means of a cork, served as a flight-hole. A small piece of honeybee foundation (wax), about an inch square, was then firmly pressed to the bottom of the nest-box near the opposite end. Around this piece of wax, a circular layer of cotton was placed as nesting-material. A tin can, about 3 inches in diameter and 2 inches high, from which the cover was first removed, was then put upside down over the honeybee foundation and cotton, after a hole, through which the queen could readily pass, had been made in the rim. Every two or three days, a fresh supply of pollen, obtained from two colonies of honeybees which had been especially secured for this purpose, was provided on the layer of wax within the cotton ring. Liquid food, consisting of about half water and half honey, was supplied daily in a porcelain dish, about  $\frac{1}{2}$  inch high, outside of the tin can. In order to keep the nest-box as sanitary as possible, a small pile of dry sand was put in one of the corners of the box.

Not being acquainted with the methods employed by Lindhard ('12), at the time my experiments were carried out, I at first placed two queens (of the same species) in each nest-box, as was done by Sladen ('12) and Frison ('18). However, like Sladen (p. 131), I found that one of the queens invariably killed the other,<sup>1</sup> sometimes within a few minutes after they had been placed together, and that the victorious queen was frequently made useless for further breeding experiments by the loss of one or more antennæ, or legs.<sup>2</sup> I therefore only placed one queen in each nest-box in subsequent experiments, and furnished each one with from one to three workers, preferably of her own species. Whenever a worker died—a rather frequent occurrence, especially as long as there was no brood—another was substituted as soon as possible. Those bumblebee “nuclei” which belonged to species easily obtainable in or near Boston, were permitted to forage out

<sup>1</sup> As already stated, Mr. T. H. Frison ('18) found that two queens of *Bremus auricomus* behaved differently in this respect, but, judging from Mr. Frison's (p. 45) account, it seems probable that one of the queens was in poor health.

<sup>2</sup> These observations were made on about twenty queens of *Bremus bimaculatus*, *fervidus*, *impatiens*, and *vagans*.



of doors, whenever the weather was pleasant, provided eggs or larvæ were present in the nest. Shortly after the first worker emerged, the tin can was removed, and the young colony, after being provided with additional nesting material, was given complete liberty.

Several nuclei, instead of building the first cells on the honeybee foundation, started their nests on the floor of the box, outside of the tin can. The behavior of such nuclei was as follows: The queen and workers, with outstretched abdomens, nestled closely to the floor of the nest-box about a certain spot which they had cleared of all foreign matter. As a result of this behavior, the chosen spot was gradually (sometimes within a day or two) coated with a layer of wax, and at this place the first cell was built. On several occasions, I tried to discourage the bees from starting their nest outside the tin can, by placing sand on the spot which they had selected. However, this did not disconcert them, for they immediately began to push the sand aside, picking up the larger grains with their mandibles and carrying them to the periphery of the wax-covered area. This experiment was repeated several times with a nucleus of *Bremus bimaculatus*, but the bees could not be persuaded to start their nest under the tin can until a layer of soap was substituted for the wax which they had deposited.

Having given a general account of the methods used in rearing bumblebee colonies from confined queens, I shall now proceed to discuss my own experiments somewhat more in detail. In order to make these data as complete as possible, a brief account of what is known concerning the nesting habits of our New England species has been added, with the exception of those cases where this has already been done in a previous paper ('22b). Since all of these breeding experiments were carried out during the spring and summer of 1922, the year has been omitted from most dates, 1922 being understood, unless otherwise indicated.

#### *Terrestris* GROUP.

##### 1. *Bremus affinis* Cresson.

From the latter part of May until the end of June, several queens of this species were confined in separate nest-boxes with



two or three workers. Although two of the queens constructed egg-cells and oviposited, the young larvæ died, apparently because they were not fed.

That it is possible to rear some, if not all, of our American *Bremus* species by the methods which were finally adopted by Lindhard ('12), is shown by the following incident, and another which will be discussed in connection with *Bremus vagans*. On May 26, a queen and two workers of *Bremus affinis* were confined together. Two days later, the queen constructed an egg-cell and oviposited, but on May 30, it became apparent that she had forsaken her brood. She was therefore set free from a third story window of one of the Bussey buildings, about noon on the following day, and her eggs and the two workers were turned over to another *affinis* queen. About five hours later, an *affinis* queen was noticed examining carefully several second story windows of the building referred to above, whereupon she mounted to the third story window from which the *affinis* queen had been liberated, and attempted to get in. I hastened upstairs, opened the window, and tried to catch her with my insect net, but missed her, and she flew away. About 11 A.M. on the following day (June 2), she again appeared at the third story window, but left as soon as I opened it, and, to my knowledge, did not return.

The nesting habits and disposition of *Bremus affinis* have been discussed in a recent paper ('22b), but the following incident seems worth recording. On June 2, a nest-box containing a queen, three workers, and an egg-cell of this species was accidentally jarred. Both, the workers and the queen immediately began to buzz angrily and rush out from beneath the tin can. In doing so, the queen accidentally encountered one of the workers, seized the latter and stung it to death.

## II. *Bremus terricola* Kirby.

A queen of this species was confined on May 26, and a few days later three *terricola* workers were associated with her. Shortly after the introduction of the workers, the queen oviposited, and on June 16, the nest contained several small larvæ. About two weeks later (July 4), two exceedingly small *terricola* workers were noticed on the sand pile. They were unable to crawl, and had probably been released from their cocoons by the



queen or workers and then dragged to the sand pile. No other brood being present in the nest, this nucleus was combined with another *terricola* colony which had been taken on the preceding day.

The nesting habits of this species have been dealt with in another paper ('22b).

#### *Borealis* GROUP.

##### I. *Bremus borealis* Kirby.

Four queens of this species, captured in or near the Arnold Arboretum, were experimented with. As no *borealis* workers could be obtained in the vicinity of Boston, two workers of *Bremus fervidus* were given to queen No. 1 (confined May 29), and three workers of *Bremus impatiens* to queen No. 2 (confined June 6), but neither one of the queens would coöperate with the foreign workers. On June 25, queen No. 1 was found dead in the nest, whereupon the workers of both queens were liberated, neither queen having started a nest. A somewhat different method was then resorted to. On June 26, about a dozen cocoons of *Bremus impatiens* from which workers were just beginning to emerge, were given to *borealis* queen No. 2. She immediately adopted both, the cocoons and the workers, and on June 28 constructed an egg-cell and oviposited. Two days later, this mixed colony was permitted to forage out in the open, after a small notch had been made in one of the wings of the queen. When the nest was examined on the following day, the queen was missing and did not return.

*Borealis* queen No. 3, captured July 2, was confined with sixteen worker cocoons of *Bremus impatiens*, which she adopted at once. On the following day, she built an egg-cell and oviposited. By July 5, four workers of *Bremus impatiens* had emerged, and this *borealis-impatiens* colony was also given complete liberty; but eight days later, the queen was found dead in a corner of the nest-box, probably as a result of an encounter with the workers.

Queen No. 4 was confined on July 8 with fourteen worker cocoons of *Bremus fervidus*, which were adopted immediately. She laid a batch of eggs on the following day, and another on



July 11. Meanwhile several *fervidus* workers had hatched, and, beginning July 13, the colony was allowed to forage in the open. For a time, this *borealis-fervidus* colony seemed to get along very well, but on July 19, it was noticed that the *fervidus* workers kept the *borealis* queen from the comb most of the time by daubing her with honey, a habit which has been described in another paper ('22a). In spite of this treatment, the queen lingered about the nest until August 18, when she died. During the first part of August, several *fervidus* males hatched in this nest, but no adult *borealis* were obtained from any of these mixed colonies.

As has been pointed out in another paper ('22b), practically nothing is known concerning the nesting habits of this species.

#### *Pratorum* GROUP.

##### I. *Bremus bimaculatus* Cresson.

After losing several *bimaculatus* queens through dueling, a queen of this species was confined alone on May 20. Two days later, a *bimaculatus* worker was given to her, but she squirted the latter with faeces,<sup>1</sup> and showed her hostility in other ways. On the following day, another *bimaculatus* worker was substituted for the first. With this second worker she soon became friendly, and by the next morning a honey-pot and a cell containing eggs were present in the nest. On May 26, two more *bimaculatus* workers were added to this nucleus. The first batch of larvæ—twelve in number—grew rapidly and began spinning their cocoons about June 7, and the first adult—a male—emerged on June 18. The bees which hatched from the remaining eleven cocoons, as well as those which emerged later, were likewise males. It is evident, therefore, that this *bimaculatus* queen had not been fertilized the preceding fall, and that, in some instances, bumblebee males may be produced as early in spring as workers, a fact which has been overlooked by other bumblebee students (cf. Dahlbom ('32, pp. 9, 10), Schmiedeknecht ('78, pp. 317, 320, 323), Hoffer ('82/83, p. 15), Wagner '07, p. 126), Sladen ('12, p. 49), and Stellwaag ('15, pp. 466, 467)).

<sup>1</sup> This method of warfare is also employed by the queens and workers of other American species, e.g., *Bremus impatiens*. In Europe, Wagner ('07, p. 82) observed a similar behavior in the case of *Bremus variabilis*, a queen of this species squirting the liquid for a distance of more than 35 cm.



Another *bimaculatus* queen and two workers were confined together on May 26. The first eggs were laid on June 5, and the first worker emerged on June 29, whereupon the colony was given complete liberty. On July 17, this colony consisted of the queen and 23 workers, a number of queens and males being produced later. The colony had completely died out by August 15.

The nesting habits and disposition of this species have been dealt with in two other papers ('22, '22b).

## II. *Bremus impatiens* Cresson.

After several queens of *Bremus impatiens* had killed each other, a queen of this species was confined alone on May 20. By May 27, she had constructed an egg-cell and oviposited, and on the following day she proceeded to build a honey-pot, all of this work being done without the assistance of workers. On May 31, June 2, and June 3, respectively, three workers of *Bremus impatiens*—the first obtainable—were given to her, and by June 4, another honey-pot and two additional batches of eggs were present in the nest. On June 9, the first batch of larvæ—eight in number—were almost full-grown, and ten days later the first worker emerged, whereupon the flight-hole was left open permanently. When this colony was examined on August 15, it consisted of the queen, 122 workers, and a considerable quantity of brood. The colony broke up toward the end of September, after having produced a large number of males and young queens.

The nesting habits and temper of this species have been discussed in several other papers ('22, '22a, '22b).

## III. *Bremus perplexus* Cresson.

A queen and two workers of this species were confined on June 6, and another queen and three workers on June 11. Both nuclei began nest-building on the day on which they were confined, but on June 17, queen No. 2 and one of her three workers were found dead in the nest, whereupon the remaining two workers and brood were given to queen No. 1. The larvæ grew rapidly, and on June 29,—twenty-three days after queen No. 1 was confined—the first worker emerged.<sup>1</sup> Several others hatched during

<sup>1</sup> This confirms the observations of Sladen ('12, p. 31) and Frison ('18, p. 47), who found that it takes from 22 to 25 days for the workers to emerge, from the time the eggs are laid. Hoffer's (82-83, p. 28) claim that the development of the workers, from egg to adult, takes a month, is therefore incorrect.



the next few days, and on July 1, the colony was given its liberty. In order to give it a better start, about twenty cocoons of *Bremus impatiens* were placed in the nest. The workers of the two species showed no hostility toward each other, and everything went well until July 14, when the *perplexus* queen was found dead in the nest. Like *borealis* queen No. 3, she probably was killed by the *impatiens* workers. During the first half of August, this *perplexus-impatiens* colony produced several males of both species, but by August 20, the colony had completely broken up.

What is known about the nesting habits of *Bremus perplexus*, we owe to Franklin ('12/13, pp. 347-348). Some years ago, this author took two nests in early August, in Vermont. Both nests were situated in the walls of houses, and were made of wool. One of the nests contained 5 queens, 1 male, and 9 workers; and the other, 8 queens and 33 workers.

In the vicinity of Boston, *Bremus perplexus* is very rare. Judging from the early appearance of the workers (the first one was taken on May 28), some queens of this species must appear as early as May 1, and most nests are probably started during that month. The sexual forms seem to be produced chiefly during July and August. The nests probably break up in September.

Regarding the disposition of *Bremus perplexus*, Franklin (p. 348) has the following to say: "This is the gentlest and least ready to sting of all the bumblebee species which I have had to deal with in the living condition. This seems peculiar, as *B. vagans*, which seems to be its nearest ally, is exceedingly ferocious." I have already ('22b) taken exception to the last part of this statement. According to my observations, *Bremus perplexus* and *Bremus vagans* are similar in disposition, both species being comparatively gentle.

#### IV. *Bremus ternarius* Say.

Of this species, two queens were taken on *Rhododendron*, in the Arnold Arboretum, on June 6, and June 8, respectively. Besides having lost much of her pile on the dorsal side, *ternarius* queen No. 2 had a very distended abdomen, suggesting that she probably had already started a colony. She was therefore set free a few minutes after she was captured with the hope that she might furnish workers for *ternarius* queen No. 1. As queen No. 1



showed no interest in the nesting material, three workers of *Bremus bimaculatus* were placed in her nest-box on June 14, but she would have nothing to do with them, and three days later, two small workers of *Bremus impatiens* were substituted for the three *bimaculatus* workers. With these two workers, the *ternarius* queen made friends and a few days later began nest-building. The first batch of eggs was laid about June 21, and the first *ternarius* worker hatched on July 14. By July 16, six more workers had emerged. The two workers of *Bremus impatiens* were then removed, and the young *ternarius* colony was left to shift for itself. By August 13, the number of workers had increased to seventeen, and a few weeks later, several newly-hatched *ternarius* males were present in the nest. At the beginning of September, the queen showed signs of becoming feeble, and on September 10, disappeared from the nest. The last workers died during the first week of October.

Very little is known concerning the nesting habits of *Bremus ternarius*. During the summer of 1863, Putnam ('64) took a nest<sup>1</sup> of this species at Bridport, Vt., on the borders of Lake Champlain. It was situated either under an old stump or under the clapboards of a house.

In the vicinity of Boston, *Bremus ternarius* is exceedingly rare. The queens, like those of *Bremus vagans*, seem to leave their winter quarters comparatively late in the spring. Most nests are probably started between the 15th of May and the 15th of June. If this assumption is correct, the first workers ought to appear shortly after June 1. As in most other New England species, the males and queens are probably produced chiefly during August and September.

Putnam (p. 99) states that *Bremus ternarius* is far more savage than *Bremus fervidus*, the latter species, according to this author, being "of quite a gentle disposition." However, I found both of these species to be extremely vicious. In other respects, the behavior of *Bremus ternarius* reminds one very much of that of *Bremus perplexus* and *Bremus vagans*.

<sup>1</sup> The other nest which Packard ('64) and Putnam ('64) considered as belonging to *Bremus ternarius*, according to Franklin ('12/13, pp. 444-445), was probably a nest of *Bremus rufocinctus*.



V. *Bremus vagans* Smith.

As in the case of *Bremus bimaculatus* and *Bremus impatiens*, several queens of this species were at first lost through dueling. The two queens from which self-supporting colonies were obtained, were confined—each with three workers—on June 8, and 11, respectively. Within a week, both nuclei had begun nest-building, and by the end of June the first batches of larvæ were spinning their cocoons. The first worker of nucleus No. 1 hatched on July 13, and the first one of nucleus No. 2, on July 14, whereupon both nuclei were given continuous liberty. The two colonies prospered and did not break up until the latter part of September, each having produced a number of queens and males.

As already mentioned in connection with *Bremus affinis*, a confined bumblebee queen, if liberated, may return to an artificial nest after she has oviposited in it. From the following incident, it will be seen that *Bremus vagans* is no exception to this rule. On June 22, the weather was exceptionally pleasant, and *vagans* nucleus No. 2 having small larvæ, the flight-hole of the nest-box was opened at about 9 A.M., in order to give the bees a chance to forage. When the nest was examined at noon, the queen, as well as the workers, had disappeared. At 2 P.M., none of the bees had returned, and, believing they had forsaken the brood, the nest-box was removed with the intention of turning the young larvæ over to *vagans* nucleus No. 2. However, about 5 P.M., *vagans* queen No. 1 was found eagerly searching about the place where the nest-box had been. She was captured, and upon being placed in the nest-box, quickly went to her brood. The workers did not return, and three others were substituted on the following day.

The nesting habits and disposition of *Bremus vagans* have been discussed in two recent papers ('22, '22b).

*Auricomus* GROUP.I. *Bremus auricomus* Robertson.

This is one of the two species which I was unable to obtain in the vicinity of Boston. All that is known concerning the nesting habits of *Bremus auricomus*, we owe to the efforts of Mr. Theodore



H. Frison ('17, '18, '21). In addition to the colony which he reared artificially, Mr. Frison ('17, '21) had under observation several nests of natural origin, one of which was taken on September 6, 1917. It was situated in a hollow cement block in the foundation of a small cabin, and contained 3 young queens, 3 males, and 15 workers—10 living and 5 dead—besides several others which were out foraging when the nest was taken. Another nest, examined July 26, 1919, at Clyman Junction, Wis., was situated about  $1\frac{1}{2}$  ft. below the surface of the ground, and contained the old queen, 12 workers, 15 eggs, and some larvæ and pupæ of *Bremus auricomus* as well as a disabled *Psithyrus laboriosus* queen. In addition to these three colonies, Mr. Frison ('17) had under observation another which was started in an artificial nest which had been placed in a clay embankment.

According to Mr. Frison ('18), *Bremus auricomus* is rather gentle in disposition. Concerning the colony which he took on September 6, 1917, he has the following to say: "The bumblebees were very docile when the nest was removed, for instead of flying angrily from the nest, the most they did was to run excitedly about on the comb and buzz loudly."

#### *Fraternus* GROUP.

##### I. *Bremus rufocinctus* Cresson.

As in the preceding case, I was unable to obtain queens of this species in the vicinity of Boston. Comparatively little is known about the nesting habits of *Bremus rufocinctus*. According to Franklin ('12/13, pp. 444-445), Putnam ('64) took a nest of this species at Bridport, Vt., in September, 1863. It was probably situated under the clapboards of a house, about eight feet from the ground, and contained 28 adult bees and 35 cells with young.

Judging from Putnam's (p. 99) account, *Bremus rufocinctus* is one of the more savage species.

##### II. *Bremus separatus* Cresson.

On May 15, a queen of this species which had been captured at Peabody, Mass., was turned over to me by Dr. L. H. Taylor. She had lost a part of one of her antennæ and, although given a *separatus* worker, refused to take any interest in the nesting material. She was found dead in the nest-box on June 9.



Queen No. 2 was taken on June 3. Having lost both of her antennæ, she took little interest in life and died five days later.

Queens No. 3 and No. 4 were taken in the Arnold Arboretum on June 8, and June 16, respectively. They were confined separately, and each one was given three workers. Both of these nuclei at once started nest-building, and toward the end of June each had large larvæ. The first workers emerged on July 9, and 11, respectively, whereupon both colonies were given their liberty. A few days later, queen No. 4, returning from a foraging trip, by mistake entered a nest of *Bremus affinis* and was stung to death. Her brood and workers were given to *separatus* colony No. 3. This colony prospered and produced a number of males and queens in August, but had completely died out by September 10.

According to Putnam ('64), *Bremus separatus* builds its nests "under old stumps and in other situations similar to those in which the nests of *B. fervidus* are found."

In regard to the disposition of *Bremus separatus*, Putnam (p. 101) has the following to say: "This species is nearly as ferocious, on being disturbed, as *B. ternarius*," a statement which is corroborated by my own experience.

#### *Dumoucheli* GROUP.

##### I. *Bremus fervidus* Fabricius.

After losing several queens of *Bremus fervidus* by dueling, a queen of this species was confined alone on May 24, but she refused to start a nest. On June 2, three *fervidus* workers were associated with her, and three days later the nest contained two honey-pots and a closed egg-cell, but the larvæ which hatched from the eggs died, apparently because they were not fed by the adults. Several other *fervidus* nuclei which were started later, likewise paid no attention to their larvæ.

The nesting habits and disposition of *Bremus fervidus* have been discussed in several recent papers ('22, '22a, '22b).

##### II. *Bremus pennsylvanicus* De Geer.

A queen of this species was confined on May 29, and six days later, three workers of *Bremus fervidus* were given to her, but she remained restless and would have nothing to do with them.



Another *pennsylvanicus* queen was therefore put in her place on June 5. Although hostile to the *fervidus* workers, queen No. 2 constructed an egg-cell and oviposited on June 11. But, as in the case of the *fervidus* nuclei, the larvæ were not fed and died shortly after hatching. Both *Bremus fervidus* and *Bremus pennsylvanicus* are Pocket-makers, *i.e.*, they feed their larvæ, at least those of the workers,<sup>1</sup> through one or more pockets which they make at the side of each group of larvæ. On returning from the field, the foraging bee deposits its load of pollen directly into these pockets, through which the latter reaches the larvæ. It seems probable, therefore, that the Pocket-makers let their worker larvæ die, whenever they cannot feed them in the usual way. If this supposition is correct, it will be impossible to rear colonies of the Pocket-makers from confined queens, unless the latter are permitted to collect pollen from flowers.

Since the methods employed in rearing colonies of other species yielded no results in the case of *Bremus fervidus* and *Bremus pennsylvanicus*, and as I was anxious to obtain a colony of the latter species, a different method was resorted to. On June 26, about a dozen cocoons of *Bremus impatiens* were given to *pennsylvanicus* queen No. 2, which she adopted immediately. She showed no hostility toward the young workers which emerged, and two days later constructed an egg-cell and laid a batch of eggs. On July 2, this mixed colony was placed out of doors so that the workers could forage. Everything went well until July 6, when the queen was found dead in the nest, having probably been killed by the *impatiens* workers.

On July 26, a third *pennsylvanicus* queen was confined with a *pennsylvanicus* worker and sixteen cocoons of *Bremus fervidus*, and on August 2, another *pennsylvanicus* worker was added to this nucleus. The cocoons were adopted immediately, as were the *fervidus* workers which hatched from them. On August 3, the queen built an egg-cell and oviposited, and two days later this *fervidus-pennsylvanicus* colony was given its liberty. *Pennsylvanicus* worker No. 1 did not return, but No. 2 and several of the *fervidus* workers brought in one load of food after another.

<sup>1</sup> The queen and male larvæ of *Bremus fervidus*, and probably also those of *Bremus pennsylvanicus*, are fed, at least toward the end of their development, like the larvæ of those bumblebees which do not feed their larvæ through pockets.



For several days, everything went well, but on August 10, it was noticed that the *fervidus* workers were daubing the *pennsylvanicus* queen and worker with honey, a habit which has been referred to before. On the next day, the *pennsylvanicus* worker failed to return, but the queen, although her pile was constantly soaked with honey, lingered about the nest until August 21, when she disappeared. A few days later, several *fervidus* males hatched in this colony, but no adults of *Bremus pennsylvanicus* were obtained from any of these mixed colonies.

The nesting habits of *Bremus pennsylvanicus* have been described by Franklin ('12/13) Howard ('18), and Frison ('16, '17, '18, '21). Judging from the data published by these authors, the nests are usually situated on the surface of the ground, but occasionally also in the ground, or in birds' nests. The largest nest taken by Franklin (p. 405) contained 1 queen, 23 males, 53 workers, and 78 cells with larvæ in them, of which 18 were queen cells.

In the vicinity of Boston, *Bremus pennsylvanicus* is comparatively rare. The queens are the last to appear in spring, the first one in 1922 being seen on May 29, and the first worker on July 22. Most nests are probably started in June. A number of males of this species were taken in September, and therefore the colonies, like those of *Bremus fervidus*, probably do not break up until the latter part of September or the beginning of October.

According to Mr. T. H. Frison ('17, '18) and Mr. Court W. Ranslow (cf. Howard, '18), the workers of *Bremus pennsylvanicus* are rather vicious. After they had oviposited, this was also true of the *pennsylvanicus* queens used in my breeding experiments. On several occasions, they seized my forceps, tried to sting them, and clung to them so tenaciously that they could be lifted out of the nest-box.

While these experiments were in progress, a number of other observations were made which will be presented in another paper.

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