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THE MORPHOLOGY OF THE EYE MUSCLE NERVES

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NINE PLATES AND FOUR TEXT FIGURES

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INTRODUCTION

An advocate of the orthodox view of the vertebrate head as a structure derived from segmented pre-vertebrate ancestors is likely to meet with an incredulity or an indifference quite unknown to morphologists of the last generation. The reasons for this are several. In the first place special creation has rested its case and evolution no longer feels the urgent necessity of strengthening the argument that the vertebrate head or any other organic structure has had a past history. In view of the general acceptance of the verdict in favor of evolution it appears to be a matter of minor importance just what this history has been, whether annelid, tunicate, arachnid or what-not.

Furthermore, the conflict with accumulating evidence and the disagreement in opinion among vertebrate morphologists; the divergence in results based upon the study of the ontogenesis of different vertebrates; the increasing conviction of the plasticity and mutability of ontogenesis; doubt regarding the actual specificity of the germ layers; all have tended to undermine the faith of our scientific forebears in the validity of the fundamental law of biogenesis except in its most general features. Faith in phylogenetic inductions based largely on embryological data has been greatly weakened. A distinguished embryologist (McMurrich '12) recently expressed the opinion that "more reliable results are to be obtained in the majority of cases from comparative anatomy." We must concede that the results of embryological investigation have disappointed those who anticipated general agreement regarding the phylogenesis of the vertebrate head.

Civil war in the camp of the morphologists between anatomists and embryologists has led some to exclaim, "a plague on both your houses!" Morphology is the loser when embryologists and anatomists attack each other instead of morphological problems. The pot has called the kettle black and the luster of neither has increased. There seems to be no particular advantage in discussing the question whether comparative anatomy or embryology has been able to make the more valuable contribution to the solution of morphological problems. It appears not unreasonable to think that, notwithstanding frequent divergence of theoretical conclusions, the key to the solution of morphological problems lies in the hand of neither alone. The two sciences must develop together. The results by one method need to be 'controlled' by the other.

The elaboration of other than the annelid theory of vertebrate ancestry upon which the orthodox conception has been largely based, has tended to throw doubt upon the view that the segmentation of the vertebrate head is an ancestral segmentation. A morphologist of the last generation might have thought it necessary to apologize for advocating the view that the vertebrate head is an unsegmented portion of an unsegmented ancestor. No apology would be required today. As a matter of fact, after a century of vertebrate morphology, we actually do not know whether vertebrates came from a segmented or an unsegmented ancestor; whether or not the present segmentation in the head region is a true metamerism; whether the pre-otic portion of the head is a coenogenetic addition to a primarily segmented body, or a palingenetic remnant of an unsegmented ancestor which has secondarily acquired a segmented trunk. Even among those who agree that the present segmentation is ancestral there is little accord as to the nature or number of these somites.

A grouping of morphological opinion, however, with reference to the extremes of expression shows a strong tendency on the part of morphologists to regard the head as metameric. The curve of variation in opinion is decidedly skew in the direction of morphological orthodoxy. Among the many papers dealing

directly or indirectly with the problem of the morphology of the vertebrate head since the opening of the twentieth century, that of McMurrich ('12) is the only one adverse to the view that the head was primitively metameric. Vertebrate morphology, however, is deeply indebted to the skeptical but stimulating spirit which has constantly compelled orthodox opinion to reestablish its fundamental tenets.

To a considerable degree the skepticism regarding the comparability of head and trunk metameres may be traced back to the demonstration of the differences between cranial and spinal nerves, the serial homology of which seemed unquestionable to the earlier morphologists. The discoveries in the field of nerve components and of their various central and peripheral relationships seemed to many greatly to increase the difficulty of comparing the nerves of head and trunk. In consequence more stress came to be laid upon longitudinal columns in the central nervous system than upon a hypothetical primitive metamerism or neuromerism.

Nevertheless, the theory of the primitive metamerism of the head is "noch nicht aus dem Welt geschafft." The last decade has not seen a diminution in the numbers of those undertaking a reinvestigation of this perennial problem, notwithstanding the attractive fields in other departments of biology which have opened up during the period. With whatever incredulity or indifference schemes of head segmentation may meet as they appear, there are indications that students of head morphology are reaching an agreement with regard to the fundamentals of the metamerism of the head. The reinvestigation of many ontogenetic and histogenetic problems, concerning which earlier investigations had led to divergent results, have led to a remarkable agreement in conclusions and have tended to remove the prejudice against ontogenetic foundations for phylogenetic conclusions. It is now seen that much of the divergence in results of earlier embryological research was the result of inadequate methods or materials—of generalizations based upon insufficient data. There is good reason for thinking that further investigation of nerve histogenesis will show a similarity between

cranial and spinal nerves which had been rendered doubtful by the incomplete researches of earlier students of nerve development. The recent elaborate studies of the histogenesis of the trochlear and oculomotor nerves by Dohrn ('07) and Gast ('09) have tended greatly to strengthen the conviction of morphologists that these nerves are comparable with spinal nerves. Belogolowy's ('10) careful monograph on the cranial nerves of the chick leads to the same conclusion. It is the purpose of the present study to make a contribution to the same end.

The writer takes pleasure in expressing his appreciation of the aid given him during the prosecution of this research by Professor John Sterling Kingsley, Director of the Harpswell Laboratory, during the summers of 1906-1913 and for the unlimited supply of embryos of *Squalus acanthias* which residence at this laboratory has rendered available. He also acknowledges gratefully a grant of \$50 by the trustees of the Elizabeth Thompson Fund, used in the attempt to get embryos of stages between 50 and 100 mm., not obtainable off the Maine coast during the summer months.

1. The importance of the eye-muscle nerves as criteria of the metamerism of the head

In a recent paper dealing with the head problem Ziegler ('08, p. 674) writes that, since he holds that the eye muscles are relatively young muscles which have not arisen directly from segmental muscles, he is of the opinion that one cannot make use of their innervation for phylogenetic conclusions. Ziegler does not give his reasons for regarding the eye muscles as relatively young muscles. Moreover, he does not prove that they have not arisen directly from segmental muscles, and in drawing conclusions concerning the phylogenesis of the head he makes use of the relations of the oculomotor as an essential connecting link.

Cole ('98, p. 237) also refers to the eye-muscle nerves as the "the least primitive of the cranial nerves" without giving any proof of the assumption.

On the other hand, Gast ('09, p. 423) asserts that the eye-muscle nerves appear very conservative in their relations to the neuromeres, notwithstanding the considerable dislocation of their muscles. He therefore concludes that the eye-muscle nerves supply most important material for the study of the primitive metamerism. However profound the changes in their terminal-organs, the central relations remain unchanged, so that they serve as important criteria of the nature and number of pre-otic segments. "Die drei Augenmuskeln Nerven haben eine hohe phylogenetische Bedeutung." The writer would emphasize their importance even more than Gast has done, for it seems to him that the demonstration of the serial homology of head and trunk metameres depends largely upon the proof of the resemblance of eye-muscle and spinal somatic motor nerves.

The reason for such an opinion is plain when we consider that other evidences of cephalic segmentation, such as cranial ganglionic nerves and visceral arches, appear limited to the head region and to have no exact homologues in the trunk. The presumption that head and trunk were primarily undifferentiated must come not from such evidence but from structures which may be more readily compared. The eye-muscle nerves in connecting somites and neuromeres, are related to structures which extend through head and trunk. Moreover, in their central relations the eye-muscle nerves resemble spinal somatic motor nerves. Therefore, failure to convince morphologists of their meristic homology with spinal nerves would tend to undermine the foundation of the traditional conception of the head.

Consequently, the divergence of opinion regarding their true nature, as evinced by their ontogenesis in Selachians, has tended to obscure the more important issue of the history of the head. The repeated attempts to compare the trochlear and oculomotor with such nerves as the trigeminal and the facial, notwithstanding their obvious differences in the adult animal, suggests the necessity of renewed investigation of their histogenesis. Recent exhaustive papers by Dohrn ('07) and Gast ('09) have appeared in response to this need. In many matters of fundamental importance this paper confirms their results. The theoretical

conclusions are not so fully in accord, but all agree that the ancestral vertebrate was a metameric acraniote, and there is no suggestion that the meristic elements of the head have had an exogenous source.

2. Technical methods

Considerable investigation upon the histogenesis of nervous structures has been made upon material prepared by methods unsuited to the special requirements of neurological investigation. To this may be attributed many of the fallacious inferences that have delayed our knowledge of the true method of nervous differentiation. Failure to use suitable neurological methods is no longer excusable, however, since the advances made by Cajal, Paton, Held and others. It is necessary for one who attempts today to base morphological conclusions upon the study of nerve histogenesis, to make sure by proper technique that he is dealing with real nervous structures and not with pseudo-nerves. Not every cellular strand or spindle-shaped cell, however closely associated with the central nervous system or its derivatives, is necessarily a nervous anlage. Some criterion by which a structure in the embryo may be identified as nervous is needed. Hence the importance of stains which will differentiate the neurofibrils from the time of their first appearance.

Equally important in addition, is the use of a method which will demonstrate cell boundaries and relations, especially in those early stages when nervous connections between nerve center and end-organ are established. Since no single method is known which will effect both of these results with equal precision, different methods for comparison and control of results appear necessary. Trust in a single neurological method, however highly perfected, may mislead as much as a less specific stain has done. The importance of methods of preservation and staining is evinced by the great divergence in the results, both technical and theoretical, of Paton ('07) and Held ('09), each of whom worked with a highly perfected technique.

Therefore, while it may be granted that the older embryological methods are no longer adequate for the purposes of neu-

rological investigation, the results by no single neurological stain, however specific, may be wholly trusted. The methods suited to demonstrate the neurofibrils in vertebrate embryos give very unsatisfactory pictures of cell boundaries and relationships. But, as a basis for theoretical conclusions, the cellular relationships are quite as important as phases of differentiation of neurofibrils. Moreover, it must not be forgotten in the enthusiasm created by the newer discoveries that, provided embryological material be abundant, the identification of an embryonic structure as nervous may be determined by tracing its fate through successive stages of development. The demonstration of neurofibrillae therefore is not absolutely indispensable in the determination of its nervous character.

In the course of the present study different methods have been used. Among those which have given the best results are Cajal's nitrate of silver, Paton's modification of Bielchowsky's method, Held's molybdic acid-hematoxylin stain and vom Rath's picro-acetic-osmic-platinic chloride-pyrogallie acid treatment. All the drawings in plates 1 to 8 of this paper were made from preparations by the latter method. They have been confirmed by observations upon material in which the neurofibrillae were specifically stained.

The Vom Rath ('95) method is as follows:

1. Fix in the dark for one to three days in the following mixture (use plenty and change each day):

Saturated and filtered solution picric acid.....	200 cc.
Glacial acetic acid.....	2 cc.
Platinic chloride (dissolve in 10 cc. water).....	1 gram
Osmic acid 2 per cent.....	25 cc.

Owing to the great brittleness of embryos fixed in this fluid all changes of liquid should be made with pipette in the same dish, avoiding as far as possible any movement of the embryos.

2. Stain in 0.5 per cent pyrogallie acid in the dark for twenty-four to forty-eight hours with several changes.

3. Grades of alcohol from 35 per cent slowly by the siphon capillary drop method to avoid shrinkage. Xylol, to which paraffin is added as it dissolves.

4. Imbed in rather hard paraffin of best quality.

5. Thin sections, not over 8 micra, preferably 4 to 6 micra.

Vom Rath's method is not specific for the neurofibrils, which are nevertheless deeply stained. Cell boundaries are shown with special distinctness and shrinkage is slight. The process is advantageous in demonstrating cell relations in the stages when nervous connections of tube and somite are effected.

Flemming's stronger formula gives excellent fixation of selachian embryos but does not allow the use of pyrogallie acid for subsequent staining. Fixation seems quite as faithful as in Vom Rath preparations, but cell boundaries are not so distinct as in the latter. Iron hematoxylin gives the best stain, subsequent to the use of Flemming's fluid, but it is necessary to paint the sections with 0.5 per cent celloidin in order to prevent their loss in staining on the slide.

For the specific purpose of demonstrating the neurofibrils Cajal's method has given uniformly satisfactory results, which appear somewhat less refined than those obtained by the Bielchowsky-Paton process. The Cajal method is as follows:

1. Fix in absolute alcohol and 1 per cent ammonia for forty-eight hours.
2. Wash for one-half to three minutes in distilled water.
3. Pyridine for twenty-four hours.
4. Distilled water—many changes—for twenty-four hours.
5. Two per cent aqueous solution of silver nitrate for three days at 35°C. in the dark.
6. Rinse in distilled water.
7. Four per cent pyrogallie acid in 5 per cent formalin for one to two days.
8. Paraffin sections.

The Simarro-Cajal silver reduction method, following fixation in 70 per cent pyridin, which has given such splendid results when applied to mammal and other amniote embryos has proved a complete failure in the case of *Squalus* embryos.

Excellent results in the differentiation of the neurofibrils have followed the use of the molybdic-acid hematoxylin process as developed by Held ('09). Tissues may be fixed by various methods including Zenker's fluid and Rabl's picro-sublimate. The stain is effected by a solution of molybdic acid in a 1 per cent solution of hematoxylin in 70 per cent alcohol. The stain

is better after months or years. Immediately before use, several drops of this tincture—depending on the strength wanted—are dissolved in distilled water and the sections are stained warm on the slide at 50° C., or for a longer time cold. The sections may be stained directly or they may be mordanted in iron alum.

The neurofibrils are differentiated by the Bielchowsky-Paton process but, like the Cajal method, this does not demonstrate the fibrils within the neuroblast cell in the earliest stages of histogenesis. By this method the neurofibrils are stained a dark brown or black, while other tissues are light brown or yellowish brown. In the process only tested distilled water and absolutely clean glass ware and glass or bone spatulas—no metal—should be used:

1. Fix and keep embryos in 10 per cent formalin neutralized or made slightly alkaline with magnesium carbonate.
2. Wash in running tap-water for twelve hours.
3. Wash in three or four changes of distilled water for a half-hour.
4. Place in pure 1 per cent nitrate of silver for six days in the dark at a temperature of about 70°C. Tissues must become reddish-brown in color. If they become yellowish-brown, throw away.
5. Place in a solution of silver nitrate freshly prepared as follows: 20 cc. of 1 per cent silver nitrate. Add 2 drops of 40 per cent caustic soda to form a gray precipitate. Then add 20 to 30 drops of strong ammonia, enough to dissolve the precipitate while stirring. Allow to remain at least forty-five minutes.
6. Wash quickly in two baths of distilled water and quickly place in distilled water, to every 20 cc. of which five drops of glacial acetic acid has been added. Leave in this five to fifteen minutes or until the reddish-brown becomes yellowish brown.
7. Wash quickly and place over night in 1 per cent hydroquinone containing 5 per cent neutral formol.
8. Wash quickly in distilled water, run up through alcohols rapidly and imbed in paraffin through benzole or chloroform.
9. Cut sections and fix on slide. Dry well, then paint slides with 0.5 per cent celloidin. This is followed by absolute alcohol-xylol, and absolute alcohol-xylol again. Then absolute alcohol to 95 per cent alcohol down to water (distilled).
10. Then one to two hours in 0.1 per cent solution of gold chloride neutralized with lithium carbonate. Grubler and Hollborn's gold chloride should be used (*flavum*, not *fuscum*).
11. Rinse in distilled water and fix in 5 per cent hyposulphite of soda for fifteen minutes. Wash in running tap water for two hours. Then alcohols up to absolute. Then absolute and eosin for a minute. Absolute alcohol, xylol, and mount in neutral balsam.

HISTOGENESIS OF SPINAL SOMATIC MOTOR NERVES

1. General description

Somatic motor nerves make their first appearance in embryos of *Squalus acanthias* of about 4.5 mm. and approximately 30 somites. With their advent begins the rhythmical bending of the embryo. The first pair of somatic motor nerves to appear are those of VanWijhe's seventh somites, the first somites differentiated, and those which form the first permanent myotomes.

Previous to the appearance of this first pair of somatic motor nerve anlagen, no protoplasmic connections or plasmodesms between tube and somite are discernible. In the intercellular space between neural tube and somite there is present a plasmoid substance or a liquid with a minimal amount of coagulable material, which, when treated by the usual reagents, assumes a vacuolated appearance. By the use of such fixing fluids as neutral 10 per cent formalin, followed by intense stains such as acid fuchsin it is possible to demonstrate this coagulable substance with considerable clearness. By the use of other fixing fluids—the plasmolyzing action of which is less intense than that of formalin—and the use of less powerful stains, the plasmoid material is almost invisible and has usually been ignored in embryological studies. Whatever may be the chemical nature of the granules of this plasmoid substance, it does not have the staining properties of protoplasm and its presence may not therefore be taken as evidence of a primary protoplasmic connection between tube and somite, although it is indisputably the medium through which the growing nerve anlagen find their way to the adjacent myotomes.

While the possibility that this plasmoid material may contribute to the growth of the nerve paths may not be denied, yet even so the contribution must be very slight or quite negligible, since the amount of coagulable material in it is very small. To represent it at all, as in figure 1, greatly exaggerates its distinctness and amount as seen in sections. There is nothing in its vacuolated structure that would suggest 'paths' or 'plasmodesms' suggested by the Hensen hypothesis. Moreover, the

proximity of tube and somite is so close that the absence of predetermined paths for the growing nerve is not surprising. Supporters of the Hensen hypothesis have greatly exaggerated the mechanical difficulty of the acquisition of a connection between tube and myotome in the absence of primary paths. But even the most ardent supporter of the Hensen hypothesis will admit the capacity of cells to throw out amoeboid processes into the surrounding medium, and for distances quite as great as that which separates somite and neural tube.

As a matter of fact, protoplasmic connection between tube and somite to form the anlagen of somatic motor nerves in *Squalus* is effected in precisely that way, as was described by Dohrn ('88) many years ago. The process is one of amoeboid outflow, as stated by that accurate observer, and as shown in figures 4 to 7 of this paper. Beginning with the seventh somite of the selachian embryo protoplasmic connection with each of the successive somites of the body is effected in the same way, that is, by amoeboid processes of medullary cells of the ventrolateral wall of the neural tube opposite the middle of the somites. Since the successive somatic motor nerve anlagen are successively formed, beginning with the most anterior, all stages in the establishment of these connections of nerve and muscle may be seen in serial cross-sections of *Squalus* embryos of all stages beginning with embryos of 30 somites. The process goes on in the caudal region until quite late stages are reached.

The protoplasmic connections which form the anlagen of somatic motor nerves are not formed by the differentiation of plasmodesms which have existed from the beginning as the result of incomplete cell division, but are formed secondarily by the free outgrowth of amoeboid processes of medullary neuroblasts. The process is one of protoplasmic movement, analogous to that seen in the remarkable culture preparations of Harrison and to the outgrowth of the neuraxons of the Rohon-Beard cells illustrated in plates 3 and 4 of this paper. Stages in the establishment of these connections between tube and myotome and in the growth of the nerve anlagen are shown in plates 1 and 2 of this paper.

In correlation with this movement of medullary protoplasm there occurs a similar movement of the protoplasm of the cells of the sclerotomic portion of the somite, which is inaugurated by an extension of amoeboid processes toward the neural tube (figs. 2 and 3) followed in later stages by a movement of entire cell bodies. In many cases, but not invariably, the movement of the sclerotome cells precedes that of the medullary cells. The protoplasmic material derived from the two sources in exceptional cases unites to form a protoplasmic strand or plasmodesm in which it is difficult to distinguish ectodermal and mesodermal constituents, as is shown in figures 8 and 9. This difficulty obtains, however, only in the earliest stages, if it occurs at all. Even in such cases the sclerotome cells soon separate to form a loose mesenchyma of lightly staining and much vacuolated cells, while the medullary portion persists as a compact strand of deeply staining protoplasm which quickly takes on a fibrillar appearance and also becomes cellular through a process of cellular migration from the neural tube (figs. 11 and 12).

Evidence that the cells of the somatic motor nerve anlagen of *Squalus* are chiefly, if not exclusively, of medullary origin has been given in an earlier paper (Neal '03) and need not be reviewed in detail at this time. Their medullary derivation seems sufficiently attested by evidence of continued migration in successive stages; by their close apposition to the fibrillar bundle or their inclusion within the fibrillar portion of the nerve anlage (fig. 12); by the change in the contour of the neural tube in successive stages as seen in cross-sections; and by the relations of the outer limiting membrane of the tube to the nerve anlage. If mesenchymatous cells are added to the nerve anlage at all, it appears to be in later stages of histogenesis only.

While spinal somatic motor nerve anlagen are primarily protoplasmic and non-fibrillar this stage is quickly passed and, as the anlage extends ventrad along the median surface of the myotome between the myotome and sclerotome of the somite, it assumes a fibrillar appearance. That these fibrils are neurofibrillar in the Apathy sense is proven by the fact that they stain intensely in either Cajal or Paton preparations. Evidence

has been given in an earlier paper that the cells of the nerve anlagen have no genetic relations to the fibers. The fibers may be traced to deeply-staining bipolar neuroblasts in the ventrolateral wall of the neural tube.

When sections in metameres immediately posterior to those in which protoplasmic connections between tube and somite have just been established by the outflow of medullary protoplasm are studied; that is, in metameres in which it may be assumed that protoplasmic connections are about to be established by a similar outflow, before the medullary cells have thrown out amoeboid processes, deeply staining cells may be found which may be inferred to be the neuroblasts of that metamere. Their more deeply staining properties may be ascribed to the presence of a deeply stained neuroreticulum within them. Analogy with the histogenesis of the Rohon-Beard cells favors this inference, since within the latter, from the time of their differentiation from the surrounding epithelial cells of the tube, a reticulum with a strong affinity for stains makes its appearance and seems to be genetically related to the neurofibrillae which later make their appearance within the long processes of these remarkable cells (plates 3 and 4). In Cajal preparations similar cells in similar relations show a deeply-stained reticulum. There is therefore reason for thinking that the neurofibrillae of somatic motor nerve anlagen have their origin in medullary neuroblasts and are not exogenous in their derivation as suggested by Paton ('07). In all stages the fibers of the nerve anlagen may be traced to deeply-stained bipolar cells in the somatic motor column of the neural tube. While it is difficult to obtain positive proof of the fact, the evidence, so far as it goes, favors the opinion that the cells which form the first protoplasmic connections between tube and somite are true neuroblasts and not indifferent cells which form protoplasmic paths for the nerves, as suggested by the cell-chain hypothesis or by the similar hypothesis of the Hertwigs. In short, connections between tube and somite are effected primarily by neuroblasts and not by indifferent cells. These primary connections appear protoplasmic and not fibrillar, only because

they are formed by the amoeboid terminations of the neuraxon processes. The protoplasmic and fibrillar relations are precisely analogous with those seen in a Rohon-Beard cell such as is represented in figure 21. The advancing end of the neuroblast cell is protoplasmic and amoeboid, while the more proximal portion of the cell has a neurofibrillar structure. There appears no good ground for doubting the analogy between the phenomena of growth of the Rohon-Beard cells and those of the medullary neuroblasts which form the fibrillar constituents of somatic motor nerves. The evidence of the exogenous derivation of the neurofibrils advanced by Paton seems unquestionably based on incompletely stained preparations. The assumption of the participation of indifferent cells in the formation of 'paths' for the growing nerve fibers has an equally insecure foundation.

After the fiber bundle of the dorsal (somatic sensory) nerve unites with that of the somatic motor nerve a cluster of cells makes its appearance median to the mixed bundle at about the level of the dorsal aorta, forming the anlage of the sympathetic ganglion. The derivation of these cells from the neural tube has sometimes been assumed. The majority of opinion has inclined to the view that they are derived from the dorsal ganglia. Positive proof of either inference has never been given on the basis of direct observation. Experimental data appear not absolutely trustworthy. The present paper makes no contribution toward the solution of this important question. Analogy with the formation of the sympathetic in the head appears to the writer to favor the view that the most of the sympathetic cells have their source in the dorsal ganglia. The problem, however, needs renewed investigation.

Turning now to a more detailed discussion of some of the mooted points in the problem of nerve histogenesis, we may take up first the important question of the primary connection between nerve and muscle.

2. *Are muscle and nerve connected with each other ab initio? Are protoplasmic connections between myotome and tube primary or secondary?*

With the statement of this question we plunge at once into the most controverted point in neurogenesis—the problem of how nerves become connected with their terminal-organs. The earlier students of head morphology avoided the details of nerve histogenesis. Yet it is clear that the morphological resemblance of nerves is determined quite as much by their cellular and central connections as by the character of their terminal-organs. In the adult elasmobranch and in the higher vertebrates these relationships are frequently so greatly modified and complicated that to unravel their intricacies requires the facts which the ontogenesis of the lower vertebrates alone can give. Avoidance of the details of histogenesis and reliance upon embryological procedure unsuited to neurological investigation has led to the inclusion of cell clusters or strands among cranial nerves without proof of their nervous character and to erroneous inferences regarding the phylogenesis of the head. Minot ('96) suggested this weakness of earlier researches and the path of later investigation when he wrote that “the attempt has been made to solve the most difficult questions of the morphology of cranial nerves without answering the inconvenient question of nerve fibers and their sheaths.” To-day the student of phylogenesis is no longer able to shut his eyes to the necessity of a thorough investigation of the genesis of nerves before drawing conclusions regarding the past history of the vertebrate head.

In the introduction to his masterly discussion of nerve histogenesis, Held ('09) states that notwithstanding the numerous observations of the past fifty years and the multiplicity of hypotheses, the question as to how nerves are formed in the embryos of vertebrates is one of the most burning questions of embryology:

Many new methods have been applied to its solution and a surfeit of new views have appeared, following one another in a short period of time, but as yet no agreement has been reached regarding the true principle of nerve development. This is due to a great extent to the

lack of a fundamental histological method which effects a satisfactory stain for embryonic as well as for adult nervous tissue. But the disagreement is also partly due to the fact that it has been the concept rather than the reality that has been observed. Finally the continued conflict of opinion has been due to the fact that no comprehensive observations have been brought together which make it possible to demonstrate in one and the same animal all of the essential stages in the development of a definite nerve path, and to extend in a comparative research over the chief groups of vertebrates, in order to find, in the diversity of phenomena seen in different embryos, the same fundamental principle of nerve development.

Held undertook this herculean task and has made a most valuable contribution to our knowledge of the histogenesis of the neurofibrillae, which, since the emphasis laid upon them by Apathy, have come to be regarded as the essential elements of the nerve fiber. In large measure Held's monograph is an attempt to reconcile the divergent hypotheses of nerve histogenesis.

Three chief theories of nerve histogenesis have been advanced. Chronologically arranged these are:

1. The theory advanced by von Baer in 1829 and later elaborated by Hensen ('64, '76, '08) of the primary connection of the nervous center with its later innervated terminal-organ. The fundamental idea underlying this von Baer-Hensen theory is that of the necessity of a primitive connection between the nerve and its terminal organ. In other words that it is impossible to conceive of a nerve fiber finding its area of distribution or its terminal-organ as the result of the free outgrowth of the nerve cell. Hensen brought forward the idea of primary nerve paths, which was founded on the actual observation that in the embryo adjacent organs are connected with each other by means of protoplasmic bridges. These were interpreted as remnants of incomplete cell divisions and they were supposed to form the primary nerve paths which later become transformed into the definitive nerves or are used for nerve formation. How the transformation occurs was unknown to Hensen, but he was convinced that they did not develop from cell outgrowths. The theory appeared to afford a satisfactory explanation of how nerves find their way to their terminal-organ. Chiefly on the ground of general principles, Gegenbaur ('98), Fürbringer ('97), Kerr

('04) and Braus ('05) have added to the Hensen hypothesis the assumption of an original and unchangeable connection of motor nerve and muscle.

Held ('09) suggests that the assumption of primary—*ab initio*—connection between muscle and nerve is not an essential part of the Hensen hypothesis. With this view, however, Hensen ('03) does not seem to agree. Hensen states his hypothesis in his *Vorrede* as follows: "Nervous connections are not established through the free outgrowth of the nerves in the embryo, but central and peripheral organs remain in connection with each other from the time of their formation (*Sonderung*) until the complete differentiation of the nervous trunks."

2. The second theory is that advanced by Schwann ('39) and later revived by Balfour ('77) as the cell-chain hypothesis, according to which the nerve fiber arises from the union of a series or chain of primary cells, which later accompany the neuraxon as the so-called Schwann's or neurilemma cells. According to this hypothesis nerve fibers are formed by the fusion of primary cells, whose nuclei become the nuclei of the differentiated fiber, so that the nerve fibers are in consequence multicellular in origin. This theory has been supported by Marshall ('78), VanWijhe ('82, '86, '89), Beard ('85, '88, '92), Miss Platt ('94, '96), Sedgwick ('94, with some modification), Hoffmann ('96), Kupffer ('90, '91, '94), Rafaele ('00), Bethe ('00-'07, with modifications), Brachet ('05, '07), Cohn ('05, '06, '07), Oscar Schultze ('04-'07).

According to the cell-chain hypothesis, the peripheral nerve fiber is the common product of a chain of cells, on the end of which, in the case of a motor nerve, or in an intermediate position, in the case of a sensory nerve—the larger cell forming the ganglion cell is situated. The cell chain not only forms the ganglion cell but also the cells of the neurilemma sheath, which are not merely sheath cells but also the cells that produce the fiber—as nerve-fiber cells or 'neurocytes' (Kupffer), 'nerve-cells' (Bethe and Apathy) or 'neuroblasts' (O. Schultze). The nerve fiber is thus an elongated mosaic consisting of a series of cells, each cell of which has added to what another cell before it has formed, and has continued into the following cell. Bethe ('00-

'07) has modified this view by assuming that mitotic cells form the first anlage of the nerve and the development of the nerve proceeds by cell multiplication. Not every fiber, however, arises from a series of cells, but a series of cells produces a large number of fibers.

3. The third theory of nerve histogenesis, first formulated by Kupffer and Bidder ('57), but finally developed as the neuroblast theory by His ('79), holds that the nerve fiber develops as the process of an embryonic ganglion cell. Even before this theory had been advanced it had been noted by Remak ('55) that nuclei are absent in embryonic nerves. His ('79, '88, '90, '93, '04) developed this process theory of Kupffer and Bidder as the 'neuroblast theory' of nerve histogenesis, according to which nerves are formed as processes of special cells or 'neuroblasts.' The first outgrowth of these cells forms the axis cylinder and the cell becomes a ganglion cell. A single process is formed by the neuroblasts of the neural tube, while two are produced by the neuroblasts of the peripheral ganglia. Other branched processes formed later become the dendrites of the ganglion cells. Thus every single nerve fiber represents a single and definite cell of origin which constitutes its genetic, nutritive and functional center. These nervous units do not unite with each other in protoplasmic continuity, but a transfer of impulses occurs without continuity. This view has been supported by von Koelliker ('86, '91, '92, '00, '05), Sagemehl ('82), Ramón y Cajal ('90-'07), von Lenhossek ('90-'03), Retzius ('93), Neal ('98, '03, '12), Gurwitsch ('00), Harrison ('01-'12), Bardeen ('03), Lewis ('06, '07), Dohrn ('07), Belogolowy ('10) and Burrows ('11).

Waldeyer ('91) has still further developed the neuroblast theory as the neuron theory, according to which the nervous system, both central and peripheral, consists of independent functional units—cells or neurons—which never become fused or united but transmit impulses by transfer or induction from one neuron to another.

Later investigators have advocated various modifications of these three theories, but not hypotheses that may be sharply distinguished from the three theories mentioned above. They

may be regarded as variations of a theme rather than as original themes. The brothers Hertwig, for example, on the basis of evidence derived from the study of the development of nerves in medusae, suggested ('78) the hypothesis that the terminal organ, primarily separated from the nerve center, becomes secondarily connected with it by means of a simple protoplasmic path which is later differentiated into the actual nerve. The connection between nerve center and terminal-organ is formed secondarily, is at first protoplasmic and not nervous, and is the product of indifferent cells and not those which form the nerve fibers. Such protoplasmic connections become the paths along which the nerves develop and are established when nerve center and terminal-organ are in close proximity. This hypothesis, in common with that of Hensen, lays stress upon the protoplasmic paths within which the definite nerve fibers are differentiated, but differs from the Hensen hypothesis in assuming the secondary connection between center and terminal-organ. The hypothesis, however, as Braus ('05, p. 472) suggests, may not be sharply distinguished from the cell-chain hypothesis which has been rendered untenable or unnecessary by Braus' experiments upon amphibian larvae. Braus admits, however, the possibility of the formation of protoplasmic bridges before the appearance of the neurilemma cells. But, since it has not yet been proved that the protoplasmic bridges are the products of indifferent cells—in other words, that they are not the product of the same neuroblasts which produce the neurofibrillae—the hypothesis remains little more than a speculation, and the phenomena upon which it seems to rely are equally well interpreted by the process theory.

Other investigators, such as Apathy ('97), Joris ('04), Pighini ('04, '05), Besta ('04), London and Pesker ('06), Brock and Gierlich ('06, '07), and Held ('09) have presented some divergent opinions, but no wholly distinct point of view. The peculiar views of Apathy possibly deserve special recognition since they have aroused so great an interest. According to Apathy ('97) the embryonic ganglion cells acquire their fibrillar structure, not by a process of cell differentiation, but secondarily

from special cells, which, as actual 'nerve cells'—that is, as neurofibril-forming cells—are intercalated in the path of the nerve fibers and which push their differentiated product into the interior of the muscle cell or gland cell as well as into the ganglion cell. This doctrine, which conceives of the ganglion cell and the nerve cell as entirely distinct kinds of cells has been also supported by Bethe ('00-'07). According to Bethe the neurilemma cells are the 'nerve cells' in the Apathy sense of the word. That is to say, they are the cells which have formed the neurofibrils and persist as sheath cells.

Held attempts ('09) to reconcile the divergent views of nerve histogenesis, finding something of truth in them all. In common with Hensen he emphasizes the importance of protoplasmic paths which furnish material for the growing nerve; agrees with the Hertwig brothers that these may be secondarily formed by indifferent cells, and supports the Kupffer-Bidder theory in holding that the neurofibrils are the product of the neuroblast through a process of centrifugal growth. While his observations seem to support the process theory in most details, he denies the doctrine of free outgrowth of nerve fibers.

None of these hypotheses of nerve histogenesis has been abandoned entirely by partisan supporters. With the introduction of special nerve methods the confusion has seemed to increase rather than to diminish, so that there is not a single result of the most recent investigation which is not directly opposed by another. The chief problem raised by Apathy as to the genesis of the neurofibrils, has not yet been solved; and the more important question of how muscle and nerve become connected seems as far from solution as ever. Relief from conflict of opinion has not followed recourse to experimental morphological methods as is shown by the divergence in the views regarding neurogenesis held by Harrison ('03, '04) and Braus ('05). While the former supports the process theory, the latter thinks that his results confirm the Hensen or Hertwig hypothesis of predetermined paths for the growing nerves.

Upon the answer to the question, whether or not nerve and muscle are primarily connected, depends, in large measure, not

only the answer to the problem of the morphology of the eye muscles and their nerves but also general conclusions regarding the phylogenesis of the vertebrate head. For it may readily be seen that if nerves are *ab initio* connected with their terminal-organs, all the modifications of the latter would naturally be accompanied by associated changes of the former. If, for example, a post-otic nerve innervates a pre-otic muscle, upon the assumption of an immutable connection of nerve and muscle, the inference that the muscle had migrated from behind the ear into its present position and carried its nerve with it, might appear the most reasonable interpretation of such a peculiar relationship. But if, on the other hand, a nerve is not invariably associated with a given muscle throughout its phylogenetic history, but may grow into muscular territory primitively foreign to it, such an assumption would greatly modify our views of nerve and muscle phylogenesis and possibly our views of the history of the vertebrate head.

The histogenetic problem of neuro-muscular relations therefore appears fundamental to any problem, such as that of the morphology of the vertebrate head, involving nerve and muscle in complex and obviously modified relations. Upon the answer to this problem depends, for example, the answer to the problem of the present singular muscular relations, of the trochlear and abducens nerves. While it is an unquestioned fact that nerve and muscle tenaciously retain connections once acquired, and while this conservatism of relation may be taken as a basic assumption in morphology, the possibility still remains that under changed conditions new neuro-muscular relations may be acquired. If Gegenbaur and Fürbringer be correct, such changes are as incredible as, for example, the inheritance of mutilations are believed to be. But, on the other hand, to all of those who have come to accept the process theory of nerve development and who therefore assume the secondary connection of nerve and muscle, the invasion of new territory and its piratical seizure by exotic nerves appears a possibility. A discussion of the present problem, therefore, does not lead the morphologist far afield. The history of the head must be written primarily in terms of nerve histogenesis.

The attempt has been made recently by Held ('06, '09) and Paton ('07) to combine the two views of nerve histogenesis, claiming a primary connection of neural tube and myotomes by means of protoplasmic bridges or plasmodesmata, along which, as paths, later the true nerves—neurofibrillae—by centrifugal (Held) or centripetal (Paton) growth—effect the definitive nervous connection with the muscle. True nervous connection with muscle is thus secondary, but the connection takes place along predestined paths—the plasmodesmata.

Whether the plasmodesmatous connections are secondary or primary remains undetermined by Paton. Held (p. 277) holds with the Hertwig brothers ('78) that they are secondary and he states that Hensen's hypothesis that all nerves have arisen through the incomplete separation of the origin and end cells cannot be correct. Held, however, does not appear to have established the truth of this assertion on the basis of a detailed study of their genesis in the vertebrate embryo. The theoretical importance of the manner of establishment of these 'primary' protoplasmic connections of neural tube and their genetic relations to the cells which form the neuro-fibrillae is so great that it appears worth while to consider this portion of the histogenetic problem with thoroughness and in great detail. The functional importance of the primary protoplasmic connections has been established by the valuable experiments of Paton ('07), who demonstrated that in selachian embryos, in stages before the appearance of neuro-fibrillae, the embryo responds by muscular movements to external stimulus. If such reactions be effected through the medium of the central nervous system, then it is certain that the efferent paths of the impulses must be the "undifferentiated protoplasmic bridges" described by Paton and identified by him as the 'plasmodesms' of Held ('06). To some this evidence might seem sufficient to prove their 'nervous' character, but not so to a disciple of von Apathy, to whom there is "no nerve without neuro-fibrillae." With regard to these protoplasmic bridges, Paton has the following to say (p. 555):

In the region of the ventral roots in a 5 mm. embryo of *Pristiurus* there is a marked bulging forward of the protoplasm and in many sections a 'bridge' about 4 to 5 μ in width similar to those shown by

Kerr ('04) to exist in *Lepidosiren* may be seen spanning the distance between the edge of the medullary substance and the inner border of the myotome. The structure of the protoplasm of which these strands are composed seems similar in all respects to that forming the general matrix and does not at any point, except at the places to be described later on, present evidence of a fibrillar structure. Less frequently strands of protoplasm are seen in other localities, as for example at the point where later in the development of the embryo processes of giant ganglion cells (Beard) emerge from the cord, or where the matrix surrounding the cells of the ganglionic masses is in contact with the periphery. Any attempt to determine the moment when these bridges appear and the manner in which they are formed necessitates the consideration of questions of fundamental importance.

Are these structures the product of a single cell or do several elements contribute protoplasm to span the interval between two given points as widely separated as the periphery and cord? Hensen's idea that these bridges are originally thrown across from one cell to another and then as the embryo grows these threads are pulled out to many times their original length, is an exceedingly ingenious and suggestive hypothesis but has not yet been proved. There cannot be any ground however for doubting the existence of these structures. The debatable point is merely in regard to the manner in which they are formed.

Further on he says:

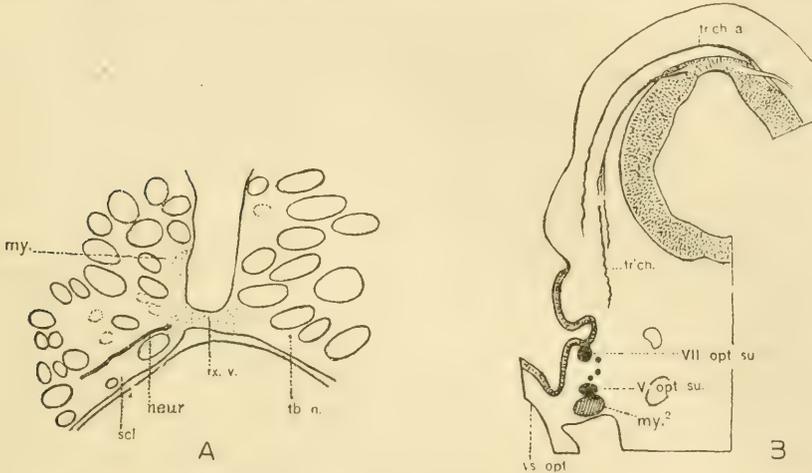
Neal ('03) in an interesting paper on the development of the ventral nerves in Selachians, says that he has been unable in any of his sections to show the existence of a protoplasmic connection 'even of the most attenuated kind between the somite and the neural tube before the first neuraxon makes its exit from the neural tube.' At first it was difficult for me to reconcile this statement with the results of my own observations as well as those of other investigators who have repeatedly observed these bridges in Selachians. At the time when Neal's investigations were conducted there was no method of staining which was capable of differentiating the component parts of the neuraxon, and it is not at all improbable that the structure which he had reason to believe was the growing end of a neuroblast was only the undifferentiated protoplasmic band or bridge. In the second place the method of fixation undoubtedly has something to do with the failure to detect the existence of these structures which are much more easily demonstrable in sections fixed in corrosive—acetic or neutral formol than they are in solutions containing picric acid. For the reasons mentioned the structures represented by Neal as neuraxons cannot be accepted as such without further proof.

Paton goes on further to say (p. 556) that:

Probably the long processes depicted by this investigator as being projected from medullary cells are in reality made up of two com-

ponents: a short process and the long undifferentiated protoplasmic strand or bridge with which it is apparently fused so as to give, in specimens stained by certain methods the appearance of a single long process. In plate 23, figure 1, one of the bridges is represented, the proximal side of which has fused with the matrix of the cord while the distal is united with that of the myotome. (See text-figure A.)

A similar condition is also depicted in figure 2. At later stages one may find connections present between the cord and group of cells, which eventually form the spinal ganglia, and between the latter and



Text fig. A Taken from Paton's figure 2, plate 23; *my.*, myotome; *neur.*, neuraxon (neurofibril, Paton); *rx.v.*, anlage of somatic motor nerve (plasmodesma, Paton); *scl.*, sclerotome (myotome, Paton); *tb.n.*, neural tube. Paton's figure 1, plate 23, is in all essentials like this, but does not show the neuraxon process (neurofibril) stained.

Text fig. B Taken from Dohrn, 1907, figure 7, plate 21; *my.*,² VanWijhe's second myotome (*m.obl.sup.*); *tr'ch.a.*, peripheral chiasma of the trochlearis; *tr'ch.*, trochlearis anlage; *vs.opt.*, optic vesicle; *V.opt.su.*, ramus ophthalmicus superficialis trigemini; *VII.opt.su.*, ramus ophthalmicus superficialis facialis.

the periphery. Further there is abundant opportunity to study these plasmodesmata (Held) in the region of the cranial nerves, where undifferentiated links of protoplasm frequently unite the existing ganglionic masses either with the central nervous system or with the periphery. In the case of the oclomotorius and trochlearis the existence of these bridges is very problematic.

The present writer ('03) made the attempt to discover whether the protoplasmic bridges of Paton were primary or secondary

connections of neural tube and myotome and he reached the same conclusion as did Dohrn ('88), namely, that they are secondary in origin and that they arise by the extrusion of processes of medullary cells which Neal regards as neuroblasts. Frioriep ('04) stated with equal positiveness that the protoplasmic bridges are connected with intramedullary cells. Against the assertions of these three observers we have the statement of Paton that "probably the long processes depicted by this investigator as being projected from medullary cells are in reality made up of two components: a short process and the long undifferentiated protoplasmic strand or bridge with which it is apparently fused so as to give, in specimens stained by certain methods, the appearance of a single long process."

This assertion taken in connection with Paton's criticism (quoted above) of my results as based upon inadequate methods of preservation and staining suggests the possibility that Paton himself has been unable to reach positive conclusions regarding the histogenesis of the 'protoplasmic bridges' and the relations of their cellular components because of too implicit reliance upon a method primarily suited to demonstrate the neuro-fibrillae but quite unsatisfactory for general cytological purposes. However adequate—or inadequate—the Bielchowsky-Paton method for demonstrating the histogenesis of the neuro-fibrillae, it appears quite unsuited for demonstrating the cellular boundaries and relations. For this purpose, the vom Rath method as used by Neal ('03) is superior. The difference between the cytological results obtained by the Paton method and those obtained by the vom Rath method are strikingly shown by a comparison of Paton's figure 2, plate 23, and figure 11 of the present paper, which represent almost exactly identical stages in the histogenesis of somatic motor nerves. The most striking difference consists in the absence of cell boundaries in the former and in their clear definition in the latter. (See fig. A, p. 25.)

But Paton's failure to determine the histogenesis of the 'protoplasmic bridges' is not wholly due to his failure to use a method of fixing and staining which demonstrates cell boundaries and relations, but there is evidence also that he did not make a

careful study of the earlier stages in the differentiation of the somite and of the 'protoplasmic bridges.' A study of those earlier stages when protoplasmic connections are formed might have convinced him that Dohrn, Froriep and Neal have stated the facts correctly. That Paton had neglected the study of the genesis of these bridges is evident from his failure to recognize the sclerotome in his sections. For had he been thoroughly familiar with the earlier stages, when the 'protoplasmic bridges' first appear, he would have seen the formation of the sclerotome associated with the development of the bridges and consequently would not have been led to the inference that the primitive fibrils of somatic motor nerves make their first appearance within the myotome. Held ('09, p. 253) has already called attention to this.

The origin of the neurofibrillae and their relation to the protoplasmic processes which form the protoplasmic bridges will be discussed in a later section of this paper in connection with the problem of the genesis of these plasmodesms. This is the essential point of difference between the results of Paton and the writer. I have regarded the medullary processes which form the 'protoplasmic paths' as neuraxon processes, since the neurofibrils are differentiated within them. These processes were therefore spoken of as neuraxons and the statement was made that "before the first neuraxon makes its exit from the neural tube, sections show no protoplasmic connection, even of the most attenuated kind, between the neural tube and the somite." Paton, holding the Apathy view of the independent and exogenous origin of the neurofibrils recognizes no neuraxon—not even an undifferentiated neuraxon—in the absence of neurofibrils. And yet, as Paton has demonstrated in the case of the Rohon-Beard cells, the neurofibrils become secondarily differentiated within the neuroblast and its process, which consisted primarily of undifferentiated protoplasm. The proof that the protoplasmic bridges are formed by neuraxon processes will be stated later in this paper.

If Paton ('07, p. 556) be correct in thinking that the trochlear and the oculomotorius acquire their nervous connections with their muscles without the participation of plasmodesmatous

bridges, a conclusion reached by Neal ('98), Dohrn ('07) and Gast ('09), there appears no good reason why he should not accept the conclusion that spinal somatic motor nerves become differentiated and connected with their myotomes in the same manner. If a nerve like the trochlear, which traverses a great distance in reaching its peripheral organ, can do so without the aid of a plasmodesmatous bridge, there would seem very little ground for assuming a different mode of histogenesis for spinal somatic motor nerves. That, as a matter of fact, their genesis is essentially alike is supported by the evidence presented in this paper,

Held ('06, '09) like Paton ('07) emphasizes the importance of the plasmodesmatous connections of neural tube and myotome, and concludes, in agreement with O. and R. Hertwig ('78), that these plasmodesmatous bridges are secondarily formed as cell outgrowths. Not only cells of the myotome and spinal cord but also those of the chorda are supposed by Held to participate in their formation. Held ('09, pp. 91-92) refers to the network between chorda, tube and myotome as 'Scilly's Faser-netz' and characterizes it as usually a cell- or nuclear-free tissue, since it consists exclusively of the basal cell processes of the germ layers united together. Secondarily, mesenchymatous cells wander into this network.

He further states (p. 281) that at the time of the appearance of the first motor nerves, which have grown from the neural tube, there is everywhere present an abundance of fine connections. The process of transformation of this—a pre-nervous plasma path—into nerves depends upon the specific power of His' neuroblasts.

With regard to their primary origin, he says (p. 277) that Hensen's hypothesis that all nerves have arisen by the incomplete separation of the origin- and end-cells cannot be correct, since it cannot be thoroughly applied to the details of the development of definite organs in their reciprocal relations. The motor spinal nerve of a frog-larva, for example, can be developed from no mitotically-formed primitive nerve. On the contrary, Held, in agreement with the Hertwigs, regards the plas-

matic connections which form the nerve paths as secondary in origin. He has, however, failed to make a detailed study of the genesis of those plasmodesmata which, according to his view, are later transformed into the somatic motor nerve Anlagen. Furthermore, one searches in vain through Held's admirable monograph for a convincing proof of the assertion (p. 274) that "the growth of the nerve substance is correlated with a resorption of the plasmatic path so that the latter passes over into the former and is utilized in its formation." Similarly unproven is the dogmatic assertion (p. 280) that the 'plasmatische Ausflüsse' of Dohrn ('88) are really not such. Dohrn's characterization of the first protoplasmic connections of tube and myotome as 'plasmatische Ausflüsse' seems admirable. That they quickly lose this undifferentiated protoplasmic character may readily be granted, so that Held's assertion is correct except for the very first stages in the appearance of the protoplasmic bridges.

A number of important questions are involved in the issue raised by Held and Paton: (1) Are protoplasmic connections between myotome and tube primary or secondary? (2) What cells participate in their formation? (3) Have these protoplasmic paths a genetic relation to the neurofibrils? That is, do the same cells which form the plasmodesms also differentiate the neurofibrillae? The writer ('03) concluded that the protoplasmic connections are secondary; that they are formed exclusively by processes of medullary neuroblasts; that within them the neurofibrils are differentiated. Paton ('07) was not able to determine whether they are primary or secondary; suggests (p. 560) that mesenchymatous cells may participate in their production; and concludes that the neurofibrils have no genetic relation with the protoplasm of the bridges, but arise independently within the myotome and grow centripetally into the tube along the bridges. On the other hand Held ('06, '09) regards the plasmodesms as secondary in origin; but holds that they are formed by the fusion of cells of chorda, tube and myotome; maintains that they have no genetic relations to the neurofibrils which grow into them secondarily from medullary neuroblasts, for which the paths provide a protoplasmic covering. The great theoretical importance

of these problems warrants a reinvestigation of those stages in vertebrate embryos which may contribute to their elucidation. Taking up these problems in order we may first ask whether or not protoplasmic connections between myotome and tube are primary or secondary.

1. Are muscle and nerve connected with each other *ab initio*? The Hensen view of the primary connection of nerve and muscle is not supported by the evidence presented by *Squalus* embryos as shown in the drawings represented in figures 1, 2 and 3, which demonstrate the relations of tube and somite in stages previous to the appearance of the anlagen of somatic motor nerves. Figure 1 may seem the most significant of the three since it is taken from a series in which no somatic motor nerve has made its appearance anywhere in the embryo. The section is typical of the trunk region of embryos of this stage and the finer histological features are in all essential respects like those presented by standard methods of preservation and staining. That the space between tube and somite is normal and not an artifact is evinced by the fact that it appears in the living embryo. In sections this region appears to be filled by a vacuolated non-staining or slightly staining plasma. The most significant fact presented in the section is the absence of protoplasmic bridges between tube and somite. Before the first anlagen of the motor roots make their appearance in later stages, it is impossible to find protoplasmic connections between the two organs. The kind of fixation or the stain used makes no difference in the phenomena, provided the space is not obliterated through shrinkage. Reagents, which like formalin cause considerable plasmolysis and vacuolization, and the use of which is likely to be followed by considerable shrinkage, may exaggerate the vacuoles and cause their confluence so that the granular material, which in Flemming or vom Rath preparations is quite evenly distributed, as shown in the drawing, becomes aggregated in denser strands and may simulate protoplasmic strands extending between tube and myotome. However it does not stain like protoplasm, even when aggregated in the manner suggested,

and for this reason has been universally ignored in all drawings. Paton ('07), for example, does not indicate its presence in the sections figured by him (figs. 1 and 2, pl. 23) although it is undoubtedly present in those stages. It may not be confused with the protoplasmic paths emphasized by him and Held ('06, '09).

The plasmodesms of Held ('09) appear to be formed by the union of the plasmoid material of the intercellular spaces with the amoeboid protrusions of the basal cells of the germ layers. At the stage represented in figure 1, protoplasmic processes are lacking in the case of the neural tube, the outside boundary of which consists of an imperforate basal membrane. The chorda is likewise without protoplasmic outgrowths. The median surface of the somite, however, shows a few short and inconspicuous amoeboid extensions. However, between the homogeneous, slightly stained protoplasmic outflows of the somitic cells and the unstained, granular material of the vacuolated plasmoid material between the germ layers, there is distinct contrast, although the two appear to connect with each other. In the drawings the plasmoid substance is greatly exaggerated in order to show it at all. As has been stated, it has been generally overlooked in most embryological studies.

Even when the films of plasmoid material coalesce as the result of reagents, they require intense stains and special illumination to make them visible. They form a most attenuated material for the production of nerves. It would seem most unlikely that growing nerves would trust themselves to such flimsy paths as guides to their destination. That students of nerve histogenesis should seriously consider such all-but-invisible films of non-protoplasmic material as the substance or path of a growing nerve suggests that followers of the Hensen hypothesis are in desperate need of a material basis for their assumptions. In no true sense do the plasmoid films constitute a primary protoplasmic connection between tube and somite. The actual protoplasmic connections are effected secondarily as the following evidence shows.

3. *What cells contribute to the genesis of protoplasmic connections between tube and somite? What cells form the first anlagen of spinal somatic motor nerves?*

In stages slightly later than those just described, protoplasmic processes are extended into the intercellular space between tube and somite by cells of neural tube and somite. In the regions in which nerve anlagen later make their appearance protoplasmic movement makes its first appearance sometimes from somitic cells and sometimes from cells of the neural tube (figs. 2, 3 and 4). In either case the ends of the projections appear connected with plasmoid films. But the first real protoplasmic connections between tube and somite are formed by the outflows of medullary cells (fig. 4).

In subsequent stages the reciprocal movement of medullary protoplasm and mesenchymatous cells (sclerotome) results in the close approximation of the two sets of protrusions and in exceptional cases, such as are shown in figures 8 and 9, the two appear indistinguishable. Such close approximation is rare and, when it occurs, appears temporary, so that the genetic relations of medullary cells to the protoplasmic bridges between tube and somite seem indisputable.

Those medullary cells, which by their protoplasmic outflow, effect the first protoplasmic connections between tube and somite usually stain more deeply than the adjacent cells, a fact to which attention was first called by His ('79). The same peculiarity distinguishes the Rohon-Beard cells during analogous stages in histogenesis; that is to say, in the stages of neuraxon production. These 'neuroblasts' of the somatic motor nerves are bipolar in shape (figs. 4-11), and within them may be detected, in suitable preparations, a neuro-reticulum with intensely stained fibrils. The reticulum does not appear to be limited to one pole of the neuroblast cell but extends around the nucleus. To its presence may be attributed the deeply staining properties of the neuroblasts. The fact that neurofibrillae make their appearance in the extended processes of these cells sufficiently evinces their neuroblastic character.

Kerr ('02) was led to doubt the hypothesis of the secondary connection of somite and tube on the ground that in the earliest stages, when somatic motor nerves are visible in *Lepidosiren* the tube and the somite are in immediate contact with each other: "*Lepidosiren* thus affords a definite anatomical basis for the view that the nervous bridge between nerve center and end-organ exists from the beginning, and that the growth of the nerve is a drawing out of this bridge as the end-organ is pushed away by the development of the underlying mesenchyma." On the basis of such evidence Kerr concluded that the Hensen hypothesis is 'almost demonstrated.'

This inference seems to be a non-sequitur from the evidence presented, since Kerr has traced the anlagen of the somatic motor nerves only to those stages when the protoplasmic connections are already established. Neumayer ('06, page 54) has already called attention to the fact that the stages described by Kerr correspond to advanced stages of histogenesis. If tube and somite in *Lepidosiren* be normally in contact in early embryonic stages, it would seem to be a form little suited to the requirements of an investigation of the primitive connections of nerve and muscle. *Squalus* embryos, in which a space is normally present, would seem much better objects of research. The normal distance between tube and myotome in *Squalus* is so small, however, that the theoretical objection that it would be difficult to explain the growth toward the muscle as a tropism is not likely to be suggested. If the direction of growth of the neuraxon processes of the medullary neuroblasts were determined chemotropically by secretions of the myotome cells there would be little chance for the neuraxons to go astray through a mixing or diffusion of specific secretions. In such a case the theoretical necessity for predetermined paths emphasized so strongly by Held ('09) does not appear very convincing. The difficulty of explaining how the extended processes of Rohon-Beard cells reach their area of distribution by a chemotropic response is much greater, but Held can hardly expect that his speculations (page 274) as to the stimulation of the neuroblast process through predetermined plasmodematous paths will be regarded as pref-

erable. But this theoretical difficulty loses its force when applied to the connections of organs in such close proximity as are neural tube and myotome in the trunk region of vertebrate embryos.

To summarize the evidence that connection between neural tube and muscle segment is formed by protoplasmic movement of medullary cells, we have first the fact that in early stages no protoplasmic connections between tube and somite are found. A plasmod substance fills the space between tube and myotome. Later, medullary cells in the ventro-lateral wall of the tube develop a neuro-reticulum and manifest deeply staining properties. This change is followed by a protrusion of amoeboid processes into the space between tube and somite. Later such protrusions become wider and more extensive, several adjacent cells adding to the size of the protoplasmic bridge thus formed between tube and myotome. The movement of medullary protoplasm is correlated with a migration of sclerotome cells into the space between tube and somite but the latter elements participate only temporarily, if at all, in the formation of the ectoderm-mesodermic connection.

In this manner are formed the anlagen of somatic motor nerves, which at first appear non-fibrillar and protoplasmic and entirely devoid of cells. These are the protoplasmic bridges of Paton ('07). Later the neuroblastic processes extend ventrad, along the median surface of the myotome and in close contact with it and the sclerotome (figs. 10-11). Medullary nuclei soon begin to wander into the anlage from the tube and the movement becomes so extensive that the form of the tube in cross section is greatly changed (fig. 12). All these stages may readily be seen in cross-sections of a *Squalus* embryo of Balfour's Stage I, by comparing sections beginning with the cloacal region and passing forward toward the head. Connections in the cloacal region at this stage are still unformed, while the movement of medullary cells has already begun in the more anterior metameres of the trunk.

4. *Have these protoplasmic connections a genetic relation to the neurofibrils?*

Paton decides adversely to the view that the protoplasmic bridges are formed by the processes of neuroblasts on the ground that they do not contain fibrillae. He does not deny, however, that "under certain circumstances a nerve cell may throw out a process of very considerable length." But he thinks that "the greatest caution should be observed in assuming that mere length of process, without positive knowledge regarding the nature of the structures contained in it, is in any sense to be considered a criterion as to whether a cellular prolongation is or is not to be called a nerve." To "refer to an undifferentiated tract as a nerve would give rise to endless confusion."

Paton ('07, p. 560) finds that in sections fixed in sublimate-acetic and stained by hematoxylin-eosin, the medullary cells appear 'faintly fibrillar;' but he thinks that this 'primitive fibrillation' has no connection with the development of the neurofibrils.

In his opinion, the conclusions of those who claim a connection between the primitive ventral root fibrils and medullary cells are based upon faulty technique. "No reliable method of staining has yet been employed that is capable of demonstrating the presence of processes in the vicinity of the distal ends of these primitive filaments." According to Paton, the first neurofibrils or 'primitive fibrils' make their appearance within the myotome at a point remote from the protoplasmic bridges, as shown in Paton's figure 2, plate 23, shown in outline in text-figure A of this paper (p. 25).

The primitive neurofibrils are coarse, deeply stained structures appearing primarily in a locality where more than in any other place the ground substance, even after sublimate fixation, seems to be granular in character, while the more delicate and attenuated filaments only become visible at later stages. . . . The apparent independence of these primitive neurofibrils in the ventral roots from cells is one of their distinguishing characteristics, but in the large cells of Beard a different arrangement exists. There the fibrils appear in the apical process of the cell close to its nucleus (compare figure 2 with figure 12, plate 23).

Thus Paton not only doubts the neuroblastic origin of the connections between the tube and myotome but also the neuroblastic origin of the neuro-fibrillae. That he has been led into error in his inference of the independent origin of the neuro-fibrils will be suggested by a comparison of Paton's figure 2 with figure 12 of this paper. Paton is mistaken in his inference, partly because of his failure to trace carefully in earlier stages the genesis of the protoplasmic bridges; partly because he has not used a method suitable to demonstrate cell boundaries, and partly because the neuro-fibrillae in his preparations are incompletely stained, as has already been stated by Held ('09). A comparison of Paton's figure 2 with figure 12 of this paper shows that the 'primitive fibril' which Paton thinks arises within the myotome actually lies between the myotome and the sclerotome. Moreover, its position corresponds with the position of the termination of the cell processes of medullary cells. The two drawings strikingly show the difference in the histological results of the vom Rath and the Bielehowsky-Paton methods. The great advantage of the former appears in the sharp definition of the cell boundaries, of the latter in the clear differentiation of the neurofibrils. The convincing proof given by Held ('09) of the endogenous origin of the neurofibrils within medullary neuroblasts certainly warrants the inference that the neurofibrils are incompletely stained by the Bielehowsky-Paton method. Later in his paper Paton admits the possibility that the neurofibrils are genetically related to the processes of the medullary cells. But he thinks that new technical methods are needed in order to solve the problem thus raised. To determine this, however, needs not so much a new method of neurological technique as a careful study of the successive stages in their differentiation by methods which we now possess, especially a method like the vom Rath which defines clearly the cell boundaries and relations. Paton's procedure admirably supplements such a method.

Held ('06, '09) has made a most thorough and painstaking investigation of the histogenesis of the neurofibrils on the ground that, as stated by Max Schultze, von Kupffer and von Apathy, these are the distinctive structures of the nerve fiber. In agree-

ment with Apathy, Held is of the opinion that the solution of the problem of the development of nervous tissue depends primarily upon the demonstration of the place and manner of origin of the neurofibrillar substance. He concludes that this important nervous element is differentiated within the neuroblasts of His, which are thus the essential nervous cell centers. These results are in harmony with the experimental results of Harrison, Lewis and Burrows.

Held finds that the first and surest histological characteristic of a cell of neuroblastic tendency lies in the occurrence of a specific network or neuro-reticulum which appears in a circumscribed region of the neuroblast in the vicinity of the nucleus. In the course of its development this network undergoes an extraordinary complication and extension of its substance. The observation of the primary appearance of the neuro-reticulum within the neuroblast had previously been made by Besta ('04).

Contrary to the opinion expressed by His, Held affirms that it is not the outflow of protoplasm into a cell process, but the definitely directed growth of a new and special cell substance which originates the first nerve trunks in the embryo. The process is not that the cell produces a protoplasmic elongation of the cell body in which subsequently and secondarily a fibrillar substance appears. On the contrary there are inner changes within the protoplasm of a neuroblast that lead eventually to the differentiation of a neurofibrillar substance which, while primarily of a minimal extension within the fibrillogenous zone of the neuroblast cell, later in the course of its special growth undergoes a mighty extension in the body after it has produced externally the pear-shaped form of neuroblast first observed by His. This fibrillar structure of the neuroblasts is 'neurofibrillar' in the von Apathy sense of the word.

Held's conclusions do not agree with those of von Apathy ('98) who holds that the ganglion cells produce no neurofibrils but are secondarily penetrated by them. According to Apathy, the neurofibrils are produced by other and special cells—the 'nerve cells'—which are not to be confounded or identified with 'ganglion cells.' The 'nerve cells' produce, ac-

According to von Apathy, the conducting or neurofibrillar substance just as muscle cells produce muscle fibers. From these 'nerve cells' the conducting substance grows on the one hand toward the center in the 'ganglion cell' and on the other hand toward the periphery into the sensory cell or muscle cell and so forth. The nuclei of the nerve cells lie in the course of the nerve fibers themselves and form in vertebrates the nuclei of the neurilemma sheath. The protoplasmic body of the nerve cell is, in general, spindle-shaped so that it may be called the nerve spindle. Its membranous boundary is the neurilemma.

For every motor nerve fiber of a vertebrate, on the other hand, according to Held, there may be distinguished a central and peripheral nerve stretch. The external limiting membrane of the neural tube divides the two. The neurofibrillae of the central stretch are formed before the peripheral. They arise through the unilateral growth of the neurofibrillar substance of a unipolar or bipolar neuroblast which proceeds basalwards in the direction of the Rabl's 'chief axis' and soon transcends the outer limits of the neural tube in its growth toward the terminal organ. The central stretch of the nerve varies with the position of the neuroblast cell. In its extent through the marginal zone the nerve fiber becomes secondarily surrounded and enclosed by glia cells.

Held distinguishes three chief stages in the development of the peripheral stretch of the nerves:

1. That of the outgrowing nerve itself, which has not yet reached its terminal organ with its specific substance, but nevertheless is connected with its terminal organ by means of a simple undifferentiated plasmodesmatous strand. Theoretically this stage is the most important. The His doctrine, according to which the amoeboid neuroblast processes extend through open spaces in the tissues, cannot be correct, since in the Anamnia the growing point of the motor nerve is connected with portions of the epithelium, that is by plasmodesmatous connections, and in the Amniota on the other hand with parts of the mesenchyma.

2. In the second stage the nerve has reached its muscle anlage, which it not only touches superficially, but cells of which

it penetrates with its neurofibrils. At this stage the motor nerve anlage in Anamnia is non-cellular while in Amniota it is cellular. The cause of this difference is as follows: In case the nerve anlage makes its appearance while the germ layers and the anlagen of the organs are still epithelial as in the Anamnia, the peripheral neurofibrillar tract lies in nuclear-free and net-like plasmodesms, which connect the related epithelial surfaces with each other. If, on the other hand, a connective tissue is already developed, then such nerve fibers extend through a variable number of cell-bodies. In the first case the nerve appears primarily without nuclei, while in the second it appears as a nucleated structure. The difference is due to a difference in the relative time of differentiation of the neuroblasts.

3. The third stage is a transition to the condition in the adult nerve with its cellular neurilemma sheath, and arises through the emigration of medullary cells into the fibrillar nerve and their differentiation as sheath cells.

Held summarizes his views regarding the histogenesis of motor nerves as follows:

The origin of motor nerves rests upon the peripherally directed growth of the specific cell substance of definite neuroblasts of the neural tube and which proceeds in the direction of the chief cell axis of the neuroblasts, and, for definite but unknown reasons, transcends the outer boundary of the embryonic neural tube. A stage of development precedes the formation and growth of the nervous substance itself, by means of which the neural tube and its motor neuroblasts, through net-like arranged paths of a simpler and not yet neurofibrillar substance, is brought into connection with the peripheral muscle anlage by complicated processes of outgrowth.

Such connection paths, which in Anamnia are simple and epithelial and in the Amniota, on the contrary, are a complicated connective tissue, are in the Hensen's sense of the word 'used' by, and in some sort of an unknown manner fused with, the nervous substance growing from the neuroblasts. The growth of the nerves to the terminal organ does not proceed in the liquid-filled vacuoles of the intercellular spaces as affirmed by His, since the growing points of the nervous substance are connected both laterally and at the outer extremity with the farther-reaching plasmodesms of the surrounding tissues. Later, by means of complicated processes of multiplication and movement of medullary cells, which wander secondarily into the primarily non-nuclear, or nucleated, nerve-path as cell-elements organically connected with it, the motor nerve becomes the multinucleated strand supplied with

neurilemma cells. The cell-chain hypothesis has entirely looked upon and conceived the beginning of this third stage as the actual beginning of the specific formation of nervous substance. Actually, however, this condition belongs to that period of the development which transforms the embryonic type of nerve into the adult and differentiated structure.

In presenting evidence of the secondary connection of nerve and muscle, and perhaps more convincingly, evidence of the endogenous origin of the neurofibrils and their genetic relation to the medullary neuroblasts, Held supports the essential points of the process theory of nerve development. His conclusions, however, differ from those usually held by supporters of the Kupffer-Bidder theory in regard to the origin of protoplasmic connections of nerve center and end-organ. In this regard Held considers himself an advocate of the Hertwig theory, since he holds that protoplasmic connection of tube and myotome is formed, not by the neuroblasts which form the neurofibrils, but independently as plasmodesms of indeterminate and multicellular origin.

There is nothing in Held's monograph, however, to indicate that he has given as careful attention to the development of these plasmodesms as to the histogenesis of the neurofibrils. The evidence presented by the writer ('03 and in the present paper) supports the view of His, von Lenhossek, Cajal that the same cells which form the neurofibrils also form the protoplasmic connections between tube and myotome. In other words, the neuroblasts themselves form the protoplasmic connections of tube and myotome. As shown in plates 1 and 2, the processes of medullary neuroblasts extend along the median surface of the somite between myotome and sclerotome and within these processes (which may be traced to their connections with medullary cells as stated by Froriep, '04) neurofibrils make their first appearance as is clearly shown in preparations made by suitable methods of staining. In the light of Held's results, it seems probable that a neuro-reticulum is present within the neuroblast cell, but no method sharply differentiates it in Selachians in early stages of histogenesis. Sometimes in Bielchowsky-Paton preparations a fibrillar network appears in certain medullary cells in stages before protoplasmic connection with the myotome has been formed. That this is neu-

rofibrillar has not been demonstrated, however, although the analogy of the histogenesis of the Rohon-Beard cells would favor this opinion.

Although the evidence presented in this paper is not sufficient to demonstrate positively that the neurofibrils of somatic motor nerves arise endogenously within those medullary cells which form the protoplasmic connections of tube and myotome, the conclusion that such is the case appears justified on the following grounds:

1. The evidence goes to show that medullary cells, by a process of outgrowth, form the first protoplasmic connection of tube and myotome.

2. In subsequent stages these processes extend ventrad along the median surface of the somite between myotome and sclerotome.

3. Within these processes which in vomRath preparations may be traced to their connection with medullary cells, the neurofibrillae make their appearance.

4. Since, as shown by Paton ('07) the neurofibrils arise endogenously within the processes of Rohon-Beard cells, and Besta ('04) and Held ('06, '09) have demonstrated a similar histogenesis in somatic motor neuroblasts of Amniota, analogy would seem to support a similar origin in Selachians.

Coghill ('13) has been able to demonstrate that in *Amblystoma* the neurones of the somatic motor column become well differentiated and typically oriented in the spinal cord before the ventral roots appear at the corresponding level. The earliest demonstrable root fibers arise as collaterals from these neurones, usually from descending processes.

5. *Is the neuraxon of a somatic motor fiber multicellular in origin, or is it the process of a single medullary cell?*

Since the beginning of the twentieth century the Schwann-Balfour 'cell-chain' theory of nerve development has been supported by Rafaelle ('00), Bethe ('00-'07), Oscar Schultze ('04-'07), Brachet ('05-'07), Cohn ('05-'07) and possibly von Apathy ('07). From its inception the cell-chain hypothesis has depended

for its support largely upon the well established fact of the cellular structure of embryonic nerves. It was this fact that caused Kupffer finally to abandon his process theory. Then also von Apathy's important discoveries of the intercellular relations of the neurofibrils have seemed to some to favor this view of neurogenesis, so that the theory appears to have a new lease of life. Bethe ('03) is of the opinion that he has established the fact of the dependence of the axis cylinder upon the 'nerve cell'—in the Apathy sense—because he finds that in his preparations the mitotic division of a nucleus interrupts the nerve fiber. Held ('09) suggests that this evidence might warrant another inference not complimentary to the quality of Bethe's preparations. Taken as a whole, the arguments in favor of this much discussed hypothesis, which has derived the larger part of its support from preparations unsuited to the requirements of neurological investigation, seem most unconvincing.

Held's ('09, p. 51) discovery of the polyneuroblastic origin of nerve fibers should not be taken as a confirmation of the cell-chain hypothesis, since it does not involve the idea of a cell-chain but asserts the formation of some neuraxons by the fusion of the processes of adjacent neuroblasts.

Bardeen ('03, p. 255) and the writer ('03) have advanced arguments against the claim that the cells of embryonic nerves participate in the formation of the fibers of the nerves. In this connection, Bardeen says that "in an early embryonic nerve of moderate size one finds many hundred fibrils enclosed by a sheath of flattened cells, but with no cells among them. In such nerves one can most easily see that the fibrils are not differentiated parts of cells lying in the nerve."

The phenomena of motor nerve histogenesis in *Squalus* affords no support to the cell-chain hypothesis, since somatic motor nerves in this animal acquire connection with the myotomes and a fibrillar structure before cells make their appearance within the nerve anlage. The cellular structure emphasized by the advocates of the hypothesis appears only in somewhat advanced stages in histogenesis. Evidence that the cell elements present in the nerve anlagen have a genetic relation to the fibers or neu-

rofibrils is wholly lacking. Proof that they form the neurilemma has been given by the writer ('03) and by Carpenter ('06).

6. *How does the elongation of the neuraxon take place?*

Does the elongation of a nerve involve simply a migration of protoplasm from the neuroblast cell as the recent experiments of Harrison ('04, '11), Lewis ('06, '07) and Burrows ('11) seem to prove? Or does the neuraxon fiber grow by the progressive differentiation of a primary plasmatic connection between tube and myotome as Sedgwick ('94) and Held ('07) have suggested?

Are we to accept Cajal's evidence of the free termination of the neuraxon fiber in the intercellular spaces of the embryo? Or shall we agree with Held ('09) that "in the growing nerve we have to do with an organically advancing growth, and not with the pushing through of a cell process?"

Although the raising of these questions takes us back to the time of von Baer and the beginnings of embryology, it cannot be said that they have been finally answered. Indeed, the problem of how the elongation of the neuraxon process is effected remains one of the most vexed questions of histogenesis.

Cajal ('07) describes the nerve fibers as advancing and growing through the mesoderm, using the cellular interstices. To him the 'Leitzellen' and their anastomosing expansions are always situated on the sides of the axons which, as the result of the disturbing action of the reagents (pyridine, alcohol), attach themselves to, or partially unite with, the mesodermic framework. To Held ('09) such evidence of nerve terminations ending freely in the intercellular spaces is a result of the rupture of the plasmodesms, with which he affirms the growing tip is normally connected. The growth of the nerve substance he states (p. 274) is correlated with a resorption of a plasmatic path so that the latter passes over into the former and is utilized in its formation.

Harrison ('01) states that the sensory nerves of *Salmo salar* possess fine lateral branches "which might easily be confused

with coagulation threads, which are everywhere present," but, unlike Held, finds no genetic relations between such coagulation threads and the elongating neuraxon process. Later ('07) Harrison writes that "it is by no means certain that the plasmodesms are not artifacts—products of coagulation," and von Lenhossek ('06) offers the same criticism.

Such criticisms suggest to Held ('09) that Cajal and Harrison have not seen the true plasmodesms, which are not only very easily ruptured but appear everywhere different from coagulation threads. They may be covered with coagulation bodies, or not; that varies with individual preparations. If, however, one has ever seen these fine plasmodesmata branch out in characteristic fashion from subdividing and branching cell processes, it is impossible longer to hold the view that these fine and definitely branching threads are coagulation products or artifacts.

Held emphasizes the delicacy of the plasmodesmata and the ease with which in fixation or imbedding and sectioning they may be ruptured and lost. While this is unquestionably true, it is also a fact that the plasmodesms become more conspicuous in preparations in which there has been excessive plasmolysis and vacuolation, as in many preparations made for special neurological purposes. On the other hand, the more faithful the cytological fixation, the less conspicuous becomes the coagulable substance found in the intercellular spaces where the neuraxon processes of medullary neuroblasts and those of Rohon-Beard cells grow towards their end-organs.

While Held (p. 297) admits that Harrison's experiments prove that processes may grow out from neuroblasts in suitable fluids, that nerve elongation may occur exclusively by protoplasmic movement and therefore does not necessarily involve the participation and differentiation of plasmodesms, nevertheless he maintains that a normal nervous system oriented to the terminal-organ is formed only with the participation of plasma connections already present. If, however, it may be assumed that the experiments of Harrison ('04-'11), Lewis ('06, '07), and Burrows ('11) throw any light upon normal processes of histogenesis—a conclusion which Held does not seem willing to admit

—they tend to show that however important the plasmodesms may be as guides for the growing nerve, their share in its production is negligible. They prove as convincingly as experimental data possibly can the logical fallacy of Held's inference, that because the growing tip of the neuraxon is connected with branched plasmodesmatous processes that therefore these processes have a genetic relation to the neuraxon. The significant fact in this connection is that Harrison's preparations show these same processes at the termination of the neuraxons growing freely in salt solutions! The genetic relations are here precisely the opposite to those inferred—or assumed—by Held in the normal growth of the neuraxon. Even if it be admitted that Held's assertion is correct that the growing and more or less branched point of growth of the nervous substance at its extremity possesses, in advance of its momentarily attained length, a projecting extension which connects (as an undifferentiated plasmatic mass between the termination of the growing nerve and the end-organ), the neuroblast with the terminal-organ, his conclusion, already quoted, that the plasmodesm is utilized in the formation of the nerve is a logical non-sequitur. Nowhere in his excellent monograph does Held give adequate evidence to prove this assertion, and the experimental evidence seems to make the assumption unnecessary. Furthermore, the assertion that the growing termination of the neuraxon is connected by a strand of undifferentiated protoplasm with the end-organ has not been demonstrated.

As further evidence in favor of the view that neuraxons and neurofibrils are differentiated within plasmodesmatous or protoplasmic strands, and consequently in conflict with the idea that they grow 'naked' into the vacuolar spaces of the embryo, Held emphasizes the fact that from their first appearance the neurofibrils appear surrounded by a layer of granular protoplasm, a relation better seen in cross-sections than in longitudinal ones. On the other hand, von Lenhossek ('06) states, on the basis of observations on the histogenesis of spinal nerves in the chick, that "first and foremost is it untrue that the young fibers are embedded in protoplasm" but that on the contrary, "the fibers

lie definitely free, and in the spaces between them there is not the slightest amount of protoplasm to be seen. Held denies the accuracy of this description as based upon unsatisfactory methods of preservation and staining. Cajal ('06, '07) in Held's opinion has been led into error for the same reason. On the other hand, Kölliker ('05) finds the fibers of the embryonic trochlearis nerve in a calf surrounded by sheaths of an intermediate substance. Gurwitsch ('00) mentions a lamellar network which grows into the bundle of 'naked' fibers from the surrounding mesenchyma sheath and divides up the nerve bundle, while according to Kappers ('04) the nerve bundle is coarsely split or divided by fine processes of the neurilemma cells.

In his search for evidence to support the hypothesis of the plasmodesmatous origin of nerve fibers, it seems not to have occurred to Held that the protoplasmic envelope of an embryonic nerve fiber may be produced by the same neuroblast that formed the fiber. The simple fact of a protoplasmic sheath around the neurofibrillae no more proves the existence of nerve 'paths' than it does the hypothesis of neuroblastic outflow. The evidence is in harmony with either supposition. In *Squalus*, embryonic nerve fibers are 'naked' in the sense that primarily they have no cellular sheaths. But it is also a fact that they have granular, protoplasmic sheaths, visible, not only in sections, but in cover glass preparations of the living nerve fiber. The substance of the sheath stains lightly under the same treatment which stains the fiber intensely. For this reason the protoplasmic envelope is easily—and has been generally—overlooked. The thickness appears to vary in proportion to the length of the neuraxon process. In nerve Anlagen, like the trochlearis, for example, in which the fibers are especially long and slender, the protoplasmic sheaths of the fibers are proportionally thin. In spinal somatic motor nerves the sheaths are relatively thick, becoming thinner as the nerve fibers increase in length. The ingrowth of processes of neurilemma cells described by Gurwitsch ('00) and Kappers ('04) occurs in later stages and adds to the interfibrillar protoplasmic substance.

The intraplasmatic position of the nerve fiber or neurofibrillar bundle, therefore, instead of proving the doctrine of primary plasmatic nerve 'paths,' is equally in harmony with the process theory of nerve development. An examination of *Squalus* embryos shows that, sooner or later, within the plasmatic neuroblast process which effects the first connection between tube and somite, the neurofibrillar bundle is differentiated as an axial fiber surrounded by the undifferentiated protoplasm of the neuroblast process. The advancing end of the nerve consists of undifferentiated protoplasm and is amoeboid in appearance. The phenomena in sections of preserved embryos are essentially identical with those in vitro of the living nerve fiber. The elongation of the neuraxon, therefore, instead of involving the use and incorporation of primary plasmodesmatous paths into the growing nerve, on the contrary is effected by a movement of the protoplasm of a neuroblast cell and the endogenous differentiation of the neurofibrils. With especial clearness are the phenomena strikingly shown in the giant cells of Rohon-Beard.

a. The histogenesis of the cells of Rohon-Beard. The conclusions based upon the study of the histogenesis of spinal somatic motor nerves are greatly strengthened and confirmed by the phenomena presented by the development of the cells of Rohon-Beard. These phenomena are presented in figures 13 to 22. The close proximity of the nerve center (neural tube) and the terminal-organ (myotome) in the case of the spinal somatic motor nerve makes it very difficult to find wholly convincing evidence of the extension of the neuraxon process, and of the secondary nature of the connection between the two organs.

In the case of the Rohon-Beard cells, however, the neuraxon process, in reaching its peripheral termination, grows into and through spaces where mesenchymatous cells are entirely absent. As shown in figures 13 to 16, the neuraxon process appears primarily as an amoeboid extension of a large, deeply stained cell, lying in the dorsal wall of the neural tube. As the neuraxon process becomes further extended, its peripheral termination shows many pseudopodia-like extensions. In some cases

(e.g., fig. 21), as the neuraxon process reaches the apex of the myotome, some of the pseudopodial processes extend median and some lateral to the myotome. It appears to be undetermined whether, in its further extension, the neuraxon may grow between the myotome and the neural tube or between the myotome and the ectoderm. The significant fact presented by such a section and by all the sections which show the cells of Rohon-Beard with their greatly elongated processes, is that there is not the slightest suggestion of a direct primary connection between the cell and its later connection with the ectoderm in the extra-embryonic blastoderm.

Such relations as are shown in figure 20, afford still further evidence of the genetic relations of ganglion cell and neuraxon. The section shows a Rohon-Beard cell lying at the apex of a sensory ganglion, and which has reached its present position by migration from the dorsal wall of the neural tube. The continuity of the cell body and of the neuraxon and evidence of their genetic relation to each other is more clearly shown than in the case of sections where the Rohon-Beard cell lies within the wall of the neural tube. The objection to the inference that somatic neuraxons are formed as elongated processes of medullary neuroblasts, on the ground that it is impossible to trace the protoplasm of the neuroblast through the wall of the neural tube into the growing nerve anlage is met by the evidence presented in this figure.

Doubt remains only as to the inference that the process is formed by a migration of protoplasm from the neuroblast into the neuraxon. Why not infer that the neuraxon is formed from intercellular bridges in situ? In answer to this question, it is sufficient to state that in the earlier stages when the neuraxons of the Rohon-Beard cells first appear, there are no protoplasmic bridges extending in the direction taken later by the growing neuraxons, but simply a non-staining plasma. Furthermore, it is as logical to conclude that the body of the Rohon-Beard cell shown in figure 20 was differentiated in situ as that its neuraxon was formed in situ. On the contrary, if there be good reason for inferring that the Rohon-Beard cell has reached its present

position as the result of protoplasmic migration from the neural tube, there is equally good reason for the conclusion that its neuraxon has been formed by the same method.

7. What is the source of the cells of somatic motor cells?

The cellular structure of embryonic somatic motor nerves has been emphasized by many embryologists since Schwann ('39) advanced the cell-chain hypothesis of neurogenesis and since its subsequent revival by Balfour ('77). The question of the source of these cells has never been fully determined. Are they medullary or mesenchymatous in origin?

Balfour ('75) was the first to infer the migration of medullary cells into somatic motor nerve anlagen and this conclusion has been confirmed by many investigators including Dohrn ('88), Beard ('88), Herrick ('93), von Kupffer ('94, '95), Hoffmann ('97), Harrison ('01), Bardeen ('03), Neal ('03), Schultze ('04-'07), Held ('06, '09), Carpenter ('06), Carpenter and Main ('07) and Kuntz ('10-'12). His ('89) and von Kölliker ('92), on the other hand, have denied the derivation of these cells from the neural tube. The former concluded that the cells which appear to be in the process of migration from the tube, are later enclosed by the 'Randschleier' which prevents their escape. If it were otherwise, he thinks, the process would lead to the formation of motor ganglia. To His it appeared quite inconceivable that connective tissue elements could come from the nervous system. The mesenchymatous origin of the cells of somatic motor anlagen has also been assumed by Vignal ('83), von Lenhossek ('97), Kolster ('99), Gurwitsch ('00) and Bardeen ('03). Von Kölliker ('05) came finally to accept the probability of the medullary origin of the neurilemma. According to Marcus ('09), the cells of somatic motor nerve anlagen come from the neural crest.

The fact that sclerotome cells are in close proximity to the somatic motor anlagen has led some students to infer that the cells present in later stages within the nerve have had a mesenchymatous derivation. In fact, the mesodermal origin of all connective tissue cells has seemed so well attested that many

have taken for granted the mesenchymatous nature of all cells associated with motor nerve anlagen. Kerr ('04) seems to have made this assumption for the cells associated with the somatic motor anlagen of *Lepidosiren*. He says (p. 121) that "richly yolked masses of mesenchymatous protoplasm become aggregated around the nerve, which till now has been quite naked." Again ('02) he writes that "in tracing back the motor trunks of the spinal nerves I reached a stage in which the faintly fibrillated trunk was ensheathed in a nucleated mass of protoplasm of mesenchymatous origin." The mesenchymatous origin of these cells has seemed so self-evident to Kerr that the problem of tracing them to their source does not seem to have occurred to him, and in consequence he advances not a particle of evidence that he has traced their genesis.

That the cells of somatic motor nerve anlagen in *Squalus* are largely, if not exclusively, of medullary derivation seems demonstrated by the following facts:

First, when cells make their earliest appearance in the nerve anlagen they lie partly in and partly outside of the neural tube. Then later, when cells are found definitely within the anlagen, as seen in figure 12, they appear at the base of the anlagen and near the tube. As cells grow more numerous in the anlagen in later stages more nuclei appear to be in the process of migration from the tube. As a result of the migration, the contour of the tube as seen in cross-section becomes changed and the nerve anlage greatly thickened. Furthermore, the nerve anlage shows a limiting membrane continuous with that of the tube, which makes it possible to distinguish the boundary of the nerve anlage and to infer its independence of mesenchymatous cells in the immediate vicinity. Moreover, the boundaries of the cells of the anlagen retain their smooth epithelial contour, while the mesenchymatous cells assume characteristically irregular, branched outlines. Whereas at first the cells of the sclerotome from which the mesenchyma of the region is derived, are closely apposed to the nerve anlagen, as seen in the figures on plates 1 and 2, they soon lose contact with the nerve and with each other and become metamorphosed into a loose connective tissue, but still retain

connection with each other by fine plasmodesmatus threads. Thus the study of serial sections of *Squalus* embryos in closely related stages of histogenesis permits no doubt that the cells of somatic motor nerve anlagen are in large part migrant medullary elements.

From an estimate of the number of migrating medullary cells and a comparison with the estimated number in adult nerves the writer ('03) concluded that the neurilemma receives accessions in later stages from the mesenchyma and that the mesenchymatous participation in the formation of the sheaths of the adult nerve is greater than is the medullary contribution. The reasons for this inference are more convincing if, as has been suggested by Harrison ('01) and others, some of the migrant medullary elements form the sympathetic anlagen. The writer agrees with His, Jr. ('97) that at least a part of the cells of somatic motor anlagen have a mesenchymatous derivation.

8. *What is the fate of the cells found in somatic motor nerve anlagen?*

Three views have been advanced as to the fate of the cells of somatic motor nerve anlagen:

1. They are 'nerve-forming cells' and they secrete the neurofibrillae.

2. They form the neurilemma cells.

3. They become the ganglion cells of the sympathetic.

According to the first view the cells of somatic motor nerves anlagen form the nerves, either by fusion into cell-chains as Schwann ('39) and Balfour ('78, '81) suggested and has since been maintained by Marshall ('78), Van Wijhe ('82, '86, '89), Beard ('85, '88, '92), Beraneck ('87), Goette ('88), Dohrn ('91), Miss Platt ('94, '96), Sedgwick ('94) with modifications, Hoffmann ('96), Kupffer ('90, '91, '94), Capobianco e Fragnito ('98), Rafaele ('00), Bethe ('00-'07) with modifications, Brachet ('05, '07), Cohn ('05-'07) and Oscar Schultze ('04, '07); or as the 'nerve cells' which secrete the neurofibrillae and which attain connections with ganglion cells and muscle fibers along plasmodesmatus paths without the participation of cell-chains.

By his discovery of a 'ventral neural crest' in *Ammocoetes* Kupffer was led to abandon the cell-process theory of nerve development and to adopt the cell-chain hypothesis. According to Sagemehl ('82) and Held ('09), however, somatic motor nerves in *Petromyzon* are primarily fibrillar. Consequently, von Kupffer's inference may be regarded erroneous as the result of defective observation.

Schultze ('04-'07) has adopted a modified form of the cell-chain hypothesis from his observation of the growth of nerves in amphibian embryos, in which he finds the nerves primarily cellular. He consequently regards the nerves as a syncytium of peripheral neuroblasts, on the assumption that the cells are the formative ones which secrete a nerve. Thus Schultze regards the neurilemma as ectodermal in origin.

According to Held ('09), however, Schultze has failed to see the earlier stages of nerve histogenesis before the anlagen have acquired a cellular character. Held ('09) regards the cells which find their way into embryonic nerves as 'Leitzellen,' and holds that they have nothing to do with the formation of nerve fibers more than to furnish the paths in which the neurofibrils are driven forth from the neuroblasts. But Schultze claims that the fibril-free cells (Held's *Leitzellen*) are as much neuroblasts as muscle-forming but primarily fibril-free cells are myoblasts.

The writer ('03) argued on the following grounds that the cells of somatic motor nerve anlagen have nothing whatever to do with the formation of neuraxons:

1. In the earliest stages of histogenesis, when the number of fibers increases most rapidly in the nerve anlage, the cells of the anlage are distinctly peripheral in relation to the fibrillar bundles (Bardeen, '03, has also emphasized this point).

2. None of the ventral nerve cells at any stage of histogenesis show the deeply-staining properties of neuroblast cells such as appear in the ganglia of the dorsal (somatic sensory) nerves. Without exception the cells of the somatic motor nerve anlagen are vacuolated, granular and lightly stained.

3. While marked changes in size and shape (correlated with the growth of the neuraxons) appear in the neuroblasts of the

sensory ganglia, no such changes appear in the cells of the somatic motor nerves.

4. While the long axes of the neuroblasts of the sensory nerves correspond, and are parallel with the direction of growth of the neuraxons, no such relation is seen in the cells of the somatic motor nerves in those early stages when the fibers increase in number most rapidly.

5. The neurofibrils do not lie within, nor are they directly connected with, the protoplasm of the cells of the somatic motor nerve anlagen. The cells in the earlier stages of histogenesis are clearly peripheral to the neurofibrillae. On the basis of such considerations, it appears extremely doubtful if the cells of somatic motor nerve anlagen have genetic relations to the nerve fibers. The cells must have some other fate.

On the other hand, Cajal ('08) thinks that he has been able to discover nerve-forming cells in a bipolar phase in the somatic motor nerves of the chick.

9. What is the histogenesis of the neurilemma?

A second, and practically unanimous opinion is that the cells of embryonic nerves form the neurilemma sheaths. Indeed, this has been the opinion of investigators, whatever their creed as to the histogenesis of nerve. According to supporters of the cell-chain theory (e.g., Dohrn, '91), that portion of the peripheral protoplasm of the nerve cells which does not enter into the formation of the neuraxon becomes the neurilemma sheath. The same opinion is shared by supporters of the process and of the plasmodesm theory. Difference of opinion centers chiefly about the problem of the origin of the cells.

Harrison ('06), on experimental grounds, concluded that the neurilemma cells of the frog are exclusively derived from the neural crest and this opinion is shared by Held ('09) who thinks that the neurilemma cells have a similar origin in *Axolotl* and *Triton*. According to Held the neurilemma cells are peripherally emigrated glia cells which have secondarily followed neurofibrillar tracts already laid down. Their source varies in different vertebrates. In *Petromyzon* and the *Selachii* the source

is exclusively medullary. In the trout some of the cells possibly come from the dorsal ganglia, while in the Amphibia they come exclusively from this source. In reptiles, birds and mammals they appear to come chiefly from the sensory ganglia.

According to the writer ('03) the cells of somatic motor nerves of *Squalus* have a medullary origin. Figures 12 to 32 (pls. 23-24) show the gradual transformation of cells into the neurilemma of the adult. Since the fact is unquestioned there appears no reason for repeating the evidence upon which this conclusion is based. Bardeen ('03), Carpenter ('06), Carpenter and Main ('07), also drive the neurilemma from migrant medullary cells. Kuntz ('10) confirms this conclusion.

But is it not possible that the medullary elements contained in somatic motor nerve anlagen enter into the formation of the sympathetic? Such a fate has been claimed for them by more than one competent investigator.

10. To what an extent do the emigrated medullary elements enter into the formation of the sympathetic ganglia?

In recent years the Remak-Kölliker view that the sympathetic system is of mesodermal origin has been shared by few investigators. Onodi ('86) considered such an origin as possible for the peripheral sympathetic plexuses and both Paterson ('90) and Fusari ('90) have held the same view of the genesis of the elements of the sympathetic.

Balfour ('78) and Schenk and Birdsall ('78) led the way to the present understanding of the ectodermal origin of the sympathetic. Balfour did not decide whether the sympathetic cells were derived from dorsal or ventral roots, but Schenk and Birdsall inferred their origin from the dorsal ganglia, a conclusion since drawn by Onodi ('86), VanWijhe ('89), His ('90), His, Jr. ('91, '97), von Kölliker ('94), C. Rabl ('97), Kohn ('05, '07), Lillie ('08), Held ('09) and Marcus ('09).

Hoffmann ('00) inferred a double source of origin of the sympathetic elements as derivatives both of the dorsal and ventral roots, an opinion shared by Neumayer ('06) and Kuntz ('11).

An exclusively medullary origin of the sympathetic elements has been inferred as a possibility by Harrison ('04) on experimental grounds, and Froriep ('07) draws the same inference. Kuntz ('10, p. 250) who had stated in an earlier paper that Froriep's conclusion "is probably correct with regard to the neurones in the sympathetic trunks and the prevertebral plexuses," in a later paper ('11) supports the view of the double source of the sympathetic elements.

Held ('09) draws the conclusion that the cells of the motor roots have nothing to do with the formation of the sympathetic system, on the following grounds: First, the anlage of the sympathetic ganglion in *Selachii* lies entirely in the axis of the sensory nerve root and not in that of the motor root. Second, the motor root has fewer cells, is nowhere thickened, and is nowhere united with the sensory nerve by means of cell-strands. Moreover, the ventral nerve extends along the myotome parallel with the sensory nerve, but its cellular material is not mixed with that of the latter. Also, the motor root lies lateral to the sensory root, nearer the myotome and farther from the sympathetic. The two are not united by cell-strands. Finally, the two unite ventral to the sympathetic anlage.

With Kohn and Froriep, Held admits the principle of a free cell migration of purely nervous cell clusters, in the formation of the sympathetic, but thinks that the sympathetic ganglia arise by the budding of chain-like connected cell masses and strands containing both ganglion cells and sheath cells.

Kuntz ('11) is the most recent student of the histogenesis of the sympathetic in *Selachii*, and his is the latest presentation of the case in favor of the medullary origin of some of the elements of the sympathetic. Kuntz's argument that the cells of the ventral roots as well as those of the dorsal participate in the formation of the sympathetic appears to consist of the following considerations: In sections of earlier stages (11 mm. *Squalus* embryos) scattered cells of large size and deeply staining nuclei appear in the mesenchyma between the myotome and the dorsal aorta. Because of their staining properties and evidence of cellular migration along the nerve anlagen, Kuntz assumes these

cells to be derived from the dorsal ganglia and the ventral nerve roots. In sections of later stages (15 mm. embryos) the anlagen of the sympathetic ganglia may be seen as clusters of similar cells attached to the nerves at the level of the aorta. The deeply staining cells of the mesenchyma are now less numerous. He therefore infers that they have entered into the formation of the ganglia. Proof that the sympathetic anlagen are not the product of the continued migration of cells from the dorsal and ventral roots is not presented. As to the evidence of migration, Kuntz is able to obtain such kinetoscope effects as the following: "numerous cells (in a 10 mm. embryo) push out from the ventro-lateral angles of the neural tube and migrate peripherally along the paths of the motor nerve-roots." That such cells enter into the formation of the sympathetic ganglia he thinks is proved by the following considerations (p. 206):

As my observations on the early stages of embryos of *Acanthias* have shown, numerous cells migrate peripherally both from the neural crest and from the ventral part of the neural tube before the fibers of the ventral nerve-roots can be traced peripherally to the level at which the sympathetic trunks arise. These cells later become aggregated to form the anlagen of the sympathetic trunks. In view of these facts it can not be doubted that in embryos of *Acanthias* many cells which have their origin in the ventral part of the neural tube enter the anlagen of the sympathetic trunks.

While there seems little reason to doubt that cells of somatic motor nerve anlagen in *Squalus* participate in the formation of the neurilemma, convincing evidence that they migrate into the anlagen of the sympathetic is wanting. The assertions of Kuntz in this connection appear quite unconvincing. In the numerous papers by this investigator which have appeared in rapid succession in recent years, covering the development of the sympathetic in all classes of vertebrates except amphibia, observations and conclusions, sense impressions and inferences are indiscriminately advanced as 'observations.' Kuntz, for example, does not bring forward the slightest evidence to prove the assertion ('11, p. 183) that "the cells which migrate peripherally from the neural crest and the neural tube . . . become scattered in the mesenchyma of the region lying between the lateral mus-

cle plates, the notochord and the aorta." The fact that some nuclei found in the mesenchyma have a more deeply staining quality certainly does not prove such an unqualified assertion. That emigrated medullary elements enter into the sympathetic is possible, but Kuntz has presented no facts that make this inference seem more certain.

So far as the evidence from sections of *Squalus* embryos goes, it seems rather to favor the view that the sympathetic anlagen receive their cellular elements largely if not exclusively from the sensory ganglia, as inferred by investigators upon all classes of vertebrates from Schenck and Birdsall to Held and Marcus. In the first place there appears no reason to doubt the ventral movement of the cells which enter into the formation of the dorsal ganglia. The entrance of cells into the sympathetic anlagen would involve only the continuation of this migration on the part of some of these cells. The masses of cells which constitute the anlagen of the sympathetic appear somewhat more closely connected with the sensory bundle of the embryonic nerve than with the motor bundle. Such relations appear in frontal sections at the level of the ganglionic anlagen (fig. 30).

In *Squalus*, however, the separation between sensory and motor bundles in the mixed spinal nerve, on the median side of which the sympathetic anlagen first make their appearance, does not appear as distinct as described by Held for other Selachii. In such sections cells lie between the sensory and motor bundles, and protoplasmic strands connect the motor bundle with the ganglia, so that the possibility that medullary elements enter the ganglia from the motor root does not seem excluded. Connection, on the other hand, by no means proves a migration. Under the circumstances inferences appear most uncertain.

But if one admit an analogy between the development of cranial and spinal somatic motor nerves, there are facts connected with the development of the oculomotor and the trochlear which favor the inference of the predominantly ganglionic origin of the sympathetic anlagen. For the first clusters of cells associated with the anlagen of these nerves are derived from the neural crest. These cell clusters, in their relations and—in the case of

the ciliary—in their adult structure, appear to be sympathetic. Their derivation from the neural crest favors the inference that the sympathetic anlagen of the trunk have a similar origin.

It may be further urged in favor of the ganglionic origin and against the medullary derivation of the sympathetic anlagen in *Squalus* that the medullary migration into somatic motor nerves, like the abducens and the hypoglossus which have no sympathetic anlagen, is as striking as in those nerves associated with sympathetic ganglia. The migrant cells of the hypoglossus and abducens can have no other destination than the neurilemma. On the other hand, sympathetic cells do migrate along the vagus nerve, the ganglionic nerve of the hypoglossus segments. So the evidence seems to favor the opinion that in *Squalus* the sympathetic anlagen are derived from neural crest elements and not from emigrated medullary cells.

11. Summary of the histogenesis of spinal somatic motor nerves

The more important features of the histogenesis of spinal somatic motor nerves in *Squalus* are shown in figures 23 to 30, and may be briefly summarized as follows:

Nerve and muscle are not primarily connected with each other in *Squalus* embryos. Previous to the establishment of protoplasmic connection, the space normally found between somite and neural tube is filled by a vacuolated, non-staining, non-protoplasmic liquid containing a relatively small amount of coagulable material.

Protoplasmic connection of somite and tube is established by an amoeboid protoplasmic extrusion from cells in the ventrolateral wall of the neural tube forming the 'protoplasmic bridges' of Paton ('07) or 'plasmodesms' of Held ('06). The cell processes which form these connections extend gradually along the median surface of the somite between myotome and sclerotome.

Within these processes the neurofibrils soon make their appearance, as shown in Cajal and Bielchowsky-Paton preparations. This evidence demonstrates the neuroblastic nature of the cells

which form the protoplasmic connections between tube and somite and their processes are therefore to be regarded as neuraxons.

The cell-chain hypothesis of neurogenesis receives no support from the evidence presented in sections of *Squalus* embryos. The neurofibrillar structure appears in the nerve anlagen before any cells are present in them.

The growth of a nerve fiber toward its terminal-organ does not involve the use and resorption of primary plasmatic paths but simply the movement and differentiation of the protoplasm of the medullary neuroblast. The most convincing demonstration of the truth of this assertion is afforded by the growth and extension of the processes of Rohon-Beard cells.

The numerous cells which, in somewhat advanced stages of histogenesis, make their appearance in the ventral nerve anlagen are not of mesenchymatous but of medullary origin; exclusively so in the earlier stages of development. That mesenchymatous cells are added to the growing nerve in more advanced stages to form the connective tissue sheaths seems probable.

The cells of the motor nerve anlagen have no genetic relations to the neurofibrils or neuraxons. In other words they are not 'nerve cells' in the von Apathy sense, nor do they unite in chains to form the neuraxons or neurofibrils with their sheaths. Whether or not they participate in the formation of the sympathetic is an open question. The evidence on the whole favors, but does not prove, the conclusion that most of the cells of the sympathetic have their source in the dorsal ganglia. That the cells of the motor nerve anlagen in *Squalus* for the most part form the neurilemma cells can be convincingly demonstrated.

Thus the phenomena of spinal motor nerve histogenesis in *Squalus* support the Kupffer-Bidder-His theory of nerve histogenesis, which has recently been strongly confirmed by the brilliant experiments of Harrison and Lewis.

HISTOGENESIS OF THE EYE MUSCLE NERVES

Does the histogenesis of the eye-muscle nerves resemble that of spinal somatic motor nerves, and are they therefore morphologically comparable? Upon the answer to this question depend in very large degree our views of the morphology of the vertebrate head.

1. General description of the histogenesis of the eye muscle nerves

The three eye-muscle nerves make their appearance at different stages in the development of the embryo; the oculomotor in a 9.5 mm. embryo; the abducens in a 10 mm. embryo; and the trochlearis in a 19 mm. embryo. They arise like spinal somatic motor nerves as plasmatic protrusions from the wall of the brain. Their terminations are amoeboid and remain so until connection with the muscle is established. The primary anlagen possess a peripheral layer of clear protoplasm of somewhat granular structure, within which may be detected an axial deeply-stained fiber (fig. 32). The thickness of the peripheral protoplasmic sheath varies in inverse ratio with the length of the fiber. As the nerve anlage elongates the granular sheath becomes exceedingly thin. The trochlear, from the time of its first appearance, in contrast with the abducens and oculomotor, appears fibrillar rather than protoplasmic, owing to the delicacy of the plasma film surrounding the axial fibers (figs. 49 and 50). This difference may be attributed to the extended course of the fibers of the trochlear within the brain wall.

Evidence of a primary plasmatic path extending from the amoeboid terminations of the eye muscle nerve anlagen to the myotomes with which they are later connected is lacking. The demonstration of their presence or absence is rendered difficult, however, as a result of the presence of a loose mesenchyma in the spaces traversed by the elongating nerve anlagen. The anlagen assume close relations with mesenchyma cells and their processes, but so far as may be observed these connections are secondary and are independent of the process of elongation of the nerve anlagen. In all cases where it is possible to identify

the growing tips of the nerve fibers the amoeboid terminations have no intimate relations with plasmodesms extending toward the muscle anlage, although they indisputably have more or less indirect connections with the processes of adjacent mesenchyma cells. Evidence that these connections are utilized in the extension of the nerve anlage has not been discovered, and the burden of proof rests upon those who assert the absorption of these plasmodesms into the nerve path.

The relations of the nerve fibers with bipolar neuroblasts in the somatic motor column of the brain are as easily demonstrable in *Squalus* embryos as in the adults and are especially clear in Cajal preparations. Even in Vom Rath preparations it is possible to follow with certainty the deeply stained processes of the neuroblasts into the roots of the nerve anlagen. There appears no good reason to doubt the genetic relation of the neuroblasts to the nerve fibers. The fact that the anlagen of the eye-muscle nerves are primarily fibrillar and not cellular, and that the cells are secondarily added to the anlagen and remain for some time distinctly peripheral in relation to the bundle of fibers, and that none of these cells assume the appearance of neuroblastic cells, constitutes evidence quite irreconcilable with the cell-chain hypothesis.

The cells which make their appearance in relation with the anlagen of the eye-muscle nerves are partly of mesenchymatous and partly of medullary derivation, like those associated with spinal somatic motor nerves. Cells are also added to the distal portion of the trochlear and oculomotor anlagen from the superficial and profundus branches of the trigeminal nerve, forming the anlage of the ciliary ganglion in the case of the former, and of a transient (?) ganglion in the case of the latter. Thus the relations to sympathetic ganglia of these two nerves seem to be the same as those of spinal somatic nerve anlagen. The abducens assumes no connections with ganglionated nerves and (therefore?) is associated with no sympathetic ganglion. Whether any of the medullary cells associated with the oculomotor and trochlear anlagen participate in the formation of the sympathetic ganglia is uncertain, as no criterion of distinction between the

various cell elements derived from different sources has been found. The clusters of ganglion (?) cells associated with the trochlear anlage, which the writer interprets as homologous with sympathetic ganglia, are clearly derivatives of the superficialis nerve and not migrant medullary elements. Most of the cells associated with the anlagen of the eye-muscle nerves may be traced into the neurilemma cells of the differentiated nerves. Their gradual penetration into the fiber bundles of the anlagen, beginning with their proximal and distal extremities, may be easily followed in successive stages.

The nidulus of the oculomotor is limited to the somatic motor column of the midbrain vesicle (neuromere II) and presents no evidence of subdivision into two or more niduli. The nidulus of the trochlear lies in the somatic motor column of the first hindbrain neuromere (neuromere III). That of the abducens is post-otic and extends through two post-otic neuromeres (VII and VIII). These relations afford important clues to the primitive segmental relations of the eye muscle nerves, and present an insuperable difficulty to the hypothesis that the eye muscles have migrated into their present relationships from a post-otic source.

2. *Does the histogenesis of the oculomotorius resemble that of a somatic motor spinal nerve?*

a. *Are the muscle and nerve, that is, midbrain and somite 1 of VanWijhe, connected with each other ab initio?* Among the opinions in regard to the histogenesis of the oculomotor nerve no one has maintained upon an observational basis that the nerve is ab initio connected with the first somite of VanWijhe. Not even Sedgwick ('94), who regarded himself as a supporter of the Hensen theory of the primary connection of nerve and muscle, was able to maintain this view by the histogenesis of the oculomotor; for he stated that the oculomotor "is differentiated from the ciliary ganglion to the floor of the midbrain." It appears, therefore, justifiable to infer—in view of the failure of all students of this much investigated nerve to demonstrate a

primary protoplasmic path connecting its nidulus and its end-organ—that in this respect the histogenesis of the oculomotor resembles that of a spinal somatic motor nerve as described above. The evidence about to be presented goes to show that the midbrain vesicle becomes connected with the first head-cavity by means of the movement or continuous extension of the protoplasm of neuroblasts situated in the somatic motor column of the midbrain.

There is, however, a slight difference in the conditions under which connection of tube and somite are effected as compared with those which obtain in the growth of spinal somatic motor nerves. As a result of the late appearance of the oculomotor anlage, relatively to the formation of a loose mesenchyma in the head, the region between midbrain and somite, which had been earlier filled with an unstaining plasmoid substance, becomes filled with a loose mesenchyma of uncertain origin, and protoplasmic connection of tube and somite is effected by growth in the midst of loose mesenchyma. Since it is almost as difficult to find the oculomotor anlage in the midst of this connective tissue, especially in preparations made by the older non-neurological methods, as to find the proverbial needle in a haystack, it is not surprising that the discovery of the true manner of growth of the nerve has been delayed and that difference of opinion has arisen.

Since, however, today all investigators, whose observations are sufficiently extended to cover this point, agree that midbrain and the first head cavity become connected with each other secondarily, and since the opposite view is, for this nerve, an assumption without a single drawing or demonstration to support it, there seems to be no good reason why an affirmative answer should not be given to the question: Are protoplasmic connections between somite 1 and midbrain primary or secondary? Held's ('09) assertion, that the neurofibrillae of the oculomotor always appear as intra-plasmatic fibrils, affords no foundation for inductions regarding the genesis of these protoplasmic paths. Held himself admits that he does not know whether the paths are primary or secondary.

Gast ('09, p. 428) expresses himself as agreeing with Dohrn that primary paths do not exist for the oculomotor. His observations confirm those of Harrison ('07) that the first fiber Anlagen are amoeboid and those of the oculomotor are the processes of medullary neuroblasts.

In disproof of the view that the oculomotor is primarily connected with its muscle, Belogolowy (10, p. 366) mentions the fact that in embryo chicks a transient branch of the oculomotor extends to the external rectus muscle, later innervated by the abducens. Belogolowy is unable to reconcile the presence of this transient and aberrant relationship with the doctrine of the fixed and unchangeable relationship of nerve and muscle.

According to Filatoff ('07) the oculomotor Anlage appears in Emys primarily as a cellular strand extending from the myotome of VanWijhe's first somite toward the base of the midbrain. Filatoff, therefore, infers the primary connection of nerve and muscle. Johnson ('13), however, finds that in reptile embryos the Anlage of the oculomotorius becomes secondarily connected with its myotome. "Filatoff's conclusions are not applicable to Chelydra."

b. What cells contribute to the formation of the protoplasmic connections between midbrain and somite 1? According to Miss Platt ('91), Mitrophanow ('93) and Sedgwick ('94) protoplasmic connection between midbrain and somite 1 is initiated by a proliferation of cells from the mesocephalic ganglion toward the brain. According to Sedgwick this migration is accompanied by a differentiation of a pre-existing syncytial strand. Ziegler ('08) also infers the centripetal growth of the oculomotor, on the basis of the evidence that in a 25 mm. embryo of *Chlamydoselachus* the oculomotor "has not extended its growth as far as the floor of the midbrain." Since the oculomotor nerve makes its first appearance in a 9-10 mm. selachian embryo and connection between brain and somite is already established in a 10 mm. embryo, Gast is undoubtedly correct in inferring that the oculomotor Anlage in Ziegler's specimen was ruptured through shrinkage and that his inference is consequently fallacious. Miss Platt, Mitrophanow, and Sedgwick were also dealing with ad-

vanced stages in the histogenesis of the oculomotor and they were consequently led astray in their inductions.

Filatoff's ('07) assertion that the oculomotor is differentiated from the myotome toward the brain in *Emys* has not been confirmed by the more recent investigation of reptile embryos by Johnson ('13). According to a second view—upheld by His ('88), Chiarugi ('97), Neal ('98), Held ('09), Gast ('09), Carpenter ('06), Belogolowy ('10)—the first protoplasmic connection between midbrain and somite 1 is effected precisely as has been found for spinal somatic motor nerves, by the continuous extension of the processes of medullary neuroblasts. Sooner or later, according to Dohrn ('88, '91), C. L. Herrick ('93), Goronowitsch ('93), Chiarugi ('97), Carpenter ('06) and Gast ('09), this protoplasmic movement is accompanied by a migration of medullary cells from the base of the midbrain. Neither Neal nor Held were able to find convincing evidence of nuclear migration into the oculomotor roots, but they did not deny the fact of migration.

The evidence presented in sections of *Squalus* embryos and represented in the figures of plate 6 of this paper, strongly confirms the opinion of those who have maintained the genetic relations of medullary neuroblasts in the ventral wall of the midbrain to the first protoplasmic connections between midbrain and the first somite. The anlage of the oculomotor makes its first appearance in *Squalus* embryos of 9 to 9.5 mm. (fig. 58), as a short, deeply-staining fiber, formed by the union of the processes of medullary neuroblasts. The evidence that this is the anlage of the oculomotor consists in the fact that the point of attachment to the brain, the direction of the long axis of the process, and the histological appearance correspond with those of the oculomotor in a 10 mm. embryo when the connection of the nerve with the myotome is already established. The nerve anlage already consists of two roots, each containing a deeply staining fiber surrounded by an envelope of granular protoplasm. At the point of union of the two roots a mesenchymatous cell appears in close proximity to the anlage. Evidence of the migration of medullary cells is wanting at this stage. The deeply staining processes of neuroblasts at the base of the midbrain

extend toward the nerve anlage and may in some cases be traced to a point outside the external limiting membrane of the brain wall.

In later stages (fig. 59) a larger number of processes of medullary cells may be traced toward the nerve anlage and a larger number of deeply stained fibers may be seen within the roots of the anlage. In this, as in all subsequent stages, mesenchymatous cells may be seen in close proximity to the nerve fibers. Some earlier stages, such as are represented in my ('98) figures *G* and *H*, pages 222-223, show no cells whatever in relation to the oculomotor anlage; the majority of sections of these early stages show cells more or less closely associated with the nerve anlage. That these cells are mesenchymatous seems indicated by the fact that there is in these stages no evidence of medullary migration and that the possibility of a derivation from the mesocephalic ganglion is excluded, since the nerve has as yet no connection with that ganglion. No evidence of a genetic relation of these cells to the nerve is discoverable. In all respects they resemble the branched granular cells of the surrounding mesenchyma. Comparison of these with later stages in the development of the oculomotor (figs. 61, 64, 70) favors the inference that the fibers of the oculomotor are of neuroblastic origin and that they have their nidulus of origin in the base of the midbrain.

The oculomotor therefore attains connection with its terminal organ secondarily, in precisely the same manner as a spinal somatic motor nerve.

c. Have these protoplasmic connections genetic relations to the neurofibrils of the oculomotor? Most embryological investigators of the oculomotorius have paid no attention to the genesis of the neurofibrils. This is true of the latest research upon the histogenesis of the oculomotor—that of Gast ('09)—as of the earlier ones. In fact, no investigation of the histogenesis of the nerve by the use of a specifically neurofibrillar stain has been made. The vom Rath method, as applied by Neal ('98) and Carpenter ('06), comes the nearest to a truly neurofibrillar stain of any which have been used upon the nerve. Of the histological

results of this method Carpenter ('06, p. 195) says that "a heavy black precipitate along the neuraxons differentiates these clearly against the less darkly colored stroma in which they appear to be imbedded." The appearances presented in sections prepared by this method resemble those in Cajal and Bielchowsky-Paton preparations in demonstrating, in the very earliest stages of histogenesis, deeply staining fibers within the nerve anlagen. And, while the results are not as specific and differential in vom Rath preparations, the staining is more complete than in either of the other two, so that the connection of the fibers with medullary neuroblasts is more clearly demonstrated. Now, since in vom Rath preparations there is no stage in the development of the oculomotor when such deeply stained fibers are absent from the nerve, and since the connection of these fibers with processes of medullary neuroblasts can be readily traced in many sections, and since there is not the slightest evidence that the cells associated with the fibrillar bundle of the nerve have any genetic relations with the fibers, it appears legitimate to conclude that the neurofibrils are differentiated within the processes of the midbrain neuroblasts, which, by their peripheral extension, form the protoplasmic connection between brain and somite.

Vignal ('83), Bardeen ('03), Carpenter ('06) and Paton ('09) have called attention to the coarseness of the primary fibers of embryonic nerves as compared with those seen in later stages of histogenesis and have inferred a process of splitting of the primitive fibers into true fibrils, an inference that seems to have been convincingly demonstrated by Miss Dunn ('02). The present paper makes no contribution to the discussion of this important histogenetic problem.

In the neuroblastic origin of its fibrils the oculomotor resembles histogenetically a spinal somatic motor nerve.

d. Are the neuraxons of the oculomotor multicellular in origin or are they the processes of medullary neuroblasts? The evidence that the neuraxons of the oculomotor are formed as the result of protoplasmic movement of neuroblasts in the somatic motor column of the midbrain consists, first, of the fact that the growth of the nerve is centrifugal as stated for various vertebrates by

His ('88), Dohrn ('91), Chiarugi ('97), Neal ('98), Carpenter ('06), Gast ('09), Belogolowy ('10). Appearing at first as protoplasmic outgrowths from the base of the midbrain, the anlage of the nerve elongates in later stages in the direction of their terminal organ—the first somite—with which it quickly becomes connected.

In the second place, the nerve, in all stages of differentiation in embryonic material, manifests a fibrillation in the form of coarse, deeply-staining fibrils which appear connected with the deeply staining processes of midbrain neuroblasts. On the basis of analogy with phenomena seen in the histogenesis of Rohon-Beard cells and in preparations of the living nerve fiber in cover-glass preparations, such evidence points toward the neuroblastic origin of the neurofibrils. In no sections prepared by any of the neurological methods used, is evidence to be found of the fusion of centripetally growing fibers with processes of medullary cells in the manner suggested by Paton ('07).

Finally, the inference is further strengthened by the absence of any positive evidence in favor of the cell-chain hypothesis. It is true that cells appear along the nerve anlage from very early stages in histogenesis, but none of these cells show the familiar form and staining properties of neuroblasts. On the basis of this evidence the conclusion appears warranted that each of the neuraxons of the oculomotor is the product of one or more medullary neuroblasts and not the product of the fusion of a chain of cells. The analogy with the histogenesis of spinal somatic motor nerves is clear.

Burckhardt ('92) describes medial and lateral niduli of the oculomotor in Protopterus, confirming Ahlborn's ('84) description of the central relations of the nerve in Petromyzon. Ahlborn's assertions, however, are not confirmed by Johnston ('05) who finds the nidulus of the oculomotor to be that of a somatic motor nerve. All investigators agree that the nidulus of the oculomotor in selachians belongs in the somatic motor column.

e. By what means does increase in the length of the neuraxon take place? The oculomotor develops under conditions which differ somewhat from those which have been described for spinal

somatic motor nerves, since the region through which it grows is filled with a loose mesenchyma before the growing tip of the nerve emerges from the base of the midbrain. Under such conditions it is difficult to ascertain that processes of mesenchymatous cells make no contribution to the growth of the nerve. The burden of proof, however, falls upon those who claim that the nerve grows by the differentiation, in situ, of plasmodesmatous paths. Such proof has not been given.

As has been suggested, the presence of a granular envelope around the fibers of the oculomotor is not to be taken as evidence of primary protoplasmic paths. Were a primary protoplasmic path of the diameter of the anlage of the oculomotor present, it could easily be found and traced in the sections. The fact is, however, that in all the stages in which the nerve anlage may be traced to its peripheral termination in the stages before it reaches the myotome, the distal end shows an amoeboid character, precisely resembling the distal extremity of nerves growing in cover-glass preparations as drawn and described by Harrison, Lewis and Burrows. No direct relation with the processes of adjacent cells nor with a direct plasmodesmatous path extending toward the myotome can be detected. All of the phenomena advanced by Held ('09) in his attempt to reconcile the Hensen with the Kupffer-Bidder-Harrison theory and to establish the assumption of primary plasmodesmatous paths are fully in harmony with the process theory of the free outgrowth of nerve fibers, while the Hensen-Held theory of primary plasmodesmatous paths finds as little support in the phenomena presented in the histogenesis of the oculomotor as in the growth of spinal somatic motor nerves.

f. What is the source of origin of the cells of the oculomotor anlage? Dohrn ('91) was the first to affirm the medullary origin of the cells of the oculomotorius anlage and his conclusions have been repeatedly confirmed by later investigators, among them Goronowitsch ('93), C. L. Herrick ('93), Chiarugi ('97), Carpenter ('06) and Gast ('09). Neal ('98) and Held ('09) were unable to find convincing evidence of, but they did not deny, medullary migration into the oculomotor. Dohrn's argument

for his conclusion was that cells of the midbrain migrate peripherally and then send out processes, which unite in a network, just outside the base of the midbrain, to form the stem of the nerve. Immediately at the beginning of the plasma outflow, cells are seen half in and half out of the wall of the tube, and later, before the oculomotorius shows any connection with the mesocephalic ganglion, large deeply-staining nuclei, like those which appear in the process of emergence from the neural tube, make their appearance in the network of the roots of the nerve. In later stages the nuclei, in increasing numbers, appear to emerge from the midbrain into the nerve anlage. The phenomena of medullary migration into spinal somatic motor nerves add support to Dohrn's contention.

Evidence similar to that presented by Dohrn ('91) may be found in sections of *Squalus* embryos in stages from 10 to 15 mm., and confirms the accuracy of his description. The cells closely associated with the oculomotor in the earliest stages of development, appear to be mesenchymatous rather than medullary in origin, but it seems not unlikely that these, like similar mesenchymatous cells associated with spinal somatic motor nerves in the very earliest stages of their appearance, are not permanently associated with the nerve. Their relations may be purely topographic and transient. But there is no reason to doubt that some of the cells of the oculomotor anlage have, like those of spinal nerves, a medullary origin.

According to Hoffmann ('85), Ewart ('90), Miss Platt ('91), Mitrophanow ('93), Sedgwick ('94), Chiarugi ('94, '97), Neal ('98), and Gast ('09) cells migrate into close relations with the oculomotorius anlage from the mesocephalic ganglion. I was not sure that these cells came into permanent relations with the nerve, while Gast inferred the derivation of some of the sheath cells from them. Harrison's demonstration, through experiments upon amphibian embryos, of the derivation of neurilemma cells from the neural crest might appear to support Gast's inference, although nerve histogenesis in amphibia is not necessarily analogous in all features with that in selachians. Gast's evidence of the forward and back—centripetal and centrifugal—

migration of cells derived from the mesocephalic ganglion along the oculomotor nerve is not very convincing.

Carpenter ('06) finds evidence of free cell migration from the mesocephalic ganglion into the loose mesenchyma and towards the oculomotor anlage in the chick. Guthke ('06, p. 43) on the other hand, asserts that the ganglion of the profundus nerve (which he erroneously calls the ciliary ganglion) has absolutely nothing to do with the oculomotorius which has no connections with the profundus ganglion in *Torpedo*.

Belogolowy ('10, p. 275) infers, in agreement with Carpenter ('06), that the cells, which in the chick migrate from the brain, probably participate in the formation of the motor neuroblasts of the ciliary ganglion; and he is of the opinion that neural crest cells play an important part in the formation of the accompanying cells.

Several have inferred the participation of the mesenchyma in the addition of cells to the oculomotor anlage. Indeed that has always been the orthodox assumption of the derivation of the neurilemma of the nerve. Held ('09) was in doubt as to the actual origin of the neurilemma nuclei of the oculomotor, but regards them as belonging to mesenchymatous cells of unknown origin. There can be no doubt of the close association of mesenchymatous cells in all early stages of the histogenesis of the nerve, and it seems not unlikely that they contribute to the formation of the sheath cells of the nerve. In fact Gast ('09) confirms the assertion of His, Jr., and Romberg ('90) that neurocytes migrate freely through the mesenchyma toward the nerve anlage.

Thus, in the derivation of its cellular elements, the oculomotor of *Squalus* resembles a spinal somatic motor nerve. Part at least of its component cells are of medullary origin; some of them may be mesenchymatous; and, finally, the nerve has close association with cells derived from a cerebro-spinal ganglion, the mesocephalic or profundus ganglion.

g. What is the fate of these cells? The fate of these cells appears even less certain than their derivation. The opinions have been expressed that at least some of the cells—whether derived

from the midbrain or from the mesocephalic ganglion—are neuroblast cells which form the fibers of the oculomotor; that the cells of the anlage form the neurilemma; that they contribute to the formation of the ciliary ganglion; and that those which migrate from the midbrain, if not also those that are derived from the mesocephalic ganglion, have both fates.

Taking up these views in turn, we find that Dohrn ('91) is the only one who has attempted to support the cell-chain hypothesis on the basis of evidence derived from the study of the development of the oculomotor nerve, but as he has wholly repudiated ('07) the considerations which earlier led him to advocate the cell-chain hypothesis, it seems inadvisable to discuss his arguments in favor of the former theory.

Gast ('09) asserts that some of the migrant medullary cells become located in the 'root ganglion' of the oculomotor, where they become differentiated as neuroblasts, but the evidence upon which he bases his statement is far from convincing. It would seem necessary, even were one prejudiced in favor of such a view, to be able to demonstrate in its favor more convincing evidence than the presence of spindle-shaped or even multipolar cells in the cell mass which Gast calls a ganglion, especially in preparations made according to the usual embryological methods which do not differentiate the distinctive histological elements of ganglion cells. In a section made by usual embryological methods, how can Gast tell whether a spindle-shaped cell is a nerve cell or a sheath cell? Even multipolarity is no decisive criterion of a ganglion cell. Therefore, while there may be no theoretical reasons why the migrant medullary elements may not be expected to form ganglion cells, it would seem that some more critical evidence than form is to be taken as proof that such is their fate. None of the methods used in the present investigation upon *Squalus* embryos demonstrate the presence of neuroblasts or ganglion cells among the cells in the roots of the oculomotor. Whatever multipolar cells appear in close proximity to the nerve appear to be similar to adjacent cells of the mesenchyma.

On the other hand, Carpenter ('06) gives convincing reasons for thinking that some of the migrant medullary cells from the midbrain participate (as a group of smaller cells easily distinguishable from the larger ganglion cells derived from the mesocephalic ganglion of the chick) in the formation of the ciliary ganglion. If this conclusion be confirmed—and this has been done by Belogolowy ('10)—it appears that at least some of the medullary cells in the oculomotorius anlage are neuroblastic.

Gast ('09) is not able to confirm Carpenter's conclusion that some of the migrant medullary elements enter into the formation of the neurilemma, but is of the opinion that the neurilemma is formed by migrant cells from the mesocephalic ganglion which travel centrad along the nerve anlage as far as the roots by which it arises from the brain and thence they migrate back again into the nerve to become the neurilemma cells. As has been stated above, Gast's evidence of this forward and back migration does not appear very convincing. Analogy with the derivation of the neurilemma of motor roots in higher vertebrates from the sensory ganglia with which they are connected does not prove such a derivation in selachians.

That the migrant medullary elements participate in the formation of the neurilemma seems to Carpenter evinced by the following considerations: "Many of these nuclei, once out on the nerve, become elongated as they move away from the neural tube," and "maintain throughout development their close proximity to the nerve fibrils." Again, the absence of evidence of the intrusion of cells from the mesenchyma is further indication of the ectodermal origin of the neurilemma. Furthermore, analogy with the differentiation of the neuroglia elements within the central nervous system points in the same direction. Finally, Carpenter was able to trace these cells through successive stages until they were, in structure and relations, demonstrably the neurilemma elements of the differentiated nerve. I reached similar conclusions ('03) for the derivation of the neurilemma of spinal somatic motor cells. The evidence appears to warrant the inference. Even Kölliker, who for years main-

tained that the neurilemma was of mesenchymatous origin, finally became convinced ('05) of its ectodermal origin.

By tracing the histogenesis of the nerve through closely connected stages until adult conditions are established in *Squalus* it is possible to demonstrate that a large number, if not all, of the cells present in the anlage of the oculomotor become differentiated as neurilemma cells. Some of these stages are shown in the figures of plate 8 of this paper.

The cells, which are at first peripheral to the bundle of fibers (fig. 65), soon begin to penetrate among them (fig. 68) as stated by Vignal ('83), Gurwitsch ('00), Bardeen ('03), Carpenter ('06), and Gast ('09). Gast is probably correct in asserting the centrifugal migration of the group of cells clustered about the roots of the nerve anlage (figs. 64, 70) and their penetration into the bundle of fibers. In fact the penetration of the fiber bundle appears to occur largely in this manner, as stated by Gast.

Altogether the evidence goes to show that the neurilemma of the oculomotor has a similar derivation and differentiation to that of somatic motor spinal nerves.

h. What is the histogenesis of the neurilemma? Gast ('09, p. 428) summarizes the histogenesis of the neurilemma of the oculomotor as follows: In its proximal portion the oculomotor passes through four different developmental stages: (1) the naked processes of the central neuroblasts extend to the mesocephalic ganglion; (2) from the mesocephalic ganglion neurocytes (Kupffer '90) wander centrad along the fibrillar bundle; these lie upon and between the fibers and form about them, especially among the roots, a loose plasmatic network; (3) the neurocytes arrange themselves wholly on the surface of the nerve anlage, which at this stage consists of a central fibrillar bundle with a cellular sheath; (4) the neurocytes migrate back into the central fiber bundle and are transformed into sheath cells.

The results of a former ('98) and of the present study are in essential agreement with this description of Gast. The evidence derived from a study of *Squalus* embryos, however, seems hardly to justify the inference of a forward and backward migration of neurocytes along the nerve anlage. Moreover, it

appears to favor the conclusion that the cell clusters at the roots of the nerve anlage are of mesenchymatous and medullary derivation. The inference that cells derived from the mesocephalic ganglion migrate centrad as far as the roots of the nerve anlage does not appear to the writer to be well established. In agreement with Gast, however, I find that penetration of the fibrillar bundle begins at proximal and distal extremities of the nerve anlage, where it divides into roots and peripheral branches, and the penetration proceeds in opposite directions towards the middle stretch of the nerve. In the phenomena presented in the histogenesis of the neurilemma there appears to be no essential difference between that of the oculomotor and a spinal somatic motor nerve. In both cases the derivation of the neurilemma cells appears to be the same.

i. To what an extent do the emigrated medullary elements go to form the sympathetic? That a ciliary ganglion is associated with the oculomotor nerve in selachians seems proved by the consensus of opinion of investigators on the basis of anatomical, histological, and embryological evidence. Anatomical evidence of its presence has been presented by Schwalbe ('79), Jegorow ('86-87), and Allis ('02); on the basis of histological structure by Haller ('98); and on embryological grounds by Van Wijhe ('82), Beard ('85), Ewart ('90), Dohrn ('91), Miss Platt ('91), Hoffman ('99), Gast ('09). Physiological evidence of a functional ciliary ganglion in selachians is wanting.

Furthermore, that the ciliary ganglion belongs morphologically and physiologically with the sympathetic system has been held by many investigators since its discovery in man by Schacher (1701) and the suggestion of its sympathetic character by Arnold ('31). Later, doubt as to its sympathetic nature was raised by Schwalbe ('79), who became an exponent of the view that it is a cerebro-spinal ganglion and who initiated the long controversy regarding its nature which has not yet been ended. Carpenter ('06) has so admirably reviewed the literature dealing with this problem that it appears unnecessary to enter into an extended discussion of the various arguments for and against its cerebro-spinal character. That the ciliary ganglion is, in part

at least, sympathetic in all forms from fishes to man seems, on the basis of anatomical, histological, embryological and physiological evidence, indisputable. But the presence of bipolar ganglion cells, like those of cerebro-spinal ganglia, in the ciliary ganglion of many vertebrates has led to the general acceptance of the view of its double nature, a view first advanced by Krause ('82).

But the presence of bipolar ganglion cells within the ciliary ganglion by no means demonstrates its morphological comparability with cerebro-spinal ganglia, since ontogenetic evidence shows that the entire ganglion—whether derived from the mid-brain or the mesocephalic ganglion—has not a genetic but merely a secondary relation to the anlage of the oculomotor, a fact emphasized by Gast ('09).

While Dohrn ('91) claimed an exclusively medullary derivation of the ciliary ganglion in Selachii, Gast ('09), working upon the same material—in fact the same sections—concludes that they are exclusively derived from the mesocephalic ganglion. These diametrically opposite conclusions indicate how obscure the phenomena of migration are in sections of embryonic material. Carpenter ('06), working upon chick embryos, infers a double derivation of the cells of the ciliary ganglion.

Von Kupffer ('95), Johnston ('05), and Belogolowy ('10b) have attempted to associate the ciliary ganglion genetically with the 'thalamic nerve.' Johnston says (p. 244) that "since the profundus ganglion is distinct from the ciliary and is formed from a different part of the neural crest, it seems altogether probable that the ciliary ganglion permanently represents the N. thalamicus." Belogolowy's discovery of an anastomosis between the oculomotor nerve and the transient ganglion of the 'thalamic nerve' likewise seems to him to prove the homodynamy of the latter with the ciliary ganglion of vertebrates. In drawing this conclusion Belogolowy fails to take into account the fact that the ciliary ganglion of vertebrates is a derivative of the mesocephalic or profundus ganglion. It is not at all clear that the complicated anastomoses between the branches of the eye-muscle nerves in reptile embryos and their peculiar relations with branches of the trigeminal nerve

throw any light upon the primitive relations of these nerves in vertebrates. Johnston's inference is fallacious since his premise is incorrect. The cells of the ciliary ganglion of *Squalus* are proliferated from the medial surface of the profundus ganglion. It may be granted, however, that the demonstration that the 'thalamic nerve' represents the primitive root of the profundus nerve, would associate this so-called nerve with the ciliary ganglion as its sympathetic visceral sensory component.

Sections of *Squalus* embryos give convincing evidence of cellular migration, both from the midbrain and from the mesocephalic ganglion, into or towards the oculomotor anlage, but it is impossible to feel convinced that the migration of the medullary cells extends as far as the ciliary ganglion. In fact the evidence of the participation of medullary cells in the formation of the sympathetic is as doubtful in the case of the oculomotor as in the case of spinal somatic motor nerves. The possibility of such a derivation can not be denied, but positive demonstration on the basis of evidence obtained from serial sections has not yet been given. In this respect, that is, in the derivation of its proper sympathetic ganglion, the oculomotor resembles a spinal somatic motor nerve.

j. Summary of the histogenesis of the oculomotor and of the ciliary ganglion. In all essential respects, the oculomotor nerve resembles in its histogenesis a spinal somatic motor nerve, i.e., in its appearance as a product of the protoplasmic movement of medullary neuroblasts situated in the somatic motor column of the neural tube; in its secondary connection with a mesodermic somite, Van Wijhe's first; in the growth and extension of its fibers by the continuous movement of the protoplasm of medullary neuroblasts; in the differentiation of its neurofibrils within the protoplasm of these processes; in the migration of medullary cells into the nerve anlage to form—at least in part—the neurilemma; in the probability of the participation of mesenchymatous cells in the formation of the neurilemma; in its union with cells of a cerebro-spinal ganglion; and finally in its association with a sympathetic ganglion, the ciliary, derived mainly if not exclusively, from a cerebro-spinal ganglion as a result of cellular migration.

3. *Does the histogenesis of the trochlearis resemble that of a somatic motor spinal nerve?*

a. *Are protoplasmic connections between myotome 2 and hind-brain primary or secondary?* The distance between the point of emergence of the trochlearis fibers and the myotome which this nerve innervates is, in *Squalus* embryos, the greatest traversed by any motor nerve. This fact lends especial interest to the development of this nerve in relation to the problem of the existence of primary paths or connections between nerve center and the end-organ. This nerve would seem to afford a critical test of the truth or falsity of the Hensen-Held hypothesis of nerve histogenesis. If any nerve in the vertebrate body needs a primary path so that it may not go astray, that nerve is the trochlear. In view of this fact it is somewhat surprising that Paton ('07), who supports this hypothesis, concedes that the trochlearis (and also the oculomotor) acquires its connection with the superior oblique muscle without the participation of plasmodesmatous bridges (Paton '07, p. 556).

Some years ago ('98, p. 237) I expressed the opinion that "the possibility of a primary connection between muscle and nerve appears excluded in the case of the superior oblique muscle and the trochlear nerve" and Dohrn, later ('07, p. 410), said quite as emphatically that "Jede Möglichkeit eines uranfänglichkeit Zusammenschangs zwischen Muskelzelle und Nervenzelle ist dabei ausgeschlossen." Belogolowy ('10a, p. 380) also agrees in affirming the absolute impossibility of a primary connection between nerve and muscle in the case of the trochlearis. While Sewertzoff ('98) and Fürbringer ('02) are unable to agree with this assertion of mine, they advance no arguments in its rebuttal. It is a significant fact that none of the supporters of the Hensen hypothesis have been able to demonstrate a primary protoplasmic path connecting the isthmus or the hindbrain with the myotome of Van Wijhe's second somite.

On the contrary, and in support of the view that the trochlear nerve develops in accordance with the process theory of neurogenesis and that the connection of hindbrain and superior oblique

muscle is secondary, Dohrn ('07, p. 416) advances the following argument:

The development of the trochlearis forms a crux for the Hensen-Gegenbaur-Furbringer hypothesis of the primary connection of the nerve and its terminal organ. So long as we have to do with the connections of spinal somatic nerves with somites already formed, one may believe in the existence of plasma bridges and be willing to recognize in them at least the paths that guide the growing nerve. The separation in this case between tube and somite is the least possible and few cells lie between them. But if one extend this view to the eye muscles he at once meets insuperable difficulties. All these have a considerable distance to traverse in reaching their end-organ, and the abducens, oculomotorious and trochlearis solve the problem each in its own way. I have already pointed out ('01) that this theory must be tested, not on the spinal somatic nerves, but on the eye muscle and splanchnic motor nerves, if it is to be regarded as tenable, and I must now put the question to the defenders of the Hensen theory how they conceive of the connection of the trochlearis through plasma bridges with its terminal organ in the other antimer.

In embryos of 12 to 18 mm. the actual trochlearis fibers remain either completely within the neuroblasts from which they are to arise or they have at most made their way through the medullary wall to the chiasma, but are not yet protruded from the wall. The superior oblique muscle, on the other hand, has developed from the mandibular somite as the most dorsal of the eye muscles and has already begun to differentiate muscle fibers. What and how many plasmodesmatous connections must be assumed in order to establish the most complicated of all paths traversed by any motor nerve? How is one to demonstrate that first plasmodesms of the neural tube, then others of the mesenchyma, then—in case 'Kettenfasern' are present before the trochlearis fibers extend through their region of distal distribution—plasmodesms of the 'Kettenfasern,' and finally those of the muscle fibers of the superior oblique muscle are likewise prepared to furnish neurofibrils through further differentiation? Would this be in the least more easily conceivable than the outgrowth of the nerve from its neuroblasts to its terminal organ? It is true that the outgrowth points to problematical forces, but shall we be without a riddle if we put another in its stead—one that presents a number of unfounded assumptions?

Held ('06), who admits the insufficiency of the hypothesis of plasmodesmatous paths for the trochlearis nerve, thinks that he may assume as the direct path-determining impulse for the outgrowing neurofibrils the principle of the axial position of the neuroblast and that of the shortest distance. These obviously fail

in their application to the growth of the trochlearis. The chief axes of the neuroblasts which form the trochlearis fibers do not point toward the end-organ, neither do they follow the shortest possible course, but practically the most indirect route possible.

In *Squalus* embryos, previous to the first appearance of the trochlear anlage, the region of its future path is filled with a loose mesenchyma derived at least in part from the neural crest. It is a most important fact, demonstrated by the observations of Kastschenko ('88), Neal ('98) and Dohrn ('07), that, from the time of its first appearance to the time it attains connections with the superior oblique muscle, the trochlear anlage in some selachians shows no close associations with mesenchymatous cells, but extends as a loose bundle of fibers through vacuolar spaces (fig. 49). The trochlearis development, says Dohrn ('07, p. 413) "affords a proof that an elongated motor nerve can grow through a considerable extent of tissue without becoming attached to a single cell of the surrounding mesoderm." Evidence of a granular protoplasmic envelope surrounding the fibers, however, is not lacking, but there is no evidence that surrounding mesenchymatous cells contribute to the production of this. The descriptions of Froriep ('91) and Miss Platt ('91) relate to more advanced stages in the histogenesis of the trochlear than those described, so that their assertions of the participation of cells in the formation of the trochlearis anlage have no relevancy in connection with the problem of the formation of the primary connection between tube and myotome.

b. What cells participate in the formation of these connections? The fact that in the adults of all classes of vertebrates the fibers of the trochlearis may be traced in suitable preparations to a nidulus in the somatic motor column, posterior to the midbrain leads to the supposition that these cells are genetically related to the fibers. The further evidence that during the period of extension of the fibers toward their terminations in the superior oblique muscle, no cells which may be regarded as neuroblasts or 'nerve-cells' in the Apathy sense, may be seen in connection with the peripheral stretch of nerve anlage, points in the same direction. The connections with cell-clusters or fragments of

the neural crest as described by Hoffman ('89), Oppel ('90), Dohrn ('91), Miss Platt ('91), and Froriep ('91) are secondary and do not appear in the primary fibrillar stretch of the nerve anlage. Filatoff ('07, p. 358) agrees with the writer ('98) that the so-called primary trochlearis (Miss Platt '91) has no genetic relation with the definitive trochlearis, which develops wholly independently of the neural crest and of the trigeminal nerve, although it requires secondary connections with the latter. According to Belogolowy ('10a) no ganglion is associated with the trochlear in bird embryos, while in the turtle the so-called ganglion of the trochlearis has no connection with the trochlearis but is connected with the superficial branch of the trigeminal (p. 424). The same is true also of the relations of the ganglion in the sturgeon. The superficialis nerve is, therefore, to be regarded as the dorsal nerve of the trochlearis metamere.

Schwalbe ('79, '81), Johnson ('05), and Carpenter ('06) have asserted that the neuroblasts which form the nidulus of the trochlear are situated in the somatic motor column of the midbrain and not in the hindbrain. Such divergent opinion may possibly be explained by the fact that in the ventral portion of the brain in this region no constriction is present such as that which divides midbrain and hindbrain regions dorsally, so that it is difficult to distinguish the limit of these two brain divisions. The majority of investigators find the nidulus of the trochlear, either in the hindbrain or in the isthmus. The assertion of Martin ('90), that the nidulus of the trochlear is primarily in the visceral (lateral) column, and that it secondarily migrates medially, has not been confirmed and, like many of the other amazing assertions of this writer, should be placed in quarantine as suggested by Dohrn ('07). In *Squalus* the nidulus of the trochlear is clearly ventral and, while the fibrillar bundle of the nerve emerges dorsally from the brain wall in the region of constriction between midbrain and cerebellum, the central fiber-tract with which it is connected may be traced posteriad and ventrad to the nidulus in the somatic motor column in the anterior portion of the cerebellar neuromere (neuromere III). On the other hand, Van Walkenburg ('10) finds two trochlear niduli in human embryos.

Belogolowy ('10a, p. 367-8) describes the histogenesis of the trochlear, which may be summarized as follows: The first developmental stages of the nerve take place in the brain. Here the fibers of the brain are gradually differentiated as processes of cells in the somatic motor column of the hindbrain. The fibers grow in the direction of the dorsal side of the brain and toward the constriction between second and third brain vesicle, that is toward the basis of the cerebellum anlage. The fiber bundles may be easily traced through the brain wall and usually number three.

At the time when these bundles have reached a point about two-thirds the height of the neural tube a nidulus of large ganglion cells makes its appearance at the place of the future point of emergence of the fibers. The evidence of their ganglionic character consists in their larger size and in the outgrowth of fibers from them. (Belogolowy's figures, however, do not bear out the latter assertion.) This dorsal nidulus is gradually enclosed by the spongiosa and penetrated by the coarse fibers of the trochlear until only isolated cells remain scattered in the region of the chiasma. The bundle of fibers of the nerve anlage is at first free of accompanying cells, which appear first at about the level of the middle of the brain wall.

Belogolowy then goes on to present evidence that the accompanying cells can have nothing to do with the genesis of the fibers of the trochlear. Notwithstanding the divergence in his account in regard to the existence of a dorsal nidulus, Belogolowy does not hesitate to compare the trochlear with typical somatic motor nerves. He agrees with the conclusion that connection between hindbrain and superior oblique muscle is secondary, an inference which—in the light of the evidence now in our possession—there seems no good reason to doubt.

c. Have these protoplasmic connections a genetic relation to the neurofibrils? As first stated by Kastschenko ('88, p. 465) the trochlearis, from the time of its first appearance, is a fibrillar structure. In preparations of *Squalus* embryos by the Vom-Rath method these fibers are deeply-staining and highly refractive threads, contrasting strongly with the protoplasm of the ad-

jacent mesenchymatous cells between which they lie. In cross sections of the nerve anlage (fig. 52, p. 181) the fibers appear as black dots or granules scattered in a granular and vacuolated protoplasmic envelope. Their appearance is essentially the same in VomRath preparations as in preparations by the Cajal or the Bielchowsky-Paton methods. The identity of this bundle of fibrils with the trochlearis in advanced stages of differentiation is established, not only by its fibrillar structure, but by its relations with tube and myotome at this and all subsequent stages of development. As this anlage has been traced in unbroken continuity until the differentiation corresponds essentially to that of the adult nerve there is no good reason to doubt the identity of the fibers of the anlage with those of the adult and that the neurofibrillae make their appearance within them. It is true that the splitting of the fibers of the nerve anlage into the neurofibrils of the fully differentiated nerve is a matter of inference rather than one of direct observation, yet students of nerve histogenesis have not hesitated to make this inference which has been drawn by Bardeen ('03), Carpenter ('06) and Paton ('07), on the ground that, while the primitive fibrils of the nerve anlage are relatively coarse structures, the neurofibrillae of later stages are much finer and more numerous. There is no reason for inferring that the neurofibrils have any other origin than the coarser fibrils of the nerve anlage, which make their appearance in the nerve from its first inception in the stages when the nerve anlage is wholly free from cells. Furthermore, at no time in the histogenesis of the nerve is there any indication of a genetic relation of the cells, which later make their appearance in the nerve, to the fibers. No neuroblasts have been discovered within the nerve anlage. So that there seems to be no reason why the histogenesis of the neurofibrils of the trochlearis may not be regarded as in every respect similar to that of the neurofibrils of spinal somatic motor nerves. In other words they are to be regarded as the product of differentiation within the neuraxon processes of medullary neuroblasts situated in the somatic motor column of the neural tube.

Miss Platt ('91a, p. 96) inferred the neuroblastic origin of the fibers of the proximal portion of the trochlearis on the basis of

the evidence that "the fibrous root of the nerve cannot be composed of prolongations from the distal cells (derived from the R. ophth. sup. V), because the fibrous or proximal part of the trochlearis arises before the distal or cellular part; further, the fibrous root is the thickest as it comes from the brain, becoming gradually attenuated as it proceeds into the mesoderm." Belogolowy ('10a) likewise finds the trochlear of the chick fibrillar from the time of its first appearance. There appears no good reason to doubt that the trochlear anlage is not only 'fibrillar' but 'neurofibrillar' in the Apathy sense of the word.

d. Is a neuraxon of the trochlear anlage multicellular in origin or the process of a single medullary cell? While it is practically impossible for any particular neuraxon of any nerve to give an answer to this question upon the basis of direct observation (and for the trochlear nerve the difficulty is increased by the extended intra-medullary course of the fibers of the nerve) it is easy to disprove the assertion that the trochlearis anlage in its peripheral stretch is the product of the fusion of a chain of 'nerve' cells, since in *Squalus*, as in *Pristiurus* (Dohrn '07), the nerve anlage may be traced as a bundle of fibers, devoid of cells or nuclei, from its point of emergence from the brain to its terminal organ. If such evidence could convert Dohrn ('07) from the advocacy of the cell-chain hypothesis of neurogenesis which on the basis of evidence partly derived from the study of the same nerve he had defended for years, it would seem that little argument would be needed to convince unprejudiced students that the trochlear nerve develops by the free outgrowth of medullary neuroblasts. It appears unlikely that any student of neurogenesis will demand that a neuraxon be traced from neuroblast to muscle fiber in order to convince him of the applicability of the process theory to the phenomena of histogenesis of the trochlear. So that, while in the case of the trochlear nerve it is impossible to deal with individual cells and fibers, the fact that, from the time of its first appearance as a fiber bundle emerging from the dorsal wall of the brain, these fibers may be traced centrally to their connection with a group of deeply stained neuroblasts lying in the somatic motor column of the hindbrain, and that these are also the adult re-

lations, will seem to most students sufficient evidence to demonstrate their neuroblastic derivation.

The evidence that in early stages of histogenesis the distal fibers of the trochlear are associated with cell clusters or ganglia, as shown by Dohrn and others, affords, as stated above, no support to the cell-chain hypothesis, since these connections with the trochlearis fibers are secondary. Their relations with the trochlear anlage therefore must receive another than a genetic interpretation. Such another interpretation is given below.

e. By what means does the increase in length of the neuraxon take place? The histogenesis of the trochlearis affords no evidence in support of the Hensen-Held contention of the differentiation in situ of plasmodesmatous paths in the formation of a nerve anlage. The loose distal brush of fibers (fig. 49), in which the nerve anlage terminates in early stages, show no such relations to cells or cell processes as Held's position would require. Furthermore, while the existence of a loose brush of fibers may readily be interpreted as an advantage to a bundle of neuraxons which effect connection with their terminal organ by a process of free outgrowth, there would appear to be no meaning to the phenomenon were the Hensen-Held hypothesis correct. As soon as the connection with the myotome is effected by the fibers, they quickly unite into a compact bundle and lose their brush-like form. Therefore, while it is difficult to demonstrate the free-growing terminations of the fibers within the mesenchyma, the positive evidence, as far as it is known, is in harmony with the process theory. The assertion that the trochlear is differentiated in situ through the use and incorporation of primary plasmodesmatous paths is wholly unfounded.

f. What is the source of the cells of the trochlearis? Somatic motor spinal nerves derive their cells from the neural tube by migration along the nerve anlage, possibly from the mesenchyma, and come into close association with cells masses derived from the spinal ganglia. Do the cells associated with the trochlearis anlage have a similar derivation?

That medullary elements enter the trochlear anlage has been held by Dohrn ('07) on the basis of evidence similar to that pre-

sented in figure 53, which shows nuclei half in and half out of the brain wall at the point of emergence of the trochlearis root. Dohrn (p. 293) also called attention to the fact that the nuclei are not peripheral in position but lie between the fibers of the nerve root. Furthermore, medullary nuclei lie nearer the periphery of the brain wall in the region where the fibers of the trochlear enter the brain than they do elsewhere in the vicinity of the root, and, as development goes on, an increasing number of nuclei appear among the fibers of the nerve root. More convincing evidence of migration would be difficult to find in sectioned material.

Belogolowy ('10a, p. 375) suggests that the absence of accompanying cells in the fiber bundle of the trochlear anlage in its intracerebral and in its proximal extent proves that the accompanying cells are not medullary in origin. However, had Belogolowy studied somewhat more advanced stages with this problem in mind, it is possible that he would have found evidence of medullary migration. The writer ('98), on grounds similar to those advanced by Belogolowy, concluded that medullary elements were absent from the trochlear anlage of *Squalus*, but renewed investigation of more advanced stages proves this inference to be erroneous.

On the other hand, evidence that mesenchymatous cells attach themselves to the nerve anlage is difficult to obtain and positive proof is altogether wanting. Belogolowy ('10a) infers the participation of mesenchymatous cells in the formation of the neurilemma of the trochlear. The most convincing evidence that may be secured is possibly that mesenchymatous cells lie in various degrees of proximity to the nerve anlage (fig. 51). In other words the evidence of the participation of the mesenchyma in forming the cellular elements of the nerve anlage are quite as obscure as in the case of spinal somatic motor nerves.

Miss Platt ('91) was the first to observe evidence of migration of cells from the anlage of the superficial branch of the trigeminal into or towards the trochlear anlage. The evidence is similar to that seen in the relations of the oculomotor to the profundus ganglion. Miss Platt, after describing (p. 95) the first appearance of the trochlearis as a "small fibrous nerve growing

from the constriction between midbrain and hindbrain," says that "soon after the appearance of this small nerve, which is the root of the permanent trochlearis, cells are proliferated to meet it from the ganglion cells that lie above the superior oblique muscle." These cells cannot rise from the brain, since "no cells are found in the root of attachment." Thus the permanent trochlearis arises from two sources from the brain and from the ganglion cells."

Dohrn ('07) also inferred that the first cells connected with the trochlear anlage in *Pristiurus* are derived from the ramus superficialis V, while in those forms in which, as in the *Torpedinidae*, the superficial branch is not differentiated, but is represented by fragments of the neural crest, the trochlearis anlage acquires relations to these cell clusters, similar to those in *Squalus* and *Pristiurus*, and presents similar evidence of cellular migration toward the nerve anlage.

The evidence of cell migration from the R. superficialis V into the trochlear anlage in *Squalus* consists of the fact that cells appear primarily, not in the proximal root of the nerve nor in the bundle of fibers, but in the distal portion of the nerve in the region where its fibers cross those of the superficialis. The phenomena presented are practically identical with those described for the oculomotor in its cellular relations with the R. prof. Trig. As development proceeds, cells increase in number in the region between the two nerve anlagen, although the proximal portion of the nerve remains free from cells. Finally, cells accumulate between the trochlear and superficialis anlagen in a mass precisely comparable to the anlage of the ciliary ganglion, forming the cell cluster to which Miss Platt ('91, p. 100) refers as the ganglion of the trochlearis lying above the superior oblique muscle anlage.

Further evidence of migration into the trochlear anlage from the superficialis nerve is found in spindle-shaped cells closely applied to the fibers of the trochlear anlage in stages when cells are wanting in the proximal portion of the nerve and when evidence of additions from the mesenchyma is lacking. Similar spindle-shaped cells show connections, on the one hand with the ramus

superficialis, and on the other with the fibers of the trochlear anlage.

Thus, in the derivation of its cellular elements, the trochlear resembles the oculomotor and spinal somatic motor nerves. There is no reason to doubt the medullary derivation of some of its cells; mesenchymatous participation is somewhat doubtful; and, finally, the migration of cells from a ganglionic nerve in relation with its anlage appears unquestionable.

g. What is the fate of these cells? Dohrn ('07) makes the interesting suggestion that the manner of development of the fibers of the trochlearis in *Pristiurus* is a natural experiment, confirming Harrison's ('04) experimental demonstration of the origin of the neurilemma in amphibian somatic motor nerves from the neural crest. That is, he concludes that the cells which migrate from the superficialis into the trochlear anlage form the cells of the neurilemma. The possibility that they may be compared with the cells of sympathetic anlagen does not seem to have occurred to Dohrn. Miss Platt ('91) speaks of these cells as ganglion cells although she has made no attempt to follow their fate in later stages.

However, the similarity of the cell-cluster, associated with the trochlearis anlage and derived from the superficialis, with the ciliary anlage in its relations to the oculomotor and the profundus is so striking that the suggestion that in this mass we have a sympathetic ganglion seems permissible. The main objection to such a view, and one that may appear insuperable, is that the trochlear appears in the adult to be associated with no sympathetic ganglion. The ganglion of the trochlear anlage—not to be confused with the fragments of the neural crest associated with the trochlear anlage in the *Torpedinidae* and reptiles—is conspicuous in *Squalus* embryos of 25 mm. but has disappeared as a mass of cells in 45 mm. embryos. Since, however, many of the sympathetic anlagen of spinal somatic motor nerves similarly disappear in these stages and do not appear in the adult, the disappearance as a distinct cell-mass of the hypothetical sympathetic ganglion of the trochlear is not to be regarded as a serious objection to the hypothesis. Moreover, the possibility

is by no means excluded that sympathetic cells are associated with the adult trochlear nerve. This point needs investigation. The important fact remains that a cell cluster, associated with the embryonic trochlearis, has the same relations and derivation as the sympathetic anlagen of the trunk and as the ganglion of the oculomotor and that in this respect as in others the nerve conforms in its histogenesis with that of a typical somatic motor nerve.

h. What is the histogenesis of the neurilemma of the trochlear? More convincingly than any other nerve in the vertebrate body the histogenesis of the trochlear anlage shows that a nerve may become connected with its terminal organ without the slightest indication of the participation of a single peripheral 'nerve cell' or chain of nerve-forming cells. Dohrn ('07, p. 411) states that it was the study of the histogenesis of the trochlearis which converted him from a supporter of the cell-chain hypothesis to one of the process theory of His. There appears in *Pristiurus* (Dohrn) and in *Squalus* the decisive evidence which, in normal embryos, effectually invalidates the cell-chain hypothesis in whatever modified form it may present itself. The evidence is wholly convincing that the neurilemma cells of *Squalus* have a secondary and exogenous derivation. Primarily, instead of a cell-chain extending from nerve center to end-organ the trochlear nerve appears as a fibrillar bundle. Into this fibrillar anlage cells migrate from the neural tube and from the ramus superficialis V. Some cellular additions may come from the adjacent mesenchyma. These cells, generically independent of the fibers, soon assume an elongated form and a relation to the nerve fibers which marks their destination as neurilemma cells.

i. To what an extent do the emigrant cells go to form the sympathetic? The absence of any positive evidence of sympathetic cells in the adult trochlearis would appear to afford presumptive evidence against such a fate for any of the cells of the trochlear anlage. But the fact that sympathetic anlagen disappear ontogenetically in many spinal somatic motor nerves makes the supposition that the trochlear possesses a transient sympathetic ganglion seem not unreasonable. Moreover, until it has been

demonstrated that sympathetic cells are wanting in the adult trochlear, the possibility that the cell cluster associated with the nerve in 25 mm. embryos forms a diffuse sympathetic along the nerve in the adult is not excluded. As far as it goes, the evidence favors the opinion that a transient sympathetic ganglion, derived from the ramus ophthalmicus superficialis trigemini is associated with the trochlear anlage as in the case of certain anterior spinal nerves. There is no evidence whatever that migrant medullary elements enter into this hypothetical transient sympathetic anlage.

j. Summary of the histogenesis of the trochlear nerve. In the manner of its growth and extension to form a secondary connection with its myotome through the protoplasmic movement of medullary neuroblasts; in the source of derivation of its neurilemma; and in its relation to a ganglionic nerve and to a sympathetic anlage, the histogenesis of the trochlear resembles in all essentials that of spinal somatic motor nerves. To this extent its serial homology with them is demonstrated.

4. Does the histogenesis of the abducens resemble that of a somatic motor spinal nerve?

a. Are protoplasmic connections between hindbrain and myotome 3 primary or secondary? No investigator has attempted to support the Hensen hypothesis of the primary connection of nerve and muscle on the basis of the histogenesis of the abducens nerve. On the contrary all who have made a careful study of its histogenesis—Dohrn ('90, '91), Neal ('98), Carpenter ('06), and Belogolowy ('10)—agree that, at first, the abducens is not connected with the posterior rectus muscle, but in its early stages of development terminates freely in the mesenchyma at the base of the brain. No evidence of the participation of plasmodesmatous paths in the forward extension of the nerve has been advanced. And, while no thorough investigation of its development from the standpoint of the Hensen hypothesis has been made, it does not seem likely that demonstrable plasmodesms would have remained unperceived.

The mere presence of a loose mesenchyma in the region later traversed by the nerve anlage gives no presumption in favor of view of Hensen and Held. The possibility of the utilization of such material in the elongation of the growing nerve may readily be granted, but as yet no demonstration of such use of protoplasmic bridges has been made. On the contrary, it is possible to demonstrate in sections the free growing end of the nerve anlage and to ascertain, on the basis of actual observation, that no plasmodesm or undifferentiated protoplasmic path connects the end of the nerve anlage with the myotome. Such a growing end of the abducens is given in fig. 35, which shows on what slight evidence the hypothesis of the utilization of protoplasmic paths depends. The amoeboid terminations of the nerve fibers (neuraxons) show attenuated connections by means of fine granular threads—as seen in sections—with amoeboid processes of mesenchymatous cells. But such evidence, so far as it permits any inferences at all, favors the opinion that the fine amoeboid processes of the growing tip are derivatives of the nerve anlage itself. For, if the processes of the mesenchymatous cells are genetically related to them, there is equally good ground for thinking that the processes which extend from the end of the nerve anlage in all directions are derivatives of the nerve itself. Harrison's experimental results verify this assumption, since, in his preparations of the living nerve fibers, similar delicate extensions of the amoeboid termination of the nerve make their appearance. In such preparations there can be no question of the genetic relations of the fine threads. To call them paths as Held ('09) has done is obviously a misnomer for structures which radiate in all directions. Were a growing structure to depend upon such flimsy paths for the material for its growth, its extension would be neither fast nor far. Furthermore, neither this section nor others, in which the observer may feel confident that he is dealing with the actual termination of the growing nerve anlage, is the relation of the termination to adjacent mesenchymatous cells such as to suggest the utilization of their substance in the elongation of the nerve.

In view of the unanimous agreement of all investigators of the histogenesis of the abducens that the nerve becomes secondarily connected with its myotome, and in view of the entire absence of evidence of primary plasmatic paths within which the neuraxon might be differentiated; furthermore, in view of the fact that it is possible to demonstrate the growing tip of the abducens anlage as an amoeboid structure similar to those seen in Harrison's cover-glass preparations, there appears little reason to doubt that the abducens acquires a secondary connection with its myotome. This inference is strengthened by Belogolow's discovery that some fibers of the abducens anlage of the chick extend in their growth farther than the anlage of the posterior rectus muscle and later atrophy. It seems easier to harmonize such evidence with the hypothesis of the free outgrowth of nerve fibers than with the hypothesis of primary connection of nerve and muscle. Moreover, the fact that the nidulus of the abducens is about equidistant from post-otic and pre-otic myotomes and that, in early stages of development, its fibers grow in both directions—that is, both anteriad and posteriad—suggests that the direction of growth is chemotropically determined. The extension posteriad is, however, transient, the posterior process is soon withdrawn, and the protoplasmic movement anteriad alone continues.

b. What cells participate in the formation of the protoplasmic connections? If the possibility of the development of the abducens by the progressive differentiation of plasmodesmatous paths be excluded, the possibility remains that the abducens develops, either by the differentiation of a chain of cells, or as the product of protoplasmic movement of medullary neuroblasts. Dohrn ('90 a, '91) alone, of all of the students of the histogenesis of this nerve, has attempted to demonstrate the former mode of histogenesis. The fact, however, that later ('07) he admitted the inadequacy of the grounds upon which that opinion was based makes it unnecessary to call attention to the fallacy of his argument. Dohrn's premises were weak, not because he was a poor observer—no man has done more to enrich our knowledge of the histogenesis of nerves in selachians—but because his

preparations were not suited to demonstrate the histogenesis of the neurofibrillae.

Filatoff ('07, p. 343) finds that the anlage of the abducens in reptile embryos appears primarily as a strand of mesenchymatous cells extending from the posterior rectus muscle towards the base of the medulla and as yet unconnected with the brain. The cellular strand is not fibrillar. How connection with the brain is effected, Filatoff is not able to state.

All other students of the histogenesis of the abducens agree that the cells of the abducens anlage have no genetic relation to it but are secondary and accessory. The fibers of the anlage arise as processes of medullary neuroblasts situated in the somatic motor column of the hindbrain, posterior to the otic vesicle. All have noted the greatly elongated nidulus, which, according to the writer ('98) extends through the first and second post-otic neuromeres (neuromeres VII and VIII). Belogolowy ('10 a, p. 270) makes the interesting discovery that in a 2.2 mm. embryo chick "the roots of the abducens extend through as many as five neuromeres." Later they arrange themselves into three groups, which however show no regular relation to the neuromeres. By a comparison of the relations of the abducens anlage to the neuromeres, Belogolowy concludes that the most anterior roots of the abducens of the chick take their origin from a neuromere next anterior to the one from which they originate in the dogfish (*Squalus*). Posteriorly the roots of the embryonic abducens in the chick are connected with those of the hypoglossus. The posterior roots of the abducens show a marked tendency to grow backwards as if to join those of the hypoglossus which lie immediately behind them. Still later some of the roots degenerate. Another important discovery made by Belogolowy is that of the existence of a transient somatic motor nerve, ventral to the ramus maxillaris trigemini and uniting peripherally with the oculomotor with which the abducens also unites. The inference of the medullary derivation of the neuraxons of the abducens is based on the evidence of the connection between deeply staining cells of the medulla and the fibers of the nerve anlage, from the time of the first appearance of the

anlage (plate 8). The earliest stage represented (fig. 31) shows that the anlage, which already has two roots of origin, contains deeply staining fibers surrounded by an envelope of granular protoplasm. Mesenchymatous cells crowd thickly around the roots of the anlage so that it is impossible to distinguish their boundaries from the protoplasmic envelope. In this and later stages (figs. 32, 41, 37) the continuity of the processes of the medullary cells with the fibers of the anlage is unmistakable.

In later stages the number of roots increases to the number of five or six and there is a correlated increase in the number of medullary cells, the processes of which may be traced towards or into the roots of the anlage. In the meanwhile cells make their appearance in the nerve anlage (figs. 33, 36), but the comparison of closely connected stages indicates that these cells secondarily attach themselves to the anlage or, in advanced stages, migrate from the neural tube (fig. 39). None of them ever has the form or staining properties of neuroblastic cells, such as appear within the ganglionic nerves or within the wall of the medulla. Also, during the stages during which the fibers most rapidly increase in number these cells are distinctly peripheral in relation to the bundle of fibers. Furthermore, the fact that the fibers in successive stages grow centrifugally toward the myotome of somite 3, and that, in the earlier stages of growth, the number of fibers is greater in the proximal portion of the anlage than in the distal portion (figs. 42-44), accords with the supposition that the fibers have a medullary origin in central neuroblasts.

c. Have these protoplasmic connections a genetic relation to the neurofibrils? Students of the histogenesis of the abducens have paid little or no attention to the differentiation of the neurofibrils. There seems, however, little reason to doubt the genetic connection between the deeply stained fibers of the nerve anlage and the finer fibrils of the fully differentiated nerve. Their most important histogenetic change appears to consist in the splitting of the fibers into finer fibrils as the diameter of the fiber increases during growth. The granular envelope, so conspicuous in the anlage, gradually disappears and is replaced by

the neurilemma cells as they penetrate among the fibers in advanced stages of histogenesis.

There appears to be no reason for doubting the identity of the fibers differentiated by the Vom Rath method with those which appear in Cajal and Bielschowsky-Paton preparations. They have practically identical appearance, form and relations, both in cross and longitudinal sections. So that if it be granted that the coarse fibrils seen in preparations made by the latter methods are genetically related to the neurofibrils of the adult nerve, the reasons apply with equal force to those which appear in Vom Rath preparations.

d. Is the individual neuraxon of the abducens anlage multicellular in origin or is it the process of a single cell? Reasons have already been stated why the abducens fibers may be regarded as products of protoplasmic movement and not of cell chains, and it seems unnecessary to repeat them. All the evidence favoring this conclusion for spinal somatic motor nerves and for the oculomotor and trochlearis may be advanced for the abducens. The proof is even more convincing in the case of the abducens, since development is simplified by the absence of connection with a ganglionated nerve.

e. By what means does the increase in length of the constituent neuraxons take place? This question may be answered in the same way as in the case of the other somatic motor nerves described, and has already been stated above. The evidence upon which the hypothesis of the plasmodesmatous origin of the nerve anlage is based is no less equivocal and unconvincing than that offered by its most recent exponent, Held ('09).

f. What is the source of origin of the cells of the abducens anlage? Since the abducens makes no connection with a ganglionic nerve and therefore receives no cells from that source, the cells of the anlage must either be derived from the mesenchyma or from the medulla or from both. Neal ('98) and Belogolowy ('10) were unconvinced of the medullary origin of the cells, which both derived from the mesenchyma. But Dohrn ('91) was undoubtedly correct in inferring the medullary origin of some of the abducens cells. The evidence of migration is quite as convinc-

ing as that seen in spinal somatic motor nerves. A section through a root of the abducens is shown in figure 39 and the appearances are seen to resemble closely those of early stages of the development of ventral spinal roots. The evidence that mesenchymatous cells attach themselves to the anlage is equivocal but seems probable. Filatoff's ('07) observation of a migration of mesenchymatous cells from the anlage of the posterior rectus muscle is interpreted by the present writer as subsequent to the acquisition of connection between brain and myotome and as a part of the process of formation of the neurilemma. Filatoff advances no evidence that these cells have a genetic relation with the fibers of the definitive nerve.

g. What is the fate of the cells of the abducens anlage? As there is no sympathetic associated with the abducens in any vertebrate, the cells of the abducens can have only one fate if they persist in the adult nerve. In a non-ganglionic nerve they must form the neurilemma. With this conclusion all investigators, whatever their views regarding nerve histogenesis, agree.

h. What is the histogenesis of the neurilemma of the abducens? At the outset all of the cells of the abducens are peripheral in relation to the fiber bundle. Penetration of cells into this bundle is slow and has scarcely begun in a 25 mm. embryo. As in the case of the oculomotor and trochlearis, penetration begins in the region of the proximal roots, that is, in the oldest part of the nerve anlage. By the time the embryo has reached a length of 45 mm., however, cells have penetrated all parts of the fibrillar bundle and have begun to assume the characteristic form and relations of neurilemma cells.

i. To what an extent do the emigrated medullary elements go to form the sympathetic? The one essential respect in which the abducens differs from the other somatic motor nerves described is in the absence of the sympathetic in relation to the nerve anlage. As in the case of the hypoglossus, however, this feature appears correlated with the absence of connection with a ganglionic nerve, a correlation emphasized by Hoffmann ('00).

j. Summary of the histogenesis of the abducens. Like typical somatic motor nerves, the abducens acquires secondary connec-

tion with the posterior rectus muscle by the continuous outflow of the protoplasm of cells in the somatic motor column of the hindbrain. Its fibers are directly connected with the medullary neuroblasts from which they arise, while its cellular elements are partly medullary and possibly, in part, mesenchymatous. From them develops the neurilemma of the differentiated nerve. No evidence that they are genetically related to the neurofibrils has been obtained. No sympathetic ganglion is associated with the abducens anlage or with the adult nerve.

5. *What light does the study of histogenesis throw upon the question of the homology of pre-otic and post-otic metameres?*

The demonstration of the similar histogenesis of the eye-muscle and spinal somatic motor nerves creates a strong presumption that pre-otic and post-otic divisions of the vertebrate body are fundamentally alike. It has been found that in no essential respect does the histogenesis of eye-muscle and spinal somatic motor nerves differ. The dorsal chiasma of the trochlearis and the lack of sympathetic connection with the abducens in no way invalidates the comparison. The hypoglossus nerve differs from the typical somatic motor nerves in precisely the same way as the abducens, but its comparability with spinal somatic motor nerves is unquestioned. In fact, if the relations of somatic motor and sensory nerves in *Amphioxus* and *Petromyzon* may be regarded as primitive, those of the abducens and of the hypoglossus are more primitive than those of other somatic motor nerves in *Squalus*. The absence of a sympathetic is likewise a primitive character. The study of the histogenesis of the eye-muscle nerves favors the prevalent conception of the vertebrate head as once like the trunk.

But the proof of the morphological similarity of segmental nerves depends, not on evidence of histogenetic similarity alone or central relations with the motor nidulus, but equally upon the peripheral distribution. So that the question arises whether or not the myotomes innervated by the eye-muscle nerves are serially homologous with those innervated by spinal somatic motor nerves.

The study of histogenesis has shown that connection between muscle and nerve is not primary but secondary. This suggests the possibility that new segmental relations may be acquired by somatic motor nerves and that a nerve may invade territory foreign to it. In this way some peculiarities in the relations of eye-muscle nerves may be explained.

RELATIONSHIPS OF THE EYE MUSCLE NERVES

1. Is the musculature innervated by the eye-muscle nerves somitic musculature?

The eye-muscle nerves innervate muscles derived from Van Wijhe's first, second and third somites. The oculomotor innervates muscles differentiated from the first, the trochlear innervates that derived from the second, and the abducens is connected with a muscle formed from the third and a part of the second (Dohrn '07). Are these muscles serially homologous with those innervated by spinal somatic motor nerves? This obviously depends upon the somitic nature of Van Wijhe's three 'somites.'

They have been regarded as true somites by many morphologists including Van Wijhe ('82), Oppel ('90), Miss Platt ('91, '97), Hoffmann ('94), Neal ('96, '98), Fürbringer ('97), Koltzoff ('01), Killian ('91), Boecke ('04), Filatoff ('07) and their presence has been demonstrated in such diverse groups as Selachii (Van Wijhe '82, Miss Platt '91, Hoffmann '94, Neal '96, Braus '99, Johnston '09); Cyclostomes (Koltzoff '01); Teleosts (Boecke '04); Amphibia (Miss Platt '97); and Reptiles (Oppel '90, Filatoff '07).

On the other hand the eye muscles have been regarded as splanchnic muscles by Stannius ('51), Langerhans ('73), Balfour ('78), Marshall ('81, '82), Dohrn ('85, '87, '04, '07), Houssay ('90), Hatschek ('92), Rex ('97, '05) and Sewertzoff ('98), and the presence of true somites in the pre-otic region has been denied by Kastschenko ('88), Rabl ('92) and McMurrich ('12). Sewertzoff ('98 b) bases his objection to the inclusion of the second myotome in the series of somitic muscles upon the con-

elusion of Froriep and Miss Platt that the trochlearis is a mixed (splanchnic motor) nerve, and not upon the basis of direct evidence.

Dohrn ('04) concluded that the superior oblique muscle is splanchnic and not somitic on the evidence that a portion of the splanchnic (ventral) mesoderm of the mandibular cavity shifts during development to a dorsal position as a result of the cephalic flexure of the brain. From this displaced mesoderm, according to Dohrn arises the masseter muscle, the superior oblique muscle and a portion of the posterior rectus muscle.

Filatoff ('07, p. 354) takes exception to this inference of Dohrn on the ground that it is difficult to distinguish dorsal from ventral mesoderm in the mandibular cavity and more especially to determine what is dorsal and what is ventral during the process of shifting relations in successive stages. Filatoff finds that the superior oblique muscle in *Emys* arises from the dorsal segmented portion of the mandibular cavity.

Against the somitic value of the pre-otic mesodermic segments it has been argued that the head of the vertebrate ancestor was, like that of Tunicate larvae, unsegmented; that the supposed segmentation is not actually metameric but is the result of mechanical influence of other organ systems; that the mesodermic divisions are irregular in size and inconstant in number; that they are discontinuous with those of the trunk; that they do not differentiate sclerotome and myotome, at least in the typical manner; that the topographic relations to nerves are different from those of trunk somites. But, since renewed investigation has disproved many of these assertions, the argument in favor of the somitic value of Van Wijhe's somites seems on the basis of the following evidence, much the stronger of the two alternatives.

That Van Wijhe's somites are serially homologous with those of the trunk seems sufficiently established by the repeated confirmation of their presence in diverse groups of vertebrates; and on the ground that their segmentation is independent of the visceral segmentation; that Van Wijhe's somites form a continuous series with those in the trunk; that they are dorsal in relation to chorda and dorsal aorta; that their development is

progressive, beginning with the neck region; that they differentiate into myotome and sclerotome; that their muscles are primarily differentiated from the median wall; and that they correspond numerically, as in the trunk region, with the neuromeric divisions of the neural tube. They, therefore, constitute the best established evidence of the primitive metamerism of the vertebrate head and of its comparability with the trunk. The somitic nature of the muscles derived from them seems therefore, indisputable.

2. *Are the relations of the oculomotorius comparable with those of a somatic motor spinal nerve?*

a. *How is the relation of the oculomotor to the ramus profundus to be interpreted?* The oculomotor nerve resembles a somatic motor nerve not only in its histogenesis but also in its central and peripheral relations.

Like a spinal somatic motor nerve, it arises from a nidulus in the somatic motor column of a neuromeric segment of the neural tube and innervates muscles derived from a somite serially homologous with those of the trunk. Furthermore it becomes connected with a ganglionic nerve after the manner of typical spinal somatic motor nerves.

This connection with the ramus profundus V, however, needs closer scrutiny. True, it is a ganglionic nerve, but is it certain that all ganglionic nerves are morphologically comparable? Our present knowledge of nerve components and their differences in the various cranial nerves, as demonstrated by the investigations of Strong ('95), Herrick ('99) and Johnston ('05 a), discourages the indiscriminate comparison of nerves on such superficial grounds as the possession of a ganglion. Therefore one, who maintains the similarity of the relations of the oculomotor with spinal somatic motor nerves, is bound to demonstrate the morphological similarity of the ramus ophthalmicus profundus V with spinal somatic sensory nerves. That they are similar seems proved first by their similar histogenesis from neural crest cells; second, by their similar peripheral distribution as general cuta-

neous nerves; and third by their common classification as somatic afferent nerves.

Three objections, however, may be raised against this homology; first, that while spinal somatic sensory nerves grow median to the myotome, the ophthalmicus profundus, like other typical cranial nerves, grows lateral to the somites; second, that cellular elements enter the ganglion of the ophthalmicus profundus from the skin, while the cellular elements of spinal sensory nerves are exclusively derived from the neural crest; and third, that the profundus is not an independent segmental nerve but a sensory branch of another nerve.

The first of these objections may be met by calling attention to the fact that in some cases, for example in the post-otic region of *Ammocoetes*, typical cranial nerves lie partly lateral and partly median to the myotomes, indicating that no sharp line of demarkation can be drawn on this basis between cranial and spinal somatic sensory nerves. Such differences appear to be correlated with differences in the relative size of the somite. Furthermore, there are comparative anatomical grounds for thinking that somatic sensory nerves were primarily inter-myotomic in position. From such primitive relations the present modified relations of cranial and spinal somatic sensory nerves may readily have been derived.

The second objection is more serious, but if the comparability of the somatic sensory nerves of *Amphioxus* and *Squalus* be granted, it will be seen that the cranial somatic sensory nerves have retained the primitive relations of the former, while the absence of direct contact with the skin in the case of spinal somatic sensory nerve ganglia, may be regarded as a secondary modification. In this respect, as in respect to the retention of a mixed function, typical cranial nerves appear more conservative than spinal nerves. Therefore, while it must be admitted that the cranial somatic sensory ganglia are more complex in their derivation than are spinal ganglia and that the serial homology of the two is incomplete, nevertheless their partial homology appears demonstrable.

Finally, the fact the fibers of the ophthalmicus profundus enter the medulla in common with those of the trigeminus will not seem to morphologists a serious objection to the comparison of the profundus with spinal somatic sensory nerves, since it would seem a matter of indifference whether somatic sensory fibers enter the brain by one path or another. In the case of the union of the roots of once independent ganglia we seem to have to do with a particular case under the general principle of the centralization of function in the region of the medulla. Analogous instances may be found in all organ systems.

Taking all the facts into consideration there appears to be no insuperable objection to the view that the ophthalmicus profundus is serially homologous with spinal somatic sensory nerves.

b. How is the relation of the oculomotorius to the ciliary ganglion to be interpreted? The comparison of the profundus nerve with spinal somatic sensory nerves is still further strengthened by the evidence of the relations with the ciliary ganglion which have been found above to be those of a somatic motor nerve to a sympathetic ganglion. The facts which prove the sympathetic character of the ciliary anlage have already been stated above and need no restatement. The ciliary ganglion of *Squalus* is to be regarded as partly, if not exclusively, a sympathetic ganglion. So that in its relations with a sympathetic ganglion the oculomotor forms no exception in the series of morphologically similar somatic motor nerves.

c. How may the relation of the oculomotorius to four eye muscles be best interpreted? The distribution of the oculomotor to four muscles may appear to require interpretation. The fact, however, that all of these muscles are derived from a single myotome by a process of splitting and that this splitting is correlated with the considerable enlargement of the eye-ball; furthermore, that the innervation of more than one muscle by a single somatic motor nerve is by no means exceptional, brings these relations also into line with those of spinal somatic motor nerves. The oculomotor, therefore, in its histogenesis and in its relations to a somatic sensory ganglion, to a sympathetic ganglion and to

somatic musculature may be considered serially homologous with spinal somatic motor nerves.

d. Conclusions regarding the morphology of the oculomotor. It is scarcely necessary to say that the conclusion that the oculomotor is serially homologous with spinal somatic motor nerves has not been reached by all morphologists. It is, however, the opinion of the majority, including Van Wijhe ('82), Beard ('85), Hoffmann ('86-'00), His ('88), Martin ('90), Dohrn ('90, '91), Zimmermann ('91), Kölliker ('96), Neal ('96, '98), Koltzoff ('01), Carpenter ('06), Filatoff ('07), Belogolowy ('08) and also, on the basis of anatomical evidence, Muëk ('15), Bell ('30), Stammer ('49), Bonsdorff ('52), Budge ('55), Huxley ('74, '75), Schneider ('79), Gaskell ('86, '89), Strong ('90), Fürbringer ('97), Wiedersheim ('98), Gaupp ('99).

On the other hand, it has been regarded as a splanchnic motor nerve homologous with the trigeminal by Balfour ('78), Marshall ('81, '82), Dohrn ('85, '87), Houssay ('90), Hatschek ('92), Sewertzoff ('98), and as a somatic sensory or mixed nerve with coenogenetic atrophy of the sensory element by Marshall ('82), Rabl ('89), Martin ('90), Miss Platt ('91), Mitrophanow ('92, '93), Sedgwick ('94), Sewertzoff ? ('99), Gast ('09), and on anatomical grounds by Schwalbe ('79, '81) and Gaskell ('89).

The most recent argument in favor of the latter opinion is that given by Gast. He admits that in its first anlage, the oculomotor develops in the same way as the anlage of a somatic motor nerve. On page 420 he states that naked neuroblast processes grow from the neural tube, unite to form a naked fiber bundle, grow to a segmental ganglion, and receive their sheath cells from it. Medullary neuroblasts also migrate into the nerve anlage. Sheath and sympathetic cells wander from the mesocephalic ganglion into the nerve anlage but retain connection with their source by a ramus communicans, a phenomenon quite in line with that of typical somatic motor nerves.

On the other hand, he says, there are indications that lateral horn elements are combined with these ventral root elements. In the first place, the close relations with the mesocephalic ganglion through which the oculomotor fibers grow in some instances

confirm this view. In other cases the oculomotor assumes very close relations with the ganglionic placode connected with the mesocephalic ganglion. Again, there is evidence of the anlagen of sensory nerves in connection with the oculomotor. The proof of this (p. 401) consists in the fact that a short cell-chain, free from fibers, extends from the mesocephalic ganglion toward the oculomotor anlage. The absence of a fiber in the cell-chain appears to Gast to exclude the possibility that the cells are in the process of migration centrad along a motor fiber of the oculomotor. On the other hand, Gast (p. 402) regards the evidence given by Mitrophanow ('93) and Sedgwick ('92) in favor of the mixed function of the oculomotor as fallacious. But Miss Platt ('91) may have seen what he regards as evidence of sensory elements in the oculomotor.

While Gast admits that this conclusion appears to conflict with the evidence of the position of the nidulus of the oculomotor, he asks if it is not conceivable that Dohrn's suggestion is correct—that the lateral and ventral niduli have united together. Then there is the possibility that the separation of ventral and lateral niduli in the head region is a coenogenetic separation and that they were primitively united. Gast, however, is of the opinion that the union of lateral and ventral niduli assumed for the oculomotor is a secondary one, as Dohrn suggested.

On the basis of this supposition, Gast (p. 421) indulges in the following speculation:

The segmentally arranged mesocephalic-oculomotorius system with its sensory and motor roots becomes secondarily separated into sensory and motor elements, whereby the motor neurones of the oculomotor retain their central connections, while the sensory neurones of the mesocephalic ganglion acquire a new root. In the case of the Selachii this root formation came to pass in such a way that a commissure was formed between the individual ganglia of the anterior head region (trigeminal, trochlear, mesocephalic and the ganglia anterior to these). This commissural nerve gradually assumed the character of a root, while fibers of the segmental sensory roots proportionally degenerated. To-day, indications of these primary sensory roots are found in the oculomotor as well as in the trochlear.

The foundation for Gast's speculation consists, primarily, in the evidence of close or intimate relations with a cerebro-spinal ganglion. But if such logic were rigidly followed, it would be necessary to regard every spinal somatic motor nerve as a lateral-horn (splanchnic motor) nerve, since close and intimate relations with a cerebro-spinal ganglion is characteristic of spinal somatic motor nerves in *Squalus*.

The supposed demonstration of the participation of sensory elements in the genesis of the oculomotor is one that would satisfy only on the basis of a strong presumption in its favor. The position of the nidulus of the oculomotor and its peripheral distribution create a strong presumption against the assumption. Spindle-shaped cells lying in the mesenchyma between the profundus ganglion and the oculomotor nerve are not necessarily neuroblasts. Spindle-shaped cells may be found almost anywhere in the mesenchyma. Even if it be admitted that the evidence that these cells are in the process of migration toward the oculomotor anlage is convincing, Gast does not know their fate. They may form neurilemma or they may enter the sympathetic or what-not. Their later histogenesis is wholly unknown. Gast has not used neurofibrillar stains in order to ascertain their neuroblastic character. It would be surprising if morphologists accepted Gast's conclusion upon the basis of the slight evidence he is able to present in its favor.

The evidence in favor of the view that the oculomotor nerve is a mixed nerve homologous with typical cranial nerves such as the trigeminal is so unconvincing, while the evidence of its histogenesis and its central and peripheral relations so strongly support the supposition that it is a somatic motor nerve, as the majority of morphologists have believed, that the acceptance of the latter seems unavoidable. This view at least does not require support from such an unproved assumption as the secondary fusion of lateral and ventral motor niduli.

3. *Are the relations of the trochlearis comparable with those of a somatic motor spinal nerve?*

There is practically a consensus of opinion that in its most essential relations, namely, in its relation to a nidulus in the somatic motor column of the hindbrain, the relations of the trochlear are comparable with those of spinal somatic motor nerves. There is less general agreement, and yet a considerable majority of morphologists agree, that the trochlear innervates somitic musculature. The main objection which has been advanced against this conclusion has been that raised by Stannius ('51) and Langerhans ('73) of the different histological structure of the superior oblique muscle, which, according to these investigators, resembles splanchnic rather than somitic musculature. This objection may be met by denying the truth of the assertion as a generalization for all vertebrates. Differences in size and in detail between fibers of the eye muscles and those of the lateral trunk muscles may exist—especially in those forms in which the eye muscles are differentiated from a loose mesenchyma, but in forms like *Squalus*, there is no important histological difference between the muscles of the eye and those of the trunk. Such slight differences as do obtain may be ascribed to differences in environment. That they are not due to difference in genesis appears demonstrated by the evidence of the somitic origin of the eye muscles. Therefore, in histological structure as well as in its histogenesis and in relations, both central and peripheral, the trochlearis conforms to the type of spinal somatic motor nerves. But there are other relations of the trochlear that may profoundly affect our views of its morphology.

a. *How may the 'ganglion' of the trochlear be interpreted?* Gast ('09) thinks that the trochlear 'ganglia' described by Dohrn ('85), Hoffman ('89), Martin ('90) and Frieriep ('91), afford very clear proof that the trochlear is a 'complete segmental nerve.' Dohrn ('07, p. 396) regarded the evidence that in *Torpedo* embryos rudimentary centripetal ganglionic nerve fibers unite with the trochlearis as supporting the view that the trochlear is a lateral-horn nerve. I ('98) had interpreted the same evidence

as proof of the derivation of the neurilemma from the neural crest. Dohrn, however, thinks that the evidence of cellular migration into the trochlear anlage from the ramus superficialis indicates that genetic relations between the trochlear anlage and rudiments of ganglia still persist today, even though they are merely transient.

Dohrn's thorough investigation of the histogenesis of the trochlear in elasmobranchs, however, indicate that the so-called ganglia of the trochlear are irregular fragments of the neural crest lying in the region through which the trochlear grows; further, that the trochlear anlage in such forms as *Pristiurus* attains connection with the myotome without any relation whatever with these fragments, which develop in inverse ratio with the development of the ramus superficialis V, of which therefore they appear to be the equivalent. In other words, the relations of the trochlear with these 'ganglia' of the *Torpedinidae* in which they appear seem similar to its relations with the ramus superficialis V in the *Squalidae*. This equivalency is recognized by Gast ('09) who says that the sensory elements of the trochlear which appear as ganglia in the *Torpedinidae* are represented by the ramus ophthalmicus superficialis V in the *Squalidae*.

Whether or not this equivalency be admitted, the relations of the trochlear to the 'ganglia' of the nerve resemble those of a spinal somatic motor nerve to the ganglionic or nervous derivatives of the neural crest. Such relations disprove the somatic motor character of the nerve in question quite as little in one case as in the other.

While in *Squalus* the trochlear anlage has no such relations to irregular fragments of the neural crest as in the *Torpedinidae*, the nerve does have relations to cell masses which precisely resemble those of the oculomotor to the anlage of the ciliary ganglion, or those of a spinal somatic motor nerve to sympathetic anlagen. Such relations of the trochlear anlage are represented in figures 54 and 55. With a strong presumption in favor of the view that the trochlear is a somatic motor nerve as evidenced by its histogenesis and by its central and peripheral connections the most reasonable interpretation of the mass of cells

at the place of union of the anlagen of the superficialis and the trochlear is that it is sympathetic. Like the cells of all sympathetic ganglia those of the trochlearis appear to come from a sensory ganglion. In both instances they collect in the region where sensory and motor fibers unite. Against this view may be urged the fact that there is no sympathetic in relation to the adult trochlear nerve. But the objection loses much of its force when it is remembered that transient sympathetic ganglia are not uncommonly found in the trunk region of elasmobranchs.

This supposed sympathetic ganglion of the trochlear anlage must not be confused with those fragments of the neural crest which Dohrn ('85) and others have called ganglia of the trochlearis. Such 'ganglia' appear, as suggested by Dohrn ('07) and Gast ('09), to be comparable with degenerated portions of the superficial nerve and not with sympathetic ganglia. Belogolowy ('10 b) finds in reptile embryos a ganglion present at the point of anastomosis of the trochlearis anlage with the ramus ophthalmicus superficialis trigemini. Belogolowy expresses the opinion (p. 70) that the so-called ganglia of the trochlearis are really the ramus ophthalmicus superficialis trigemini which is represented in some forms by scattered clumps of cells. With this opinion the writer is in full accord.

b. How may the innervation of a muscle derived from a somite (Van Wijhe's second) also innervated by the abducens be interpreted? Another problem presented by the relations of the trochlear is the fact that it innervates a muscle derived from a somite also supplied by the abducens nerve. This comes about in the following manner: A portion of the myotome of Van Wijhe's second somite unites with the myotome of the third somite to form the posterior rectus muscle. Miss Platt ('91), with characteristic accuracy, observed this connection, but was led to infer its later degeneration and to confirm the conclusion of Van Wijhe ('82) that the posterior rectus muscle is derived exclusively from the third somite. Lamb ('02) reached the same conclusion. But Dohrn ('07), after a careful reinvestigation of the development of the superior oblique muscle, asserted the

persistence of that portion of the second myotome in connection with the myotome of the third somite. Dohrn has affirmed its persistence correctly. There is no doubt whatever that it persists in the superior oblique muscle of *Squalus*. Its relations are diagrammatically shown in figure 81. Thus it comes about that the trochlear and abducens nerves innervate portions of the same somite. In this way a problem in nerve relations is presented which will be fully discussed in connection with the problem of the relations of the abducens nerve. Suffice it to say here that this relationship of two somatic motor nerves to a single somite does not in any way affect adversely our views of their real morphology. As a matter of fact, emphasized by Bardeen ('04), all typical somatic motor nerves have a bimeric distribution to two adjacent myotomes. The most perplexing problem raised in this connection is that there is reason to think that the trochlear and abducens nerves do not belong to successive metameres. This question will be taken up later. But, even more difficult for one who attempts to demonstrate the somatic motor character of the trochlear, is the problem of its dorsal chiasma. In this feature the trochlear is the most peculiar nerve in the vertebrate body.

c. How may the dorsal chiasma of the trochlear be best explained? Van Wijhe ('86), in order to explain the dorsal emergence of the fibers of the trochlearis, assumed that, as a result of the extension of the anterior column to the olivary body and to the loop extending behind the corpus quadrigeminum, the root of the trochlear was drawn over the loop into its present position.

According to His ('88) the peculiar relations of the trochlear may possibly be explained as the result of the flexure of the neural tube in the region of the isthmus, a condition which he thinks is favorable to the sagittal growth of the neuraxon processes of the neuroblasts at the base of the cerebellum.

Rabl ('89), however, on the basis of observations on the selachii, birds and mammals, concluded that the roots of origin, both of the oculomotor and of the trochlear were primitively dorsal, but that gradually, through the enlargement of the peduncular paths, the root of the oculomotor was shifted to the ven-

tral surface of the brain, while the trochlear has retained its primitive position. It may readily be seen, however, that such considerations have to do with the dorsal emergence of the trochlear fibers rather than with the chiasma of the nerve.

Martin's ('90) unconfirmed and incredible assertions regarding the transmigration of the trochlear nidulus appear to deserve no restatement, although they were accepted tentatively by both Minot ('92) and Kölliker ('96) so far as they relate to a central origin of the trochlear chiasma. Kölliker, however, finds it difficult to conceive of a transmigration of a nidulus, although von Lenhossek and Ramón y Cajal have inferred a migration of neuroblasts from the ventral column of the tube into the sensory roots. An extensive migration of neuroblasts therefore appears possible.

Fürbringer ('02, pp. 134-136) has given the most thorough consideration to the problem of the chiasma of the trochlear and his hypothesis, although affected by his views of the primary and unalterable connection of nerve and muscle, seems to be the most elaborate of all that have been advanced. Following an idea advanced earlier by Hoffmann ('89), Fürbringer suggests that the trochlearis may have innervated musculature belonging to the parietal eye. According to Fürbringer the parietal eye (or pair of eyes) was situated primarily near the paired lateral eyes, which were then slightly differentiated and probably occupied a more dorsal position than later. The aberrant musculature connected with both kinds of eyes may have very early separated itself—as the course of the nerve indicates—from the dorsal portions of the neighboring myotomes and may, in correlation with the primitive condition of the eyes, have been in a very slightly differentiated and—so to speak—fluid condition, with its fibers extending in various directions. Those portions of the muscle which extended somewhat diagonally or transversely had the tendency, like other muscles extending toward the median line of the body, to migrate over into antimeric territory. There was nothing like a median fin in the region to hinder this migration, which could occur freely.

With the degeneration of the parietal eye the musculature which became associated with it disappeared. The muscles of

the lateral eyes, however, which at that time were in close proximity to one another did not degenerate but became attached to, and at the same time migrated with newly differentiated fibers, to the eye of the opposite side where they increased in size while the original muscles of that side atrophied. In this way they formed the present superior oblique muscle, the nerve of which with its dorsal chiasma—primarily peripheral—still discloses the early history of the transmigration of its muscle. Then with the higher development of the retina of the lateral eyes there came an enlargement of the centers within the brain, especially a greater development of the roof of the midbrain, which extended backward over the place of emergence of the trochlear fibers and covered it, so that a part of its root as well as its chiasma was shoved backward and at the same time enclosed within the wall of the brain. The lateral eyes were not restricted to that dorsal muscle derived from the second myomere of the opposite side but very soon acquired connections with more ventral musculature innervated by the oculomotor and abducens, and, as the eyes moved into a ventral position, the muscles became enlarged and differentiated.

In support of this hypothesis, Fürbringer advances the following considerations: First, in defense of the assumption that the parietal eye once possessed a musculature of which today there is no evidence (with possibly the exception of the unconfirmed case in mammals mentioned by Nicholas ('00) who claimed to demonstrate rudimentary striated muscles in the pineal region of the ox), he argues that the absence of parietal muscles today by no means proves that they never existed. Many skeletal structures have existed in fossil species without leaving a trace in modern vertebrates. *Typhlichthys* has no eye muscles but its ancestors must have had them. The assumption of an anti-meric transmigration of the hypothetical ancestral eye muscles has met both opposition (Dohrn '01) and support (Gaskell '01). In reply to the opposition, Fürbringer cites many cases of the extensive migration of muscles in all directions within the vertebrate body. Many instances of the transmigration of musculature on the ventral side of the body are known, as for example the muscles innervated by the facialis, vagus and hypoglossus,

as well as some parts of the *M. sternalis*. Since there is no median fin in the head region to prevent transmigration, it may have occurred there as readily as in the ventral trunk and head region.

The vagrant nature of the superior oblique muscle is evinced by the considerable variation in the origin and insertion of the muscle as well as in its extended migration in the embryo.

Several fairly obvious objections may be raised against Fürbringer's ingenious hypothesis: First, the absence of any direct evidence from comparative anatomy and embryology that any vertebrate muscle has migrated in toto from one side of the body to the other; Second, the complete failure of ontogenesis to support the hypothesis. Ontogenesis is hardly so discredited, even by Fürbringer, that this lack of ontogenetic support can be wholly ignored; Third, the total lack of evidence of an epiphysial musculature. Nicholas ('00) did not demonstrate the actual connection of rudimentary muscles with the epiphysis; Fourth, the improbability of such a swapping of muscles as is assumed in the hypothesis. Why the lateral eyes should lose muscles which they already possessed and adopt those of another degenerating organ is not perfectly obvious. Still other objections might be mentioned but it seems unnecessary to multiply them.

Of course it may be said that no hypothesis dealing with phylogenetic changes so remote as the origin of the trochlear chiasma may be advanced which will seem so compelling as to preclude criticism. To many, Fürbringer's hypothesis will appear a reasonable one, on the assumption that nerve and muscle are phylogenetically inseparable. The relations of the trochlear and superior oblique muscle have always seemed a stumbling block to the supporters of that assumption, the truth of which does not seem more certain as the result of the difficulty of explaining the chiasma of the trochlear.

Fewer difficulties, it might appear, would meet an hypothesis which assumed the secondary connection of nerve and muscle by means of the free outgrowth of the nerve fibers.

Johnston ('05, p. 210) suggests that:

The course of the root fibers dorsally through the brain wall may be due to the course of the fiber tracts through which they run. The position of the nucleus of the nerve relative to the tracts which form the ansulate commissure in typical fishes suggests strongly that the axones from the cells of the trochlearis nucleus may have followed some of these bundles as the path of least resistance. The tracts between the tectum opticum and the base of the oblongata, the tracts between the inferior lobes and the cerebellum, and others, all running more or less dorso-ventrally in the side wall of the brain and decussating ventrally at the level of the trochlearis nucleus—these bundles, which lie ectal to the nucleus of the trochlearis, may have constituted an effective barrier to the axones of the trochlearis in their attempt to reach the ventro-lateral surface of the brain. The axones may then have turned upward along the ental surface of these bundles until they reached the dorsal surface of the brain. If the fibers were thus directed in their course they would be carried to the mid-dorsal line before gaining an exit from the brain and if they then grew straight on they would pass to the opposite side.

Filatoff ('07, pp. 366-7) thinks that certain phenomena in the development of the brain may help to explain the peculiar origin of the trochlearis. At the time of the formation of the cephalic flexure the roof of the midbrain, which up to that time formed a part of the thin upper wall, thickened. The production of this thickening may be explained in the following way. By the development of the flexure the cells of the floor of the midbrain become most strongly compressed, since they come to lie directly in the place of flexure, and they seek to elongate themselves in the most direct way where the pressure is a little less strong, namely towards the upper wall. The point at which the trochlearis arises is directly determined by this elongation of the cells. The point of emergence of the trochlearis fibers is shoved from the ventral to the dorsal side.

Figure 82 of this paper suggests two possible phylogenetic stages in the development of the trochlear chiasma. An earlier stage is represented on the left of the diagram and a later stage, corresponding essentially to that seen in some elasmobranch embryos, is shown on the right. It is assumed that originally the trochlear, as a somatic motor nerve, was distributed to the myotome of the second somite of its own side after the fashion of typical somatic motor nerves, and that this myotome, when

fully developed, extended dorsally to form a union or interdigitation with the antimeric myotome. As Fürbringer has correctly stated, no skeletal structures would prevent the extension of fibers across the median plane. It may be imagined that this extension of muscle fibers across the median plane was correlated with the muscular development of the prostomial region. *Petromyzon* still shows (fig. 77) the extension of myotomes into this region. Under such conditions slight variations in the length of the nerve fibers which grow to connect with these muscles might bring about a peripheral chiasma. The possibility that such muscles had connections with the epiphysis is not excluded, but such a supposition does not seem necessary. Changes in the extension and direction of growth of muscle and nerve fibers in this region may have been correlated with the development of the cephalic flexure which would seem to require some adjustment of the muscles since the forebrain and midbrain regions were flexed into a more ventral position. The final result of the flexure, however, appears to have been a shifting of those portions of the musculature which persisted in this region into a more ventral position and a separation of the muscles which had been apposed in the median plane above the brain wall.

The growth and great enlargement of the lateral eyes also brought about changes in the (Van Wijhe's) second myotome, which became split into dorsal and ventral moities (*my. 2 v.l.*, *my. 2 m.*, *my. 2 d.l.*) in precisely the same way as occurs ontogenetically in the post-otic muscles of *Petromyzon* as a result of the growth of the otic vesicle (figs. 78, 79).

It may be assumed that, as in the latter case, the median portion of the myotome degenerated, together with its branch of the somatic motor root (trochlear nerve), while the lateral moiety became innervated by a branch of the abducens (fig. 82, *abd.*). The dorsal moiety, however, retained its connection with the trochlear nerve, and possibly also with fibers from both sides of the brain, by means of a dorsal, peripheral chiasma. Then, when later this dorsal moiety degenerated with the exception of that portion which became attached to the eye-ball to form the external oblique muscle (crossed hatched in the dia-

gram) the result would be essentially the conditions that obtain today in those forms of selachian embryos in which a peripheral chiasma persists (fig. B, p. 25), and are shown in the right hand side of the diagram. It is necessary, however, to assume that phylogenetically the chiasma came to be more and more central and that gradually the fibers of the trochlear nerve became exclusively crossed fibers. What factors determine the survival of the crossed fibers and the elimination of the direct is no less mysterious than those which have produced the ventral chasmata of the eye and the pons. Possibly purely mechanical conditions of the sort suggested by the writer ('98) and by Johnston ('05) are responsible. All who have discussed the chiasma agree in one essential point—namely, that the chiasma of the trochlear is secondary and that it constitutes a coenogenetic modification of a somatic motor nerve. Therefore, its existence does not affect our views of its morphology. All other questions are subordinate to this, and it does not appear greatly to matter whether or not the true history of the origin of the chiasma has been or ever will be told.

d. Conclusions regarding the morphology of the trochlearis. The trochlear has been regarded as a somatic motor nerve on the basis of anatomical evidence by Stannius ('49), Huxley ('74, '75), Schneider ('79), Gaskell ('86, '89), Osborn ('88), Strong ('90), Fürbringer ('97), Wiedersheim ('98), and Gaupp ('99) and on the basis of embryological evidence by Van Wijhe ('82), His ('88), Martin ('90), Dohrn ('90, '91), Zimmermann ('91), Hoffmann ('94), von Kölliker ('96), Neal ('96, '98, '12), Koltzoff ('01), Filatoff ('07), Belogolowy ('08, '10).

On the other hand it has been regarded as a splanchnic motor nerve on the basis of anatomical evidence by Bell ('30), Hatschek ('92), Haller ('98), Fürbringer ('02). Stannius ('51) and Langerhans ('73) reached the same conclusion on the basis of the histological structure of the superior oblique muscle; while Balfour ('78), Marshall ('81, '82), Dohrn ('85, '90, '04, '07), Béranek ('87), Houssay ('90), Hatschek ('92), von Kupffer ('94) and Sewertzoff ('98) supported this view on the basis of ontogenetic evidence.

Then too we have the view that the trochlear was primitively a dorsal nerve, innervating visceral musculature, advanced by Schwalbe ('79, '81), Dohrn ('85) and von Kupffer ('95); innervating somitic musculature, by Miss Platt ('91) and Hoffmann ('97, '99, '00); and finally that it was primarily purely sensory and possibly secondarily mixed but eventually losing its sensory components, by Rabl ('89, possibly), Martin ('90), Oppel ('90), Froriep ('91), Platt ('91), Mitrophanow ('92, '93), von Kupffer ('95), Hoffmann ('89, '97, '99, '00), and Sewertzoff ('98).

This brief summary of the views held regarding the morphology of the trochlearis will possibly suffice to show that opinion is about equally divided for and against the view that the trochlear is a somatic motor nerve. Since a detailed statement of the arguments, for and against, has been given by both Fürbringer ('02) and Dohrn ('07) it appears to be unnecessary to discuss slightly divergent individual opinions.

The chief argument in favor of the comparability of the trochlear with dorsal ganglionic nerves appears to be, not the evidence of its dorsal origin, since the point of emergence of its fibers is ultra-dorsal rather than dorsal and in this respect it differs as much from a dorsal nerve as from a ventral one, but the evidence of its relations with neural crest cells, either aggregated as the ramus superficialis V, or as scattered clumps of neural crest cells called ganglia by several investigators. Dohrn ('07) is right in asserting in contradiction to Neal ('98) that the relations of the trochlearis with the ramus superficialis or with transient clumps of neural crest cells have a phylogenetic significance. But the fallacy of regarding the trochlear as a dorsal nerve on the basis of such evidence has already been pointed out above. By a similar argument every somatic motor nerve of the body which becomes associated with a ganglionic nerve must be regarded as a dorsal nerve.

Dohrn is also correct in asserting that the evidence of the formation of the trochlear as a bundle of neuraxon processes of medullary neuroblasts proves that the nerve is a somatic motor nerve no more and no less than it proves the trochlear to be a

splanchnic motor nerve. It is not a little surprising, however, that Dohrn seems to have forgotten that the motor nidulus of the trochlear has the relations of a somatic motor nerve and not those of a splanchnic motor nerve. In the heat of the attempt to prove the trochlear a dorsal nerve Dohrn seems also to have forgotten that somatic motor nerves have relations with ganglia, both dorsal and sympathetic, comparable with those of the trochlear. Of his special views regarding the segmental relations of the trochlear consideration will be given later.

On the other hand it has been shown that in every essential detail of central and peripheral relationship, of histogenesis and of adult histological structure the trochlear must be regarded as a somatic motor nerve. Its peculiarity consists in one special feature—its chiasma, but in this respect it differs quite as much from a typical dorsal nerve as from a ventral somatic motor one. The interpretation of the mode of genesis of this chiasma is quite as easy upon the assumption that it is morphologically a somatic motor nerve as upon the assumption that it is a dorsal nerve, the opinion of Dohrn ('07) to the contrary notwithstanding.¹

4. Are the relations of the abducens comparable with those of a somatic motor spinal nerve?

As a purely motor nerve, with a ventro-lateral nidulus and with a distribution to somatic musculature, there seems to be no good reason for questioning the serial homology of the abducens with spinal somatic motor nerves. The absence of a sympathetic ganglion is one striking point of difference, however, which is correlated with the absence of any connection with a dorsal nerve. In both these respects the abducens resembles the more primitive somatic motor nerves of *Amphioxus* and *Petromyzon*, and the absence of a sympathetic ganglion is to be regarded as a case of the retention of an ancestral character.

¹ Dohrn ('07, p. 410) thinks that the writer's view of the trochlear as a somatic motor nerve makes the solution of the problem of the chiasma more difficult.

The second and more important point of difference between the abducens and spinal somatic motor nerves is its distribution as a post-otic nerve to pre-otic myotomes. Instead of innervating musculature of its own metamere, it is distributed to myotomes of anterior metameres (fig. 76). This relation, therefore, demands interpretation.

a. How may the innervation of pre-otic musculature, the posterior rectus muscle, by a post-otic nerve, the abducens, be interpreted? Are the myotomes innervated by the eye-muscle nerves post-otic myotomes which have migrated into pre-otic territory? In the attempt to solve the problem presented by the distribution of the abducens, namely, the problem of the distribution of a post-otic nerve with a post-otic nidulus to pre-otic muscle, two alternative hypotheses suggest themselves.

According to the first hypothesis, the posterior rectus muscle is to be regarded as a post-otic muscle which has migrated in the course of phylogeny into pre-otic territory, carrying with it the associated nerve, the abducens. As would be expected the nidulus of the abducens has retained its primitive position in the medulla, posterior to the otic capsule. One of the conditions which has brought about the migration of the posterior rectus muscle may have been the reduction and final disappearance of the pre-otic muscles, through the development and hypertrophy of the sense organs, cranial ganglia and cartilage cranium. Then, after the atrophy of the pre-otic muscles, post-otic myotomes invaded the territory in the same way as occurs ontogenetically in the case of the anterior trunk somites of *Petromyzon* (figs. 77 and 78). This evidence from *Petromyzon* meets an objection which may be raised against the theory, namely, that it is unreasonable to suppose that if the pre-otic region became too crowded to retain its own muscles it would be able to contain muscles from elsewhere, since this is precisely what seems to occur ontogenetically in this animal. In *Petromyzon* the pre-otic somites break up into loose mesenchyma and in later stages post-otic myotomes invade the territory. A comparison of figure 77 with figure 81, shows how similar the relations of the associated nerve in *Petromyzon* (*nr.* 2, fig. 77) and of the abducens nerve of *Squalus* are.

McMurrich ('12) has recently advanced a theory (p. 175) which involves the assumption of this migration of post-otic myotomes into pre-otic relationships. Johnston ('02 and '05, pp. 230-233) has in my opinion completely refuted Fürbringer's argument in favor of the phylogenetic migration forward of trunk nerves and myotomes into the occipital region. To all who hold the view of the primary continuity of nerve and muscle the hypothesis will seem the only tenable one, in spite of obvious difficulties and objections. Belogolowy ('10 a, pp. 380-384) has recently advanced an extended argument against the hypothesis of the primary continuity of nerve and muscle.

The most obvious objection to this hypothesis is the entire lack of ontogenetic evidence in its support. Had the phylogenetic migration of muscle assumed by the hypothesis actually occurred, we should expect to find some ontogenetic evidence of it. But there is as little ontogenetic evidence that the somites from which the posterior rectus muscle develops have migrated from behind the ear as that the mandibular and hyoid arches with which they are associated topographically have migrated from a post-otic position into their present location in the embryo. Furthermore, the hypothesis cannot be reconciled with the fact that the abducens—a post-otic nerve—innervates a myotome (Van Wijhe's 2nd) of which a dorsal moiety is innervated by the trochlearis, a nerve with a pre-otic nidulus (fig. 81). The niduli of these two nerves lie in widely separated neuromeres of the brain—one of them pre-otic and one-post-otic—and yet they innervate muscles derived from the same somite. If the posterior rectus muscle were once post-otic, it is difficult to explain how the somite from which it is in part derived (Van Wijhe's 2nd) is innervated also by a pre-otic nerve with a pre-otic nidulus. If the hypothesis were true, it would be necessary to assume a migration of the nidulus of the trochlearis from behind the ear into its present position. Of such a migration of a motor nidulus from one metamere into another several segments removed, there is neither comparative anatomical nor embryological evidence. The careful comparative anatomical investigation of the nidulus of the abducens by Kappers ('10) discloses no such migration of the nidulus as McMurrich's

hypothesis requires. On the other hand, there is much evidence of the distribution of motor nerves—such as the abducens—into metameres other than those in which they have their nidulus. This fact suggests a second hypothesis of the origin of the relations of the abducens to the posterior rectus muscle.

According to the second hypothesis, the abducens has attained its present relations by a process of substitution or nerve piracy; that is to say, a post-otic nerve has, in the course of phylogeny, usurped the area of distribution of a pre-otic one. The clue to the process by which this substitution has taken place is afforded by the anterior post-otic nerves in *Petromyzon*.

Ontogenetically, the two anterior post-otic somites of *Petromyzon* divide into median and lateral divisions, of which the former lie median to the otic capsule and adjacent to the notochord, while the latter lie lateral to the otic capsule and to the large ganglia of the ninth and tenth cranial nerves (figs. 78 and 79). The lateral portions of the myotomic divisions divide (completely, in the case of the first post-otic myotome) into a dorsal division (*my. 4 d.l.*), above the otic capsule, and a ventral division, below the ear, the division occurring along the line of lateral line sense organs. The relation between the series of lateral line sense organs—including the otic capsule—and the line of cleavage of the myotomic divisions seems more than merely topographic, and there is little reason to doubt that the development of the ear and the sense organs and their related ganglia has been one of the conditions—if not the essential condition—of the splitting of the myotomes. That is to say, the splitting of the myotomes may reasonably be regarded as an adaptation to the conditions brought about by the enlargement of the sense organs and cranial ganglia. The median division of the anterior post-otic myotomes develops embryonic muscle fibers, which degenerate and disappear in relatively early embryonic stages, for they are entirely absent in a 50 mm. embryo. With them disappear, it may be inferred, the associated somatic motor nerves; although, in spite of much pains, I have been unable to demonstrate the existence of embryonic nerves associated with these myotomic divisions. The reason for this fail-

ure may perhaps be ascribed to the small size of lamprey embryos and the great difficulty of identification of embryonic nerves. The differentiation of muscle fibers, however, as Harrison has shown, does not imply the presence of nerves.

The portions of these myotomes lying lateral to the ear persist into the adult and form the anterior segments of the lateral trunk musculature (fig. 77; *my. 4 d.l.*, *my. 4 v.l.*). They are innervated by branches of nerves of posterior myotomes, namely, those of the fourth and fifth post-otic myotomes. That each of the anterior myotomes was at one time innervated by its own segmental somatic motor nerve seems indisputable, and some explanation of the present modified relationships seems required. We may assume that the nerve (*rx.v.*) (*n'v. 1* and *n'v. 2*, fig. 77) which innervates the five most anterior post-otic myotomes consists of the combined nerves of these five segments, each of which retains its primary connection with its related myotome. This assumption harmonizes with the hypothesis of the primary continuity of nerve and muscle. Were this assumption correct, however, we should expect to find the nidulus of the nerve extending anteriorly as far as the otic capsule. Of this there is no evidence. The only neuroblasts, the processes of which can be traced into the roots of this nerve, lie solely in the region of the roots.

As an alternative explanation of the nerve and muscle relations under discussion, it may be assumed that the nerves associated with the three anterior myotomes have degenerated, while their area of distribution has been usurped by the nerves of posterior myotomes. The degeneration of the median division of the first post-otic myotome may have been one of the conditions which lead to this substitution in the case of this myotome. Such an assumption of nerve substitution is in harmony with the rapidly increasing evidence in favor of the process theory of nerve development and with the limitation of the nidulus of the nerve as stated above.

Furthermore, the evidence presented by Johnston ('08) and his conclusions, support this assumption. In his discussion of the segmental relations of ventral nerves in Petromyzonts John-

ston writes (p. 584): "There seems to be no definite or constant arrangement of these motor fibers. They pass in a haphazard fashion to one or two myotomes, branch once, twice or three times, et cetera. In studying the peripheral nerves of *Amphioxus* with methylene blue I gained the general impression that the nerves in that animal showed still less regard for segmental relations." The point of these statements in this connection is, not the fact of irregularity of nerve relationships in these primitive chordates, but the evidence against the view of the inseparability of muscle and nerve, afforded by them. Such facts point unmistakably toward the possibility of changed innervation under changed conditions. Moreover, the more recent conclusions regarding the phylogeny of the nervous system (Parker '10) are against the view of the primary continuity of nerve and muscle.

Still further in confirmation of the view that the abducens has acquired its present relations by a process of substitution, is the fact to which my attention has been called by Dr. W. H. Lewis, that when the digastricus first arises in the human embryo it is innervated by the facial nerve. Later in development, the muscle divides and the anterior belly becomes innervated by a branch of the trigeminal. If such a process of nerve piracy occur ontogenetically, it is clearly possible that a similar process may have taken place phylogenetically in the case of the abducens.

b. How may the innervation of musculature derived from two somites by a single nerve—the abducens—be best interpreted? The majority of investigators have confirmed Van Wijhe's statement that the musculature innervated by the oculomotor is derived from the first or pre-mandibular somite; that innervated by the trochlear is differentiated from the second or mandibular somite; while the abducens musculature is developed from the third somite. That is, each eye-muscle nerve is distributed to a single somite. With the exception of Dohrn ('01, '04) all students of the genesis of the eye muscles including Kastschenko ('88), Miss Platt ('91), Hoffmann ('97), Neal ('98), Sewertzoff ('99) and Lamb ('02), agree upon the monomyotomic distribution of these nerves. Belogolowy ('10 a, p. 252) finds that the anlage of the

posterior rectus muscle in the chick consists of two or three masses, but he does not suggest that this division indicates a metamerism of the muscle.

Upon the basis of observations upon *Torpedo* embryos Dohrn ('90) inferred that each of the eye-muscle nerves has a polymeric origin and distribution, the abducens representing three or four metameres, the oculomotorius possibly a larger number, and the trochlear a single metamere. A year later, however, he concluded that the *m. obliquus superior* is developed from two myotomes. Regarding the number of somites in *Torpedo*, however, Killian ('91) and Sewertzoff ('99) reached conclusions divergent from those of Dohrn, and Dohrn ('91, '01, '07) has repeatedly revised his conclusions, and in his latest paper ('07) decides that the second somite of Van Wijhe represents three or four myomeres. In this connection the statement of Sewertzoff ('98) that the somites of Dohrn ('90) and Killian ('91) in *Torpedo* secondarily merge into those of Van Wijhe and that the myomeric segmentation of *Torpedo* and *Pristiurus* is identical, is important.

Miss Platt ('91) had noticed that just anterior to the anlage of the posterior rectus muscle there appears the rudiment of a large muscle derived from the posterior portion of the second (mandibular) somite. This muscle, according to Miss Platt, soon degenerates and her conclusion has been confirmed by Lamb ('02). Johnson ('13) thinks that he is able to identify the same rudiment in *Chelydra* embryos. But Dohrn ('01, '04) denies its degeneration and affirms its persistence as an integral part of the definitive posterior rectus muscle, the major portion of which is derived from the third somite of Van Wijhe.

Sewertzoff ('99) derives the posterior rectus muscle of the selachii from Van Wijhe's third somite, which he regards as the first true somite, and which he states is formed by the confluence of two primary somites. Sewertzoff makes the interesting discovery in *Torpedo* that the third and fourth somites unite to form the posterior rectus muscle. This divergence of opinion regarding the metameric relationships of the posterior rectus muscle is important and the whole problem should be reinvestigated in embryos of both *Squalidae* and *Torpedinidae*.

After a careful reinvestigation of the genesis of the posterior rectus muscle the writer is able to affirm with positiveness that Dohrn ('01, '04) is correct in claiming the persistence of the rudimentary muscle which Miss Platt ('91) thought was transient in the embryo. On the contrary, it persists and forms, as Dohrn stated, the anterior portion of the posterior rectus muscle, and is innervated by the abducens nerve.

The problem presented by the fact of the distribution of the abducens nerve to two myotomes—Van Wijhe's second and third—is more apparent than real, since, as has been shown by Bardeen ('98) for mammals and by Johnston ('08) for Cyclostomes, a bimeric distribution is the rule for somatic motor nerves. Therefore, the distribution of the abducens to two myotomes, instead of presenting a difficulty, constitutes still further evidence of its morphological similarity with spinal somatic motor nerves.

c. Is the abducens of Gnathostomes homologous with the most anterior spinal nerves of Petromyzon? The cyclostomes have no posterior rectus muscle and no abducens nerve (Johnston '05 a). Assuming the primitive character of the cyclostomes, in this feature the conclusion seems inevitable, therefore, that in the course of phylogeny one or two of the anterior spinal nerves of the Cyclostomes have been converted into the abducens of the Gnathostomata. The apparent similarity between the ventral ramus of the anterior spinal nerves of *Petromyzon* (*n.v.* 2, fig. 77) and the abducens gives sufficient grounds for raising the question whether there may not be an homology—partial or complete—between them. The position attained by the musculature (*my.* 4 *v.l.*, fig. 77) innervated by the nerve *n.v.* 2 is so close to the eye as to make possible, through slight variations in the course of phylogeny, its attachment to the eye-ball. The nidulus of origin of both nerves is post-otic and somewhat extended, while the distribution is pre-otic. But here the resemblance ceases.

Against the exact homology of these nerves, it may be urged; first, that the myotome innervated by the abducens is a pre-otic one, while that innervated by the nerve *n.v.* 2 is a post-otic myotome; second, that the nerve *n.v.* 2 is the nerve of the fourth

and fifth post-otic myotome while the fourth and fifth post-otic myotomes of *Squalus* (Van Wijhe's 7th and 8th) have their own somatic motor nerves, namely, the anterior roots of the embryonic hypoglossus (fig. 81), which must therefore be the exact homologues of the nerve *n'v.* 2.

The relations are such, however as to indicate that the abducens is the intrinsic nerve of Van Wijhe's 4th and 5th somites. The transient ramus recurrens of the abducens (VI, *rec.*, fig. 76) may possibly indicate the former relations of the abducens to the 6th somite or at least to posterior myotomes. If this conclusion be correct, then it follows that *Petromyzon* has, in the course of phylogeny, lost the homologue of the abducens with the loss of the median portions of the post-otic myotomes.

It will be recalled that the abducens has a ramus recurrens, observed by Dohrn ('90, '01), Miss Platt ('91), Neal ('98) and Belogolowy ('10), and interpreted by them as evidence of a former posterior distribution of the abducens. If this conclusion be correct, the homology of the abducens with the lost nerves of *Petromyzon* is rendered more certain.

One of Belogolowy's ('10 a) most important discoveries is the fact that in chick embryos the roots of the abducens and of the hypoglossus form members of a continuous series of anastomosing roots. By such evidence their serial homology seems clearly demonstrated. Moreover, he also finds roots of the abducens arising from the second hindbrain neuromere—a pre-otic neuromere—and infers that this root is a remnant of the primitive nerve belonging to the third somite. Belogolowy lays considerable stress upon the fact that anastomoses are formed between the abducens and the oculomotor fibers, resulting in relations resembling those between the abducens and the hypoglossus. Altogether the evidence seems overwhelming in favor of the view that all of these nerves form members of a continuous series of homologous nerves, and that the assumed distinction between post-otic and pre-otic regions of the head is arbitrary and artificial.

d. Conclusions regarding the morphology of the abducens. That the abducens is a somatic motor nerve innervating somitic mus-

culature has been held on anatomical grounds by Bell ('30), Stannius ('49), Huxley ('74, '75), Schneider ('79), Gaskell ('86, '89), Strong ('90), Fürbringer ('97), Wiedersheim ('98), Gaupp ('99), Kappers ('10) and on embryological grounds by Van Wijhe ('82), Beard ('85), His ('88), Dohrn ('88, '90), Martin ('90), Oppel ('90), Zimmermann ('91), Miss Platt ('91), Hatschek ('92), Hoffmann ('94-'00), von Kupffer ('94), von Kölliker ('96), Neal ('96, '98), Sewertzoff ('98, '99), Carpenter ('06), Koltzoff ('01), Filatoff ('07), Belogolowy ('08, '10).

Relatively few morphologists have regarded the abducens as a splanchnic motor nerve. These are Stannius ('51) and Langerhans ('73) on the basis of the resemblance of the histological structure of the posterior rectus muscle to visceral musculature and Balfour ('78), Marshall ('81), Dohrn ('85) and Von Kupffer ('94) on embryological grounds.

Therefore on the basis of the strong preponderance of morphological opinion and on the ground that in its histogenesis, in its relations to a somatic motor nidulus and to somitic musculature, and finally in its histological structure the abducens resembles a somatic motor nerve no alternative view of its morphology seems possible. In its two divergent characters, namely, its lack of connections with sensory and sympathetic ganglia, the abducens shows primitive features which do not affect our conception of its morphology.

If, on the basis of the considerations presented above, there seem good reasons for thinking that the pre-otic and post-otic regions of the head were primitively alike and segmented in correspondence with a somitic musculature, the question naturally arises whether the eye muscle nerves and their relations throw any light upon the vexed question of the number of cephalic segments. The demonstration of a segmented somitic musculature with associated somatic motor nerves in the head region would seem to warrant an optimistic view of the possibility of a definite answer to the problem with which morphology has wrestled without cessation for over a century. We may therefore turn to the following question:

5. *How many metameres are represented by the three eye-muscle nerves?*

It was the undisputed opinion of the earlier morphologists that each of the eye-muscle nerves represented a single metamere. As long as vertebrate morphology was largely based upon anatomical data, or at least the leading morphologists were comparative anatomists, there appeared little reason for assuming a polymerism of these nerves. Each was considered a segmental nerve or the ventral root of a segmental nerve and that conception met all intellectual demands.

When, however, comparative embryology developed, the discovery of a cephalic coelom and an independent mesodermic segmentation in the head region of elasmobranch embryos drew investigators away from anatomy, and the history of the head became written largely in terms of ontogenesis. Competition arose among embryologists to determine who could discover the largest number of ancestral head segments; and somites, neuromeres and epibranchial placodes became successively the favorite objects of investigation. The need of motor nerves to supply these segments soon became apparent and all evidence of polymerism of nerves was eagerly sought.

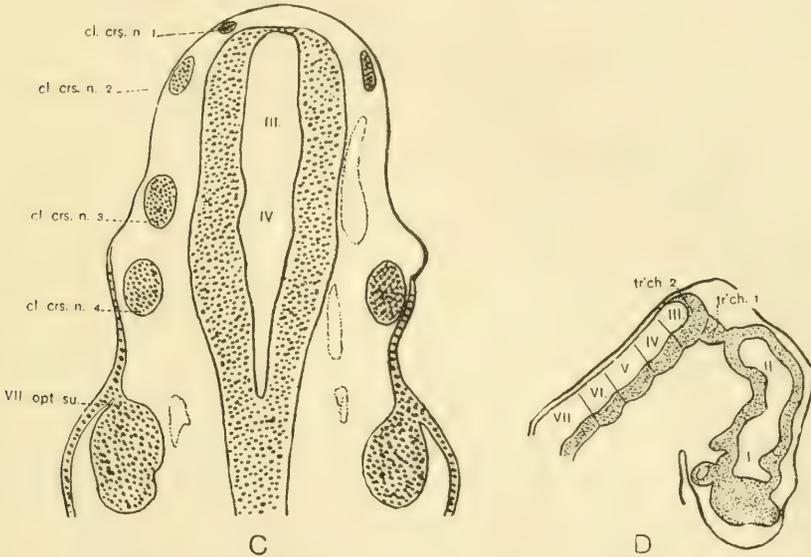
Dohrn ('90), upon the discovery of more numerous mesodermic segments in *Torpedo* embryos than had been discovered elsewhere, became a strong advocate of the polymerism of the eye-muscle nerves. For it was evident that the morphological importance and segmental value of his mesodermic segments in large measure depended upon the demonstration of a corresponding segmentation of other organ systems. However, objections were quickly raised to throw doubt upon the real metameric value of Dohrn's mesodermic segments. It was soon found that some of the microcoelic cavities which Dohrn had called somites were merely transient vesiculations of the mesoderm of the mandibular arch ventral to the somitic mesoderm; that the segments did not correspond upon the two sides of the body (an objection to which Dohrn replied that it was to be expected in degenerating structures); that the segments are not constant, as evinced

by the divergence in the results of different investigators—Dohrn ('90), Killian ('91), and Sewertzoff ('98, '99); that similar micro-coeles are seen in the trunk region but that they soon become confluent to form the definitive somites, just as in the head region they unite to form the somites of Van Wijhe (Sewertzoff '98, '99); that they lack numerical correspondence with other metameric structures; that they appear only in a divergent and highly modified group of elasmobranchs—the Torpedinidae; and that they may be considered of metameric value only by ignoring the evidence from comparative anatomy.

Notwithstanding all the objections raised against their metameric worth, Dohrn regarded them as the essential criteria of the primitive segmentation of the vertebrate head, and he considered this opinion supported by evidence of the polymerism of the eye-muscle nerves. Gast ('09) shares with him this conception of their value on the ground (p. 424) "that the polymerism of the hyoid and mandibular arches is attested not only by the different cavities of their mesodermic segments but by the strong evidence of the polymerism of the nerves associated with them." Brohmer ('09, p. 39) objected to Dohrn's conclusions on the ground that although it is necessary to assign a nerve to each 'primitive segment' of the head, Dohrn had left this anatomical standpoint out of consideration. The truth of this assertion is emphatically denied by Gast ('09) who affirms that Dohrn did not disregard the innervation of the mesodermic segments. Gast accuses Brohmer of being only superficially acquainted with Dohrn's argument for the polymerism of the trochlear and the abducens. Brohmer, however, is not the only morphologist who is skeptical of the real existence of the 'nerves' which Dohrn associates with his numerous mesodermic segments. As a matter of fact the foundation for Dohrn's assumption of the polymerism of the eye-muscle nerves, namely, the polymerism of Van Wijhe's somites, is denied.

Dohrn bases his inference of the polymerism of the trochlear on the assumption that the mandibular cavity is polymeric; that the trochlearis divides into two branches; that there are two hindbrain neuromeres corresponding to the two trochlear nerves;

and that there are in *Torpedo marmorata* two transient ganglia associated with the trochlear anlage. Text figures C and D illustrate Dohrn's argument. Corresponding with the four mesodermic segments which he claims are represented in the mandibular cavity Dohrn discovers two 'trochlear neuromeres' (*tr'ch.* 1; *tr'ch.* 2) and two trigeminal neuromeres (IV and V). The



Text fig. C Horizontal section of a 9 mm. embryo of *Torpedo marmorata*, after Dohrn ('07); *cl. crs. n. 1*, 'chiasma' group of cells; *cl. crs. n. 2*, anterior 'trochlear ganglion'; *cl. crs. n. 3*, second 'trochlear ganglion'; *cl. crs. n. 4*, Gasserian ganglion; *VII. opt. su.*, ganglion of the r. ophth. sup. facialis; *III, IV*, Neal's neuromeres.

Text fig. D A parasagittal section of an embryo of *Torpedo*, after Dohrn ('07); *I-VII*, neuromeres according to Neal ('98); *tr'ch. 1, tr'ch. 2*, Dohrn's trochlear neuromeres; *IV, V*, Dohrn's trigeminal neuromeres; *VI*, Dohrn's facialis neuromere.

two 'trochlear ganglia' found in *Torpedo* are seen in the frontal section represented in figure 3.

In support of the contention that the second myotome is polymeric and corresponds to at least two myotomes, Dohrn mentions the fact that from this myotome are differentiated two

muscles innervated by two independent nerves, the abducens and the trochlear. This evidence alone, however, does not necessarily warrant the conclusion drawn by Dohrn, since each myotome is typically innervated by two somatic motor nerves. Further, the two myotomic divisions of the mandibular cavity are not antero-posterior in their relations, as Dohrn's hypothesis would require, but are dorso-ventral in their relations to each other.

Since it would be difficult to find any somatic motor nerve which does not divide into at least two branches, this fact does not materially strengthen Dohrn's polymeric assumption.

The second of Dohrn's trochlear neuromeres is a late secondary subdivision of the most anterior hindbrain neuromere. From the evidence of a purely topographic relation with a clump of disintegrating neural crest cells, Dohrn infers that the trochlear includes the splanchnic motor niduli of two metameres. Unfortunately for this supposition, however, Dohrn is unable to demonstrate the presence of these two niduli. He states that no splanchnic motor fibers persist in connection with the second trochlear neuromere. Here again he is in error, since the splanchnic motor fibers of the ramus mandibularis trigemini have their nidulus in this portion of the hindbrain. These fibers enter the brain as the minor root of the trigeminal. If Dohrn's scheme of segmental relations were correct, two splanchnic motor nerves—his 'second trochlear' and the ramus mandibularis trigemini would have their niduli within the same neuromere—Dohrn's 'second trochlear' neuromere. These nerves actually do have their niduli in a single neuromere (neuromere III—Dohrn's first and second trochlear) but one has a nidulus in the somatic motor column while the other has a splanchnic motor nidulus. The weakest point in Dohrn's argument is its failure to take into consideration the central nidular relations. The argument in favor of the polymerism of the trochlear seems unconvincing, although it is the strongest argument yet advanced with this purpose in view. The argument in favor of the polymerism of the oculo-motor is even less adequate.

In objection to Dohrn's conclusions Belogolowy ('10 b, p. 23) advances the following considerations:

Dohrn, on the basis of the plurality of the ganglionic clumps connected with the trochlearis anlage and the subdivision of the nerve into single fibers, inferred the plurality and polymerism of the trochlearis. The discoveries of Dohrn appear to me quite insufficient to draw such a conclusion from, since it is impossible to use as a basis for such inferences the plurality of position and number of such indefinite elements. Were we to follow this method, it would be easy as the final result to consider the maximum number of branches which might be met by chance as the number of segmental nerves which have participated in the formation of the trochlearis; and the presence of clumps of neural crest cells which accompany these branches might be considered as sufficient proof of our assumption. But it appears to me at least risky to admit as a decisive criterion for our inferences the accidental occurrence of this or that number of anastomoses or ganglionic clumps.

On the other hand, there appear good reasons for regarding the abducens as a nerve representing more than a single metamere. In the first place its roots arise from at least two hind-brain neuromeres (neuromeres VII and VIII) and its nidulus is equally extensive; its transient and rudimentary ramus recurrens is suggestive of an earlier distribution to posterior myotomes; and lastly, it is distributed to myotomes other than those of its own metamere.

It might seem at first thought as if the most convincing evidence bearing upon the question of the polymerism of the abducens and the other eye-muscle nerves is the simple fact that they are distributed to three successive myotomes, Van Wijhe's first, second and third. And were this the only relation to be taken into consideration, such a conclusion would seem unavoidable. This conclusion however conflicts with the evidence that at least three neuromeres separate the niduli of the trochlear and abducens; and, further, the evidence that the abducens innervates muscles of other metameres than those of the post-otic metamere to which it belongs. Therefore the problem of the number of metameres represented by the three eye-muscle nerves cannot be solved by ignoring the neuromeric relations.

Ziegler ('08) and his pupil Brohmer ('09) have recently attempted to determine the number of cranial metameres, ignoring the neuromeric segmentation. Is neuromerism a matter of no consequence to the student of head morphology?

6. Are neuromeres satisfactory criteria of the primitive metamerism of the head?

By his discovery of symmetrical cross furrows on the widely expanded neural plate of *Salamandra atra* embryos von Kupffer became the first exponent of the view that the nervous system manifests a 'primary metamerism,' independent of the mesodermic segmentation. He was the pioneer—as he was in many other lines of morphology—among those who lay stress upon the segmentation of the nervous system as the best preserved manifestation of the primary metamerism of the vertebrate body. Repeated confirmation of the presence of neural segments prior to the appearance of mesodermic segmentation has been given by Froriep ('91, '93), Locy ('95), Hill ('00), Johnston ('05), Wilson and Hill ('07) and Griggs ('10).

At the same time it is a truism of morphology that the segmentation of the nervous system is a secondary one, determined by and dependent upon the segmentation of the muscular system. This has been especially emphasized by Mihalkowitz ('77), Ahlborn ('84 b), Froriep ('92), Kingsley ('12) and Coghill ('13). Observation, however, seems to have warranted the generalization that the nervous system is most conservative. When once it has acquired a segmentation adapted to whatever peripheral system, the segmentation is retained, even after the associated structures, sensory or muscular, have disappeared. So that when it was demonstrated beyond a doubt that the hindbrain neuromeres manifest a segmentation which could not be interpreted upon purely mechanical grounds and which appear independently of the mesodermic segmentation, the conclusion seemed inevitable that in these hindbrain neuromeres is preserved the indisputable remnants of the primary segmentation of the head. Finally when Locy ('95) claimed to have been able to trace the

'primary' neuromeres of the open neural plate of selachian embryos into the neuromeres of the closed neural tube, it seemed as if neural segmentation were the court of last appeal in all questions relating to the ancestral metamerism of the head. Few morphologists—Mihalkowitz ('77), Broman ('95), and Filatoff ('07)—have held the neuromeres to be purely the results of mechanical pressure or of growth in confined space and devoid of phylogenetic significance. This interpretation has been discredited since the demonstration that the hindbrain neuromeres of many vertebrate embryos are local thickenings of the medullary wall and not defined merely by foldings of the wall.

Johnston ('05, p. 234) probably expresses the general attitude of morphologists when he says that "nervous structures represent more segments than have been preserved in the mesoderm. In other words, there are preserved vestiges of nerve structures belonging to segments whose entodermal and mesodermal organs have disappeared for the most part. But the division of the brain wall into neuromeres gives a clue to the number of segments." The same implicit confidence in the value of the subdivisions or segments of the nervous system as criteria of the primitive metamerism is expressed by Griggs ('10, p. 434) in the conclusion that "if, as the most recent investigation seems to show, the nervous system, appearing first, presents a simpler and more unaltered condition than the other two systems, then it may well serve as a basis for the study of the segmentation of the head; and other organs should be shown to correspond to it rather than vice versa." It may well be doubted, however, if the truth of the premises of either Johnston or Griggs may be admitted.

Notwithstanding the faith inspired in these morphologists that, through the study of neuromerism the primary metamerism of the head will be ascertained, the conflict in their observations and conclusions seems hardly to justify their confidence. In the Urodeles, for example, Kupffer ('85) finds eight primary neuromeres in the region where Froriep ('91, '93) finds three, or four, or five, and an anterior unsegmented region large enough to include three or four more. Froriep, however, denies their seg-

mental value. Eycleshymer ('95) also finds only a few large segments of no metameric significance. Loey ('95) found four or five in the region and agreed with Froriep and Eycleshymer that they were of no segmental importance. Griggs ('10) recognized one neuromere in the forebrain region, two in the midbrain and one or more in the hindbrain region and regards these as the only true neuromeres. On the other hand, there are "in the closed neural tube of *Amblystoma* a series of swellings, extending from the anterior end of the brain to the otic pit, but since these divisions are of varying morphological significance they cannot rightly be called neuromeres." By what criteria shall the real neuromeres be determined? Is Griggs correct in denying neuromeric value to the forebrain or midbrain vesicles or their secondary subdivisions? Are the true neuromeres those of the open neural plate of *Amphibia*, as Griggs maintains, or are the real neuromeres those of the closed tube as held by the majority of morphologists? The difficulty of determining the real metamerism of the nervous system is increased, if morphological opinions are influenced at all by the segmentation of the brain of *Bdellostoma*.

Dr. Bashford Dean, in a letter which he kindly permits me to use, makes the following statement of the neuromeric conditions in *Bdellostoma* embryos:

If we believe that a 'neuromere' is represented objectively by a specially and definitely dilated spot in the central nervous system, expressed either in the inner lumen or in the outer wall of the medullary axis, we certainly cannot interpret the conditions in the hag-fish in terms, for example, of the shark or the amphibian. In the first place, the hag-fish embryos may show a great number of these dilated regions, as many indeed as twenty-seven or twenty-eight in the midbrain and hindbrain. They may indicate also that these dilated areas are indefinite in number; and that in relatively the same age these areas may be either very obscure, absent, or barely visible, or may be reckoned with almost mathematical precision. They are moreover, rarely symmetrical; it is curious also that their symmetry is never expressed in the same way in the large series of embryos examined. Neuromeres are difficult to distinguish in the forebrain of *Bdellostoma*. Occasionally there appear to be two, three, or four present. These can be discerned faintly on one side or the other side of the medullary axis, never paired.

In the midbrain as many as eight may be reckoned, and in other embryos none at all. A symmetrical number sometimes occurs. In the cases just examined, I find five specimens out of fourteen showing the same number of midbrain neuromeres on both sides. In one case I counted three right, three left; in another eight right, eight left; in another three right, three left; in another four right, four left, and finally one in which but a single neuromere appeared on either side. In the hindbrain, the number of neuromeres varies between three and twenty-four, and they differ in number on different sides, a difference of ten having been noted in the same individual in right and left sides of the body.

It may be objected of course, (1) That in these cases of asymmetry the neuromeres were present on the 'off' side but were 'suppressed,' and therefore could not be counted. (2) That the change in the number of neuromeres might be due to different stages in development, the neuromeres appearing most completely at a definite date of development. The fact remains, nevertheless, that asymmetry and variable numbers are present, and to an extraordinary degree, a state of affairs which does not make in the direction of clearing up our knowledge of these 'segmental' structures. (3) That the neuromeres of *Bdellostoma* are artifacts. Against this criticism I note that I have seen them clearly in living embryos.

But it may be objected that the foldings of the *Bdellostoma* brain are not the symmetrical foldings which have been recognized as neuromeres in other forms; that no one would be likely to interpret the variable and asymmetrical structures which Dean describes in *Bdellostoma* as of phylogenetic or morphological value; that real neuromeres are symmetrical and permanent thickenings of the brain wall. In reply to such objections it should be remembered that morphologists have not always insisted upon the constancy or the symmetry of problematic structures. In fact Dohrn ('04), in reply to this objection to his microcoelic mesodermic segments, replied that inconstancy and asymmetry would be expected in degenerating metameric structures like these. Out of hundreds of *Squalus* embryos examined by the writer in order to confirm Locy's results only two or three showed symmetry or regularity in the segmentation of the edges of the neural plate (figs. 2 and 3, Neal '98). Yet morphologists have not refused to accept Locy's conclusions because of this lack of confirmation of his results on selachians.

The main objection to the use of neuromeres as essential criteria of metamerism is not so much their variability in different vertebrates or the diversity of opinion among morphologists regarding them, as it is the difficulty of finding criteria by means of which the coenogenetic may be distinguished from the palinogenetic, especially in the regions of the forebrain and hindbrain. In an earlier paper ('98) the writer protested against the uncritical acceptance of all sorts of foldings of the central nervous system—dorsal, ventral or lateral—as evidence of the primary metamerism of the nervous system. Special protest was raised against the claim of Loey ('95) that he had been able to trace the 'primary' neuromeres of the open neural plate into the neuromeres of the closed neural tube. Eyeleshymer ('95) and Kingsley ('97) were likewise unable to accept Loey's assertions.

Hill ('00), however, confirmed Loey's results by his observations on teleost and chick embryos and considered Neal's objections as 'negative.' Wilson and Hill ('07, p. 147) on the other hand "cannot admit that Hill has fully and adequately met Neal's objections to Loey's interpretation of the early crenation of the margin of the cephalic plate, for example, in *Squalus*. The weightiest part of Neal's contention, as it appears to us, is not merely negative, as C. Hill represents it, but resides in the positive statement that the beaded thickenings found are not only asymmetrical but are quite variable in different specimens."

Johnston ('05), ignoring Neal's objections, accepts the results of Loey and Hill on the ground that "the work of these last two authors is evidently most painstaking and their results are so complete and so far in agreement that they may be taken to represent the present state of knowledge of the neuromeres." Yet von Kupffer ('06, p. 164), working on chick embryos, finds that Hill's 'astonishing pictures' of the neuromeres of the chick give the impression that "the subjective motive of the investigation had influenced too much the completion of the drawings." Kupffer states (p. 248) that in spite of equally extended observations he was not able to confirm Hill's results. Gräper ('13) also has been unable to find Hill's neuromeres in the chick.

Therefore, in view of the disagreement among students of neuromerism regarding the nature and number of true neuromeres, and the persistent doubt regarding the results of Locy and Hill, morphologists may still feel skeptical regarding any scheme of metamerism based upon the segmentation of the nervous system and uncontrolled by the evidence of mesodermic segmentation.

The writer finds himself in agreement with Belogolowy ('10 a, p. 510) in the opinion that the neuromeres have only a secondary importance as criteria of the primitive segmentation of the head. The latter states (p. 515) that:

Without having any organic relations to the functional activities of the nervous system, and presenting merely form changes of the neural tube, the neuromeres in my opinion, can serve at best merely as topographic landmarks in the study of the nervous centers. The complete lack of any satisfactory explanation of their appearance and the indications of the possibility of their purely secondary formation under the influence of this or that mechanical factor acting on the nervous system—as in the case of the constrictions of the spinal cord under the pressure of the somites—limits to the utmost their employment as criteria of metamerism.

Johnston's assertion ('05, p. 234) that "nervous structures represent more segments than have preserved in the mesoderm;" that "in other words, there are preserved vestiges of nerve structures belonging to segments whose entodermal and mesodermal organs have disappeared for the most part" begs the entire question. The fact that the brain shows a larger number of divisions—whether the problematical marginal beadings of Locy or the secondary subdivisions of the differentiated tube—than does the mesoderm, does not prove that the nervous divisions are ancestral or primitive. Moreover, there is no reason for assuming that mesodermic segments have disappeared in the head region of *Squalus*. As a matter of fact, the number of somites in *Squalus* corresponds with the number of primary brain vesicles. In this numerical correspondence we have the strongest proof of the metameric value of these two segmental structures. Moreover, this inference accords more fully with the conclusions

of comparative anatomy than does the assumption of more numerous pre-oral segments.

Assuming on such grounds the metameric value of those primary brain vesicles or neuromeres which correspond numerically with the somites of Van Wijhe, more metameres are found anterior to the ear than are admitted in the schemes of metamerism of Ziegler and Brohmer, but fewer than in those of Johnston and Belogolowy. In figure 76 are diagrammatically expressed the primitive segmental relations based on the assumption of a correspondence of primary neuromeres and Van Wijhe's somites. That the abducens may be regarded as the somatic motor nerve of a metamere posterior to the one whose myotome it innervates seems indicated by its relations. Neuromeric relations therefore afford an important clue to the primitive metameric relations of the head. For this reason neuromeric segmentation may not be disregarded in any attempt to define the metamerism of the head.

7. *Are these metameres serially homologous with those of the trunk?*

A summary of the evidence gathered by the last two generations of vertebrate morphologists convincingly demonstrates that the vertebrate head possesses a metamerism comparable with that of the trunk. First and most important, a true somitic segmentation occurs in pre-otic and post-otic regions alike. An identical segmentation characterizes selachian (Van Wijhe '82), amphibian (Miss Platt '97) and cyclostome (Koltzoff '01) embryos. That is to say, these craniotes pass ontogenetically through an acraniate stage, comparable to the adult form of *Amphioxus*.

The discovery by Koltzoff ('01-'02) that in embryos of *Petromyzon* a series of somites—exactly homologous with those discovered by Van Wijhe ('82) in selachian embryos—occurs, not a single one of which disappears in ontogeny so that the muscular metamerism is unbroken as in *Amphioxus*, appears to the writer to be one of the most important made during the present generation. The recent rehabilitation of *Amphioxus* as an ancestral type by Delsman ('13) seems to justify the hope that

the ancestral history of the head may yet be known and general agreement among morphologists be attained.

In both pre-otic and post-otic regions of the body the mesodermic corresponds numerically with the neuromeric segmentation. While the topographic alternation is not clear in the head as in the trunk, the relations, both nervous and numerical, indicate a primitive correspondence.

The somatic motor column continues uninterruptedly from post-otic into pre-otic regions. In both regions nervous connection with somitic musculature is effected similarly by the movement of the protoplasm of the neuroblasts lying in that column. The secondary connection of nerve and muscle in both regions affords the possibility of the acquisition of new metameric relations such as appear in the case of the abducens nerve. The relations of this post-otic nerve to pre-otic myotomes indicates that no fundamental difference distinguishes the two regions.

The somatic motor nerves acquire relations with somatic sensory nerves and with sympathetic anlagen in the pre-otic region in precisely the same manner as do spinal somatic motor nerves. The misinterpretation of these relationships has long obscured the perception of their true morphology and delayed the acceptance of the conclusion that head metameres are comparable with those of the trunk.

Typical pre-otic metameres, represented by the midbrain-oculomotor-premandibular and by the hindbrain-trochlear-mandibular segments, possess all of the essential components of typical trunk metameres, namely, myotome, sclerotome, neuromere, somatic motor and somatic sensory nerves, and sympathetic anlagen. Their morphological comparability can be doubted only by doubting facts which have been repeatedly confirmed. Neither comparative anatomy nor embryology justify the speculation that these elements are of exogenous, post-otic origin.

Over against such evidence, we have differences between pre-otic and post-otic regions such as would be expected in highly differentiated regions. But the considerations advanced above indicate that these differences are differences of detail and are not fundamental. They would appeal more strongly as objec-

tions to those not fully conversant with the embryos of selachians and cyclostomes, or those whose views are affected by some divergent view of the ancestry of vertebrates. Ontogenesis strongly favors the view that the metamerism of the vertebrate body extends throughout its length and that the metameres of the head are morphologically similar to those of the trunk.

8. The primary segmentation of the pre-otic region: Conclusions

The evidence presented in the present paper scarcely justifies the attempt to draw conclusions regarding the maximal number of pre-otic metameres. Admirable attempts in this direction have recently been made by Johnston ('05 a) and Belogolowy ('10 a). The writer is convinced, however, in the light of the evidence now at hand that we may make positive assertions as to the minimal number of pre-otic segments, and as to their essential constituent elements.

The vertebrate head anterior to the ear consists of at least five metameric divisions, diagrammatically represented in figures 73 to 76. Their more important constituents may be summarized as follows:

The most anterior pre-otic metamere contains a well defined mesodermic segment, the anterior somite of Miss Platt ('90), the early degeneration of which is correlated with the absence of a somatic motor nerve in this metamere. The homology of this somite with the adhesive organ of *Amia* (Reighard '02) does not invalidate the comparison with a trunk somite, however surprising such a modification of a somite may appear. The absence of a sympathetic ganglion is to be expected in a metamere devoid of a motor nerve. The *nervus terminalis* appears to be the somatic sensory element of the segment. The writer agrees with Belogolowy ('10 a) in regarding this as the primary nerve of the olfactory apparatus, and its ganglion the primary ganglion of the olfactory nerve.² Burekhardt's assertion that the *nervus terminalis* of selachians contains motor fibers needs con-

² Bröökover has recently confirmed this opinion on the basis of observations upon *Amia* embryos.

firmation. The neuromere of the first cephalic metamere is the primary forebrain vesicle. None of the secondary subdivisions of the vesicle are morphologically comparable with hindbrain neuromeres. Notwithstanding the fact that morphologists, including Dohrn, von Kupffer, Johnston, and Belogolowy, assume a larger number of segments in this region of the brain, the writer is unable to accept their conclusions as well-founded. They rest upon evidence of equivocal neuromeres, doubtful nerves, problematic microcoelic cavities, the walls of which Dohrn has called somites, upon the far-fetched homology of the eye with dorsal ganglia and other debatable grounds. The so-called thalamic nerve is simply a persistent strand of neural crest cells which never shows neuroblast nor fiber. If it have any phylogenetic significance at all, it is not to be regarded as evidence of an additional member of the series of somatic sensory nerves, but as the old cellular root of the profundus nerve, the fibers of which now enter the brain along the profundus commissure and through the Gasserian ganglion. An optic neuromere is recognized by Johnston and Belogolowy on the debatable ground of the homology of the optic vesicle with a portion of the neural crest. In view of the doubt regarding the phylogenesis of the paired and the pineal eyes and the great uncertainty whether the former were primitively dorsal or ventral, the comparison of these structures with the anlagen of ganglionic nerves and with each other appears decidedly premature.

The assumption of Hoffmann ('94) that the anlagen of the ganglionic nerves are hollow outpocketings of the neural tube is a concept rather than a percept. No one has ever seen in sections of well preserved embryos the neural crest appear as hollow outpocketings with a lumen continuous with that of the tube. The basis for such a conception as that of Hoffmann consists of the doubtful evidence of two layers of cells in the nerve anlagen. The lumen is a product of the imagination.

Moreover, it is not so certain as would be desirable for a confirmation of the hypothesis that the paired eyes were primarily dorsal structures. The lowest vertebrates—including *Amphioxus* in that category—show the eye as a ventral or lateral structure

and not as a dorsal one (Parker '08). Granting the fact that vertebrates have existed—and still exist—with eyes near each other and the median plane, the evidence that this was the primitive relation is wanting.

In the light of the evidence now in our possession, all that may be affirmed with assurance with regard to the metamerism in the forebrain region is that in this region we have at least a single metamere, serially homologous with those of the trunk. The morphologist who goes farther than this and affirms the polymerism of the forebrain segment is skating on extremely thin ice.

The elements of the second metamere are shown in the diagrammatic cross-section represented in figure 73. The myotome is the premandibular and the neuromere the midbrain. The somatic sensory nerve is the ophthalmicus profundus and the sympathetic ganglion is the ciliary. That the ophthalmicus profundus trigemini was once an independent segmental nerve seems evinced by its relations in cyclostomes. The secondary splitting of the premandibular myotome into dorsal and ventral moieties is evidently correlated with the development of the eyeball (fig. 81). The facts do not warrant the assumption of some morphologists that the oculomotor nerve—the somatic motor nerve of this metamere—has a bimeric distribution. No one who has made this assumption has been able to demonstrate the required two motor niduli. The premandibular somite is a single somite. The slight ventral fold in the wall of the midbrain is not sufficient evidence to establish the existence of two neuromeres. The large size of this neuromere, as well as that of the forebrain, is correlated with the functional importance of these portions of the brain. Their later subdivisions may be best interpreted as coenogenetic.

The third metamere consists essentially of the elements shown in figures 75, 76 and 81. Its myotome is the mandibular and its neuromere the cerebellar (neuromere III), within which lies the nidulus of the trochlear nerve, which is therefore the somatic motor nerve of the segment. The trochlear nerve becomes connected with the ramus ophthalmicus superficialis trigemini, the somatic sensory nerve of the metamere. There is evidence of

a transient sympathetic anlage. While the chiasma of the trochlear is an anomaly, it may be regarded as coenogenetic and its existence does not invalidate the comparison of this metamere with a trunk segment. The ramus mandibularis trigemini appears to be the splanchnic motor element of this metamere.

The fourth metamere contains the third or hyoid myotome and the fourth neuromere (second hindbrain neuromere). To this segment may be assigned as the somatic sensory nerve the major root of the trigeminal in part. Since no neural crest is proliferated from this neuromere, however, this assignment must be made with a question mark, although the major root of the trigeminal is attached to this neuromere. The neuroblasts in the somatic motor column of this neuromere do not produce a nerve. The transient nerve seen in this region in chick embryos (Belogolowy '10) may be the somatic motor nerve of this metamere which has disappeared phylogenetically. The myotome of the metamere, however, is innervated by the nerve of a post-otic metamere, the abducens. An attempt has been made above to explain this anomalous relationship which does not appear to vitiate the comparison with a trunk metamere.

The fifth and last pre-otic metamere includes the fifth neuromere and the fourth somite which is partly sub-otic, a position to which it presumably owes the loss of its myotome. To the degeneration of the myotome may be attributed the loss of the somatic motor nerve of this metamere. No sympathetic anlage develops in this segment and the somatic sensory components are also lost. But the proliferation of the cells of the facialis nerve from this neuromere justifies the inference that they once have been present in this nerve. The loss of the myotome of this and of the following somite, a loss in all probability due to the enlargement of nerve ganglia and sense organ in this region, tends to show that the preservation of the myotomes of the first, second and third somites is due to their functional relation with the eye-ball. The eye muscles are the last remnants of the lateral trunk musculature anterior to the ear. Their earlier relations with post-otic myotomes are diagrammatically expressed in figure 81.

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ABBREVIATIONS

- | | |
|--|---|
| I to VIII, neuromeres or encephalomerer | <i>cl.crs.n.</i> , neural crest cells |
| 1 to 9, somites of VanWijhe | <i>cl.n'bl.</i> , neuroblast cell. |
| 1 ^d to 6 ^d , dorsal divisions of myotomes | <i>cl.R.B.</i> , Rohon-Beard cell |
| 1 to 6 | <i>d.pro.</i> , pronephric duct |
| 1 ^v to 6 ^v , ventral divisions of myotomes | <i>ec'drm.</i> , ectoderm |
| 1 to 6 | <i>en'drm.</i> , entoderm |
| <i>V.md.</i> , ramus mandibularis trigemini | <i>fis.vsc.</i> , visceral clefts |
| <i>V.mx.</i> , ramus maxillaris trigemini | <i>gls.phy.</i> , glossopharyngeus |
| <i>V.opt.su.</i> , ramus ophthalmicus superficialis trigemini | <i>gn.dors.</i> , dorsal ganglion |
| <i>V.opt.p'fnd.</i> , ramus ophthalmicus profundus trigemini | <i>gn.opt.pf'nd.</i> , ganglion of the r. ophthalmicus profundus |
| <i>V.trec.</i> , ramus recurrens abducens | <i>gn.sym.</i> , sympathetic ganglion |
| <i>VIIac.</i> , ramus acusticus facialis | <i>gn.vag.</i> , vagus ganglion |
| <i>VIIbuc.</i> , ramus buccalis facialis | <i>hyp.</i> ¹ , first, or transient, root of hypoglossus |
| <i>VIIhoi.</i> , ramus hyoideus facialis | <i>l.</i> , lens |
| <i>VIIopt.su.</i> , ramus ophthalmicus superficialis facialis | <i>la.ct.</i> , cutis plate |
| <i>a.</i> , 'anterior' somite of Miss Platt | <i>la.mu.</i> , muscle plate |
| <i>abd.</i> , abducens nerve anlage | <i>m.</i> , mouth |
| <i>ao.d.</i> , dorsal aorta | <i>ms'ec'drm.</i> , mesectoderm |
| <i>arc.vsc.</i> , visceral arch | <i>ms'ench.</i> , mesenchyma |
| <i>ax.</i> , axone process | <i>ms'en'drm.</i> , mesentoderm |
| <i>cd.d.</i> , chorda dorsalis | <i>mb.cl.</i> , cell membrane |
| <i>ch.dors.</i> , dorsal chiasma | <i>mb.lim.</i> , limiting membrane |
| | <i>mu.hyp.</i> , hypoglossus musculature |
| | <i>mu.obl.sup.</i> , superior oblique musele |

<i>my.</i> , myotome	<i>n'v.</i> ² , second post-otic nerve in Petro-
<i>my.</i> ^{1,2,3-8} , myotomes of VanWijhe's so-	myzon
mites 1 to 8	<i>oc'mol.</i> , oculomotorius nerve anlage
<i>my.</i> ^{2dl.} , dorso-lateral division of the	<i>ot.</i> , otic vesicle
second myotome	<i>pl.</i> , plasma
<i>my.</i> ^{2vl.} , ventro-lateral division of the	<i>rx.d.</i> , fiber bundle of dorsal nerve
second myotome.	<i>rx.v.</i> , fiber bundle of ventral nerve
<i>my.</i> ^{2m.} , median division of the second	<i>scl.</i> , sclerotome
myotome	<i>so.</i> , somite
<i>my.</i> ^{4dl.} , dorso-lateral division of the	<i>sp.</i> , spiracle
first post-otic myotome	<i>subch.</i> , sub-chordal rod
<i>my.</i> ^{4vl.} , ventro-lateral division of the	<i>tb.n.</i> , neural tube
first post-otic myotome	<i>thyr.</i> , thyreoid
<i>my.</i> ^{4m.} , median division of the first	<i>tr'ch.</i> , trochlearis
post-otic myotome	<i>tr'ch.dors.</i> , dorsal branch of the troch-
<i>n.</i> , olfactory pit	learis nerve
<i>nidl.</i> , nidulus, or 'motor nucleus' of	<i>tr'ch.vent.</i> , ventral branch of the troch-
nerve	learis nerve
<i>nidl.abd.</i> , nidulus of abducens nerve	<i>tr.art.</i> , truncus arteriosus
<i>nidl.oc.</i> , nidulus of oculomotor nerve	<i>vac.</i> , vacuole
<i>nidl.tr'ch.</i> , nidulus of trochlearis nerve	<i>vag.</i> , vagus nerve
<i>n'v.</i> ¹ , first post-otic nerve in Petro-my-	<i>vn.crd.</i> , cardinal vein
zon	<i>vs.opt.</i> , optic vesicle

PLATE I

EXPLANATION OF FIGURES

All the figures of this plate were drawn with Abbe camera, one-twelfth homogeneous oil immersion objective and No. 6 compensation ocular of Zeiss. In reproduction the magnification has been reduced by one-third. The series of drawings illustrates the stages just preceding and following the appearance of the anlagen of somatic motor nerves in the trunk region of *Squalus* embryos.

1 A portion of a cross-section (DK 2-3-10) of a *Squalus* embryo with eight somites (Stage D of Balfour) in the middle trunk region showing the relations of neural tube, somite, and chorda. The absence of plasmodesmata or protoplasmic strands connecting neural tube and somite is to be noted. A vacuolated plasma fills the intercellular space between the neural tube and the myotome.

2 A portion of a cross-section (IK 3-2-37) of a *Squalus* embryo of 6 mm. (Balfour's Stage I) in the middle of the trunk region, showing the conditions just previous to the appearance of a ventral nerve anlage. The section is taken from an embryo of about twice the length of the one from which figure 1 was drawn. The section shows no indication of protoplasmic or nervous connection between the neural tube and the somite. The outflow of processes from the sclerotome cells (*scl.*) is the beginning of the movement of mesenchymatous cells

PLATE I: EXPLANATION OF FIGURES (CONTINUED)

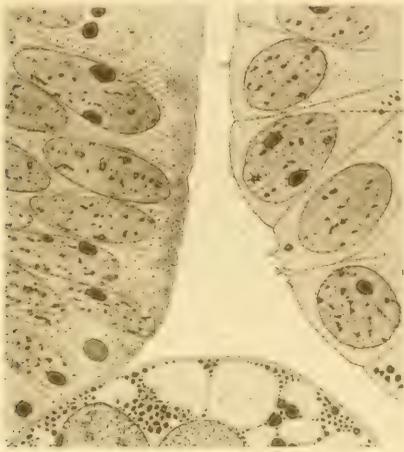
into the region between myotome and neural tube. The anlagen of the somatic motor nerves of the myotomes immediately anterior to the one shown in the section have already made their appearance as protoplasmic outflows from the neural tube, an outflow which is correlated with a movement of the sclerotome cells.

3 A portion of a cross-section (II 5-3-12) of a *Squalus* embryo of 7 mm. (Balfour's Stage I) in the cloacal region, showing the relations of neural tube and somite immediately before the appearance of a somatic motor nerve. Nervous, or protoplasmic, connection of neural tube and myotome has already been effected in the metameres just anterior to the one from which this section was taken. The deeper staining properties of the cell (*cl.n'bl.*) may indicate that it is a neuroblast about to extend a process toward the somite. The migration of the mesenchyma cells from the sclerotome has already begun. This and figures 1 and 2 show that before outflows of neuroblastic cells make their appearance there is no protoplasmic connection between the nervous and muscular systems. In the intercellular space between the two, however, may be demonstrated a dilute, plasmoid substance containing a minimal amount of vacuolated coagulable substance more resistant to stains than the cellular protoplasm. To show it at all in a drawing greatly exaggerates its visibility.

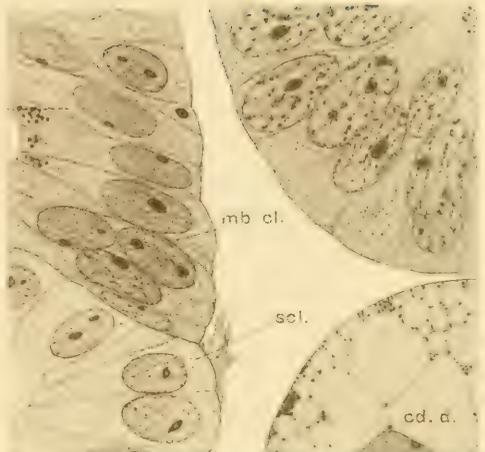
4 A cross-section (II 4-3-11) of a *Squalus* embryo of 7 mm. (Stage I of Balfour) in the trunk region just anterior to the cloaca, showing a very early stage of protoplasmic connection between neural tube and myotome, established by an amoeboid outflow of a single neuroblast cell of slightly deeper staining properties than those of the surrounding cells. The pseudopodial processes extend in various directions toward the somite and show finer branches which have connections with the vacuoles of the intercellular plasmoid substance. That the processes are genetically related to the neuroblastic cell, however, is evinced by their staining properties.

5 A portion of a cross-section (II 4-2-1) of the same embryo as the one shown in figure 4 in the second metamere anterior to it. At least two cells appear to participate in the protoplasmic outflow from the neural tube. Focussing brings out the fact that the amoeboid processes extend antero-posteriorly along the surface of the myotome as well as dorso-ventrally. The neuroblast shows the characteristic deeper staining qualities of the neuroblast cell.

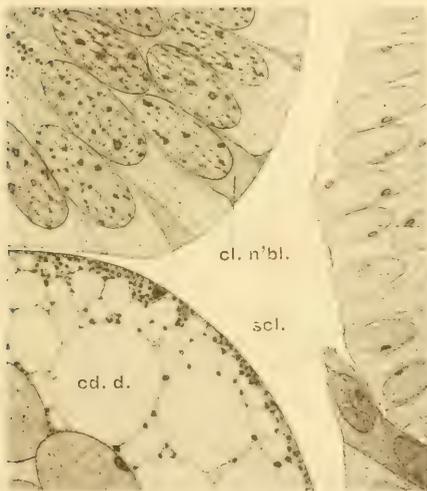
6 A portion of a cross-section of the same embryo as figures 4 and 5. The section (II 4-2-14) is through the metamere immediately posterior to that in figure 5 and anterior to that in figure 4. As compared with figure 5, the amoeboid processes seem further extended and the limiting membrane of the neural tube seems interrupted for a greater extent than in the sections anterior and posterior. The outer boundaries of at least three cells are extended beyond the limiting membrane of the neural tube.



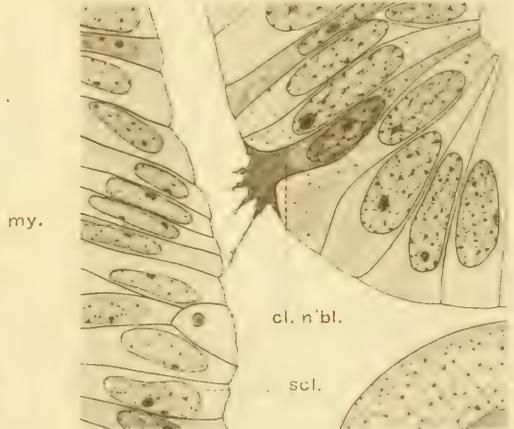
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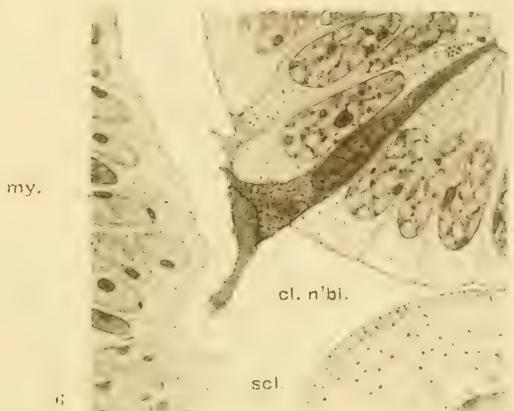
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6

EXPLANATION OF FIGURES

All the figures of this plate were drawn with the same lenses, etc., as those of plate 1. In reproduction the magnification has been reduced one-third. The series illustrates stages in the development of the anlagen of spinal somatic motor nerves in *Squalus* embryos up to the time of migration of medullary cells into the nerve.

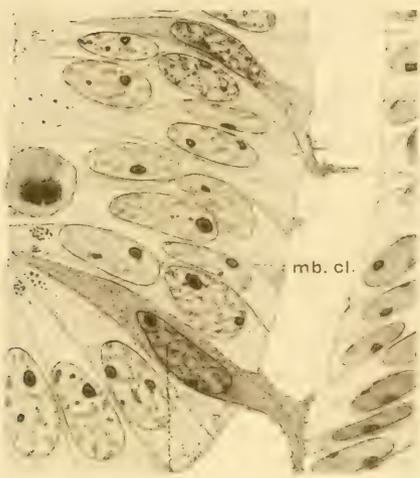
7 A portion of a cross-section (IL 4-2-17) of a *Squalus* embryo of 7 mm. (Stage I of Balfour) of the same series as those shown in figures 4, 5 and 6, showing the anlage of the somatic motor nerve of the same metamere as that in figure 6 but of the opposite side of the body and three sections posterior. The evidence that the protoplasmic connection between the neural tube and somite is a derivative of the neural tube is the same as that shown in the earlier figures. The section also shows a protoplasmic outflow from a cell in the lateral wall of the neural tube. Such outflows are exceptional and do not appear to persist in later stages.

8 and 9 Portions of two adjacent sections (II 5-2-17 and II 5-2-18) in the cloacal region of a 7 mm. embryo, showing an early stage in the development of a plasmodesm or anlage of a somatic motor nerve. The protoplasmic connection between the neural tube and the myotome consists of two elements, one derived from the neural tube, that is, a portion composed of the processes of neuroblasts (*cl.n'bl.*) labeled *ax.* in the figure, and the other consisting of migrant cells from the sclerotome of the somite (*scl.*). The two elements are readily distinguishable in the sections, since the processes of the neuroblastic cells stain more deeply than the sclerotome cells and the limiting membranes of the cells are shown distinctly.

10 A portion of a cross-section (IL 4-1-30) of a 7 mm. embryo in the middle trunk region where the myotome and sclerotome lie somewhat nearer the neural tube than in the caudal region. The protoplasmic bridge between neural tube and myotome consists of the processes of medullary neuroblasts which extend between the myotome and sclerotome along the median surface of the myotome. A comparison with earlier stages shows that the bridge is not a primary one, as thought by Held ('06) and Paton ('07), but secondary.

11 A portion of a cross-section (II 5-1-13) of a 7 mm. embryo, showing a somatic motor nerve anlage in a stage of development somewhat more advanced than that shown in figure 10. Processes of medullary neuroblasts may be traced for some distance along the median surface of the myotome, between the sclerotome and the myotome. The more deeply staining properties of the distal portions of the axone processes are noteworthy, in connection with the problem of the origin of the neurofibrillae. The stage corresponds essentially with Paton's ('07) figure 2. The difference in the phenomena and resultant difference in interpretation may be ascribed largely to the difference in the methods of staining and of preservation. What Paton regards as a neurofibril arising in the myotome independently of the nervous system is actually, as shown in sections of embryos preserved by the vom Rath method, the distal portion of the neuraxone process of a medullary neuroblast. Instead of appearing within the myotome as stated by Paton, the actual position, as seen in figure 11, is between the myotome and sclerotome. The results obtained by Paton's excellent method of staining the neurofibrillae need to be controlled by a comparison with methods which, like that of vom Rath, bring out the cell boundaries.

12 A portion of a cross-section (IL 3-2-42) of a 7 mm. embryo, showing an early stage in the process of migration of medullary cells into a somatic motor nerve anlage in the middle trunk region. Evidence has been given in a former paper ('03) that these cells are chiefly, if not entirely, concerned in the process of formation of the neurilemma of the somatic motor fibers. As shown in the figure, the breaking up of the sclerotome into loose mesenchyma has already begun. The relation of the fibrillar portion of the nerve anlage to neuroblasts in the neural tube is not shown in the section, a result of the bending of the axones upon their emergence from the neural tube.



tb. n.
mb. lim.
pl.
my.
cd. d.

cl. n'bl.

7



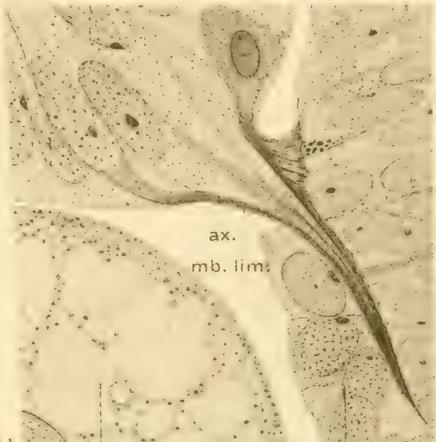
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tb. n. cl. n'bl. scl. my.



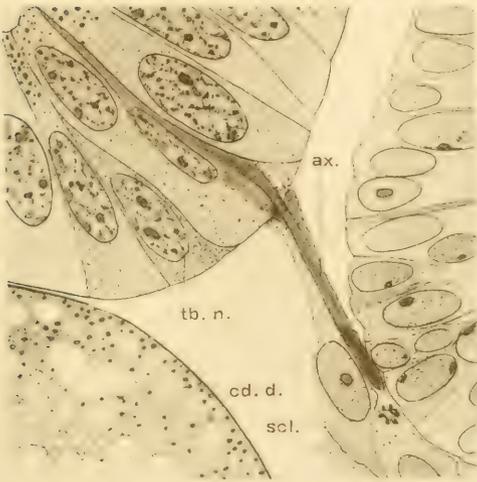
cd. d. mb. cl. scl. ms'en'drm.

8

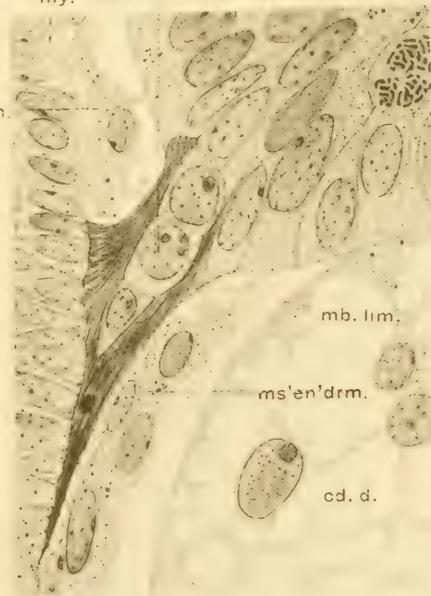


11

my. cd. d. ms'ec'drm. scl.



tb. n.
cd. d.
scl.



12

PLATE 3

EXPLANATION OF FIGURES

All the figures of this plate were drawn in the same way as those on plates 1 and 2. Magnification reduced one-third in reproduction. The series of figures on this and the following plate illustrate stages in the extension of the neuraxon processes of the Rohon-Beard cells. The phenomena appear analogous with those shown in the formation of the anlagen of ventral motor nerves (plates I and II).

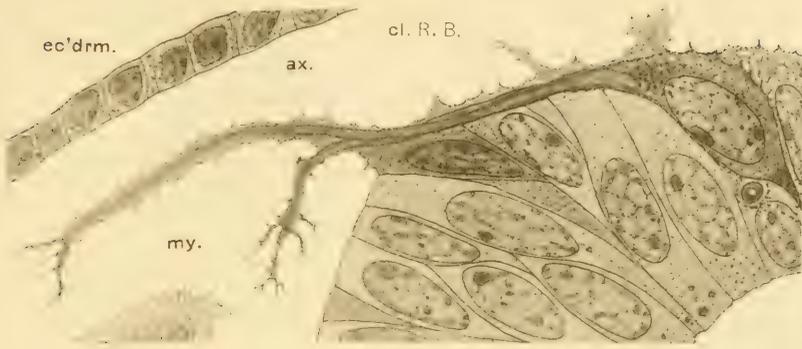
13 A portion of a cross-section (IJ 4-1-22) of a 6 mm. embryo in the middle trunk region. A portion of the ectoderm and of the dorsal wall of the neural tube are shown. The amoeboid process (*pl.*) of a medullary cell is strikingly similar to those which form the ventral nerve anlagen. Finer branches of the protoplasmic processes show definite relations to the vacuolated intercellular plasma, but no evidence that this relation is a genetic one. The phenomena are entirely in harmony with the supposition that the amoeboid protoplasmic process has a genetic relation to the medullary cell. Earlier stages show no evidence of protoplasmic continuity between neural tube and ectoderm.

14 A portion of a cross-section (IK 2-6-37) of a 6 mm. embryo in the middle trunk region, showing a portion of the dorso-lateral wall of the neural tube and a Rohon-Beard cell with deeply stained neuraxon process extending beyond the limiting membrane of the neural tube. Similar outflows of adjacent cells may also be seen, and the phenomena strikingly resemble those presented in the formation of the somatic motor nerves (plates 1 and 2).

15 A portion of a cross-section (sections IK 2-3-22 and IK 2-3-23 combined) of a 6 mm. embryo in the middle trunk region, showing a part of the dorso-lateral wall of the neural tube and the greatly elongated neuraxon process of a Rohon-Beard cell which may be traced through the wall of the neural tube into the plasma-filled space between the neural tube and the ectoderm. The pseudopodia-like extensions of the distal extremity of the neuraxon show relations to the vacuolated plasma, but such evidence does not prove any genetic relationship between the two, nor a primary protoplasmic relationship between the neuroblast cell and its peripheral distribution.

16 A portion of a cross-section (sections II 5-1-31, II 5-1-32 and II 5-1-33 combined) of a 6 mm. embryo in the middle trunk region, showing the dorso-lateral wall of the neural tube and the neuraxon processes of two adjacent Rohon-Beard cells, one of which extends into the space between myotome and ectoderm the other between myotome and neural tube, the neuroblast cell of the latter not shown. The neuraxon processes appear distinctly fibrillar only in the proximal portion. Since the distribution of these neuraxon processes in later stages is essentially the same, *viz.*, the extra-embryonic ectoderm, the different relations to the dorsal part of the myotome appears to favor the view that the path by which a neuraxon finds its way to its terminal organ is a matter of chance rather than a predetermined one along an intercellular bridge. Moreover, the amoeboid processes do not extend in one direction, as would seem demanded by the latter assumption, but irregularly in different directions.

17 to 20 (See p. 170).



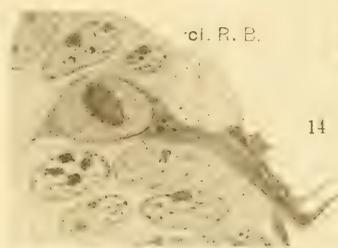
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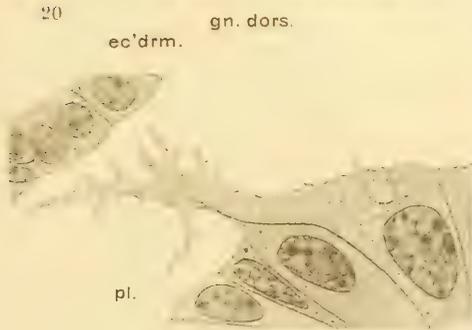
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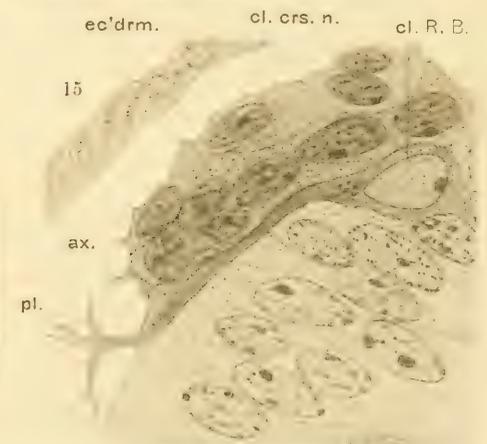
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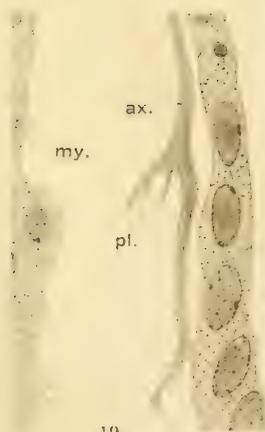
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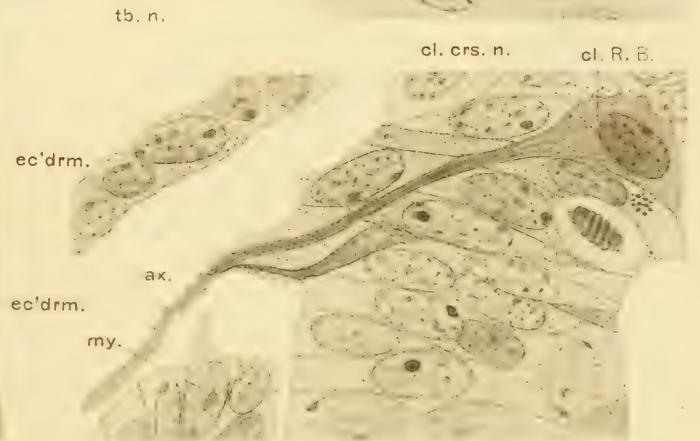
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15



19



17

PLATE 3: EXPLANATION OF FIGURES (CONTINUED)

17 A portion of a cross-section (sections IK 2-4-15 and IK 2-4-16 combined) of a 6 mm. embryo in the middle trunk region, showing the processes of two Rohon-Beard cells, one of which lies out of the plane of the section. The conditions resemble those shown in figure 16, except that the processes appear confluent instead of divergent. The neurofibrillae are well differentiated in the proximal portion of the neuraxon.

18 and 19 Portions of cross-sections (IK 2-5-6 and IK 2-5-37) of a 6 mm. embryo in the middle trunk region, showing the distal termination of the growing neuraxon process of a Rohon-Beard cell as it extends into the space between the myotome and the ectoderm. In the case of the process in figure 18 the growing end lies nearer the myotome, while in figure 19 the growing end lies nearer the ectoderm. Comparison with earlier stages shows that in this region there are no protoplasmic bridges in the region shown in the drawing—the intercellular spaces are filled with an unstained vacuolated plasma. The brush-like termination is a characteristic feature of the neuraxon process.

20 A portion of a cross-section of a 7 mm. embryo in the middle trunk region, showing a Rohon-Beard cell on the top of a spinal ganglion. Its deeply staining neuraxon process contrasts strongly with the surrounding mesenchyma, and the evidence of the genetic relation of ganglion cell and neuraxon process seems more convincing than in the case of the cells which are imbedded in the wall of the neural tube. The process may be traced to a point lateral to the myotome. The section figured however does not show the peripheral termination of the process, which bends out of the plane of the section.

PLATE 4

EXPLANATION OF FIGURES

21 and 22 Portions of cross-sections of 6 mm. embryos in the middle trunk region, showing Rohon-Beard cells in somewhat advanced stages of the extension of the neuraxon processes. Figure 21 is a combination drawing from three sections (II 4-4-26, 27, 28). Figure 22 is a combination of two sections (IK 2-6-29, 30). The presence of neurofibrillae in the protoplasmic processes evinces their nervous character. The distal extremity of these neuroblast processes, however, is granular, vacuolated and extended into pseudopodial processes. Figure 21 does not appear to harmonize with the assumption of predetermined paths for the extension of the nervous outgrowths, but rather with the view that neuraxons are pseudopodia-like outgrowths of neuroblasts. The variation in the form of the amoeboid termination of the neuraxon processes shown in the two figures is striking and significant.

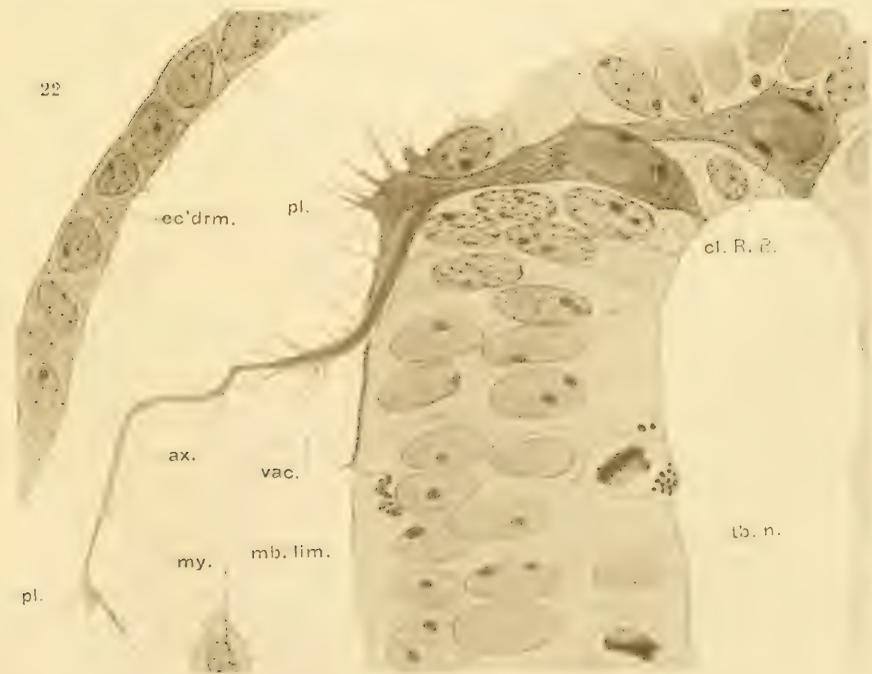
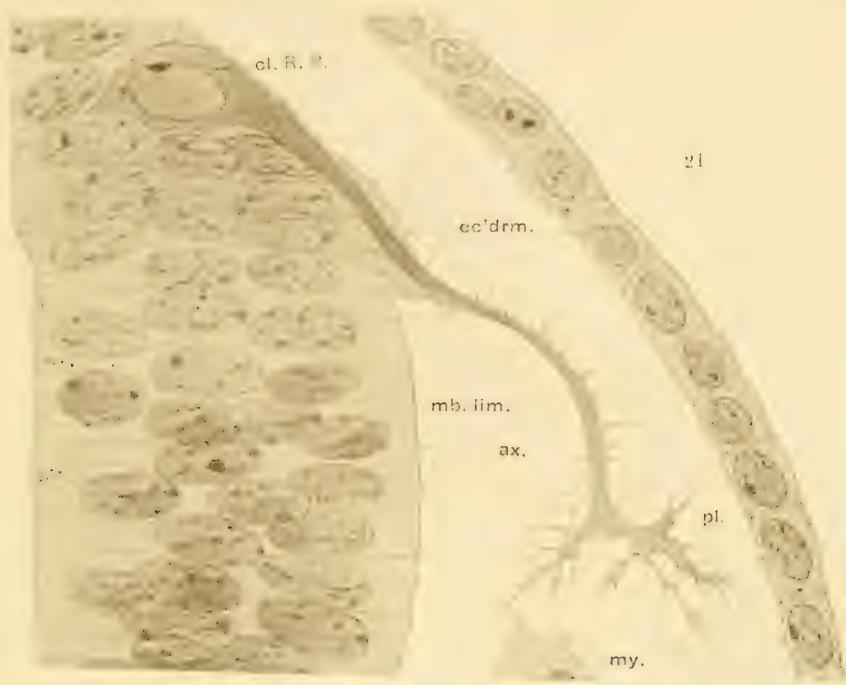


PLATE 5

EXPLANATION OF FIGURES

All the figures are based on camera drawings. Figures 23 to 28 inclusive are a series of semi-diagrammatic drawings, illustrating the more important stages in the development of spinal somatic motor nerves and summarizing the essential features of the histogenesis of spinal ventral nerves. Figures 29 and 30 are camera drawings of spinal nerves with one-twelfth homogeneous oil-immersion objective, No. 6 compensation ocular of Zeiss, and camera lucida of Abbe. Reduction one-third in reproduction.

23 A semi-diagrammatic drawing of a portion of a cross-section (IH 1-6-9) of a 6 mm. embryo in the region of the cloaca. The anlage of the somatic motor nerve is seen as a structure consisting of the protoplasmic processes of neuroblasts in the ventrolateral wall of the neural tube. The migration of the mesenchyma of the sclerotome has already begun.

24 A portion of a cross-section (IE 2-1-last), of a 9 mm. embryo in the caudal region, showing a somewhat more advanced stage in the development of a ventral nerve. The neuraxon processes of the medullary neuroblasts have extended farther along the median surface of the myotome and the mesenchyma cells have advanced to a point nearer the neural tube.

25 A semi-diagrammatic drawing of a cross-section (IE 2-7-27) of a 7 mm. embryo in the middle trunk region, showing the beginning of the migration of medullary cells into the anlagen of somatic motor nerve and neural crest. The relation of the fibrillar portion of the somatic motor nerve to neuroblasts is shown in the figure.

26 A semi-diagrammatic drawing of a portion of a cross-section (IH 2-1-20) of a 9 mm. embryo in the trunk region showing a somewhat more advanced stage in the development of a somatic motor nerve. Medullary cells are still in the process of migration from the neural tube along the nerve anlage. The ganglion of the dorsal nerve has been formed by the migration and aggregation of neural crest cells between myotome and neural tube. A Rohon-Beard cell in an early stage of formation of a neuraxon process appears in the dorsal wall of the neural tube.

27 A semi-diagrammatic drawing of a cross-section (KJ 3-4-middle) of an 11 mm. embryo in the middle trunk region, showing a more advanced stage in the differentiation of a somatic motor nerve. The emigrated medullary cells at this stage form a sheath enclosing the fibrillar portion of the nerve. Neuroblasts have become differentiated within the dorsal ganglion. A Rohon-Beard cell with neuraxon process extending between myotome and ectoderm is shown.

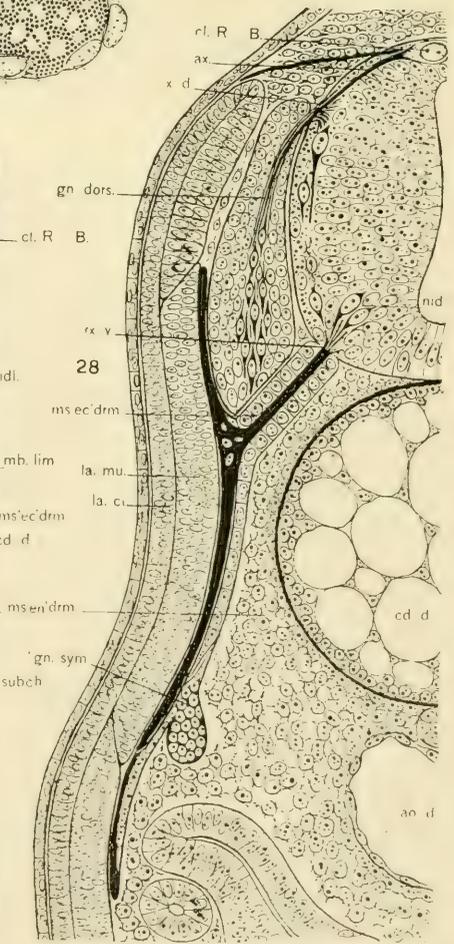
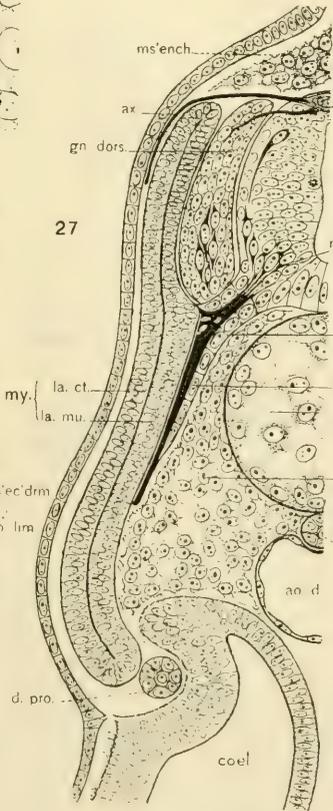
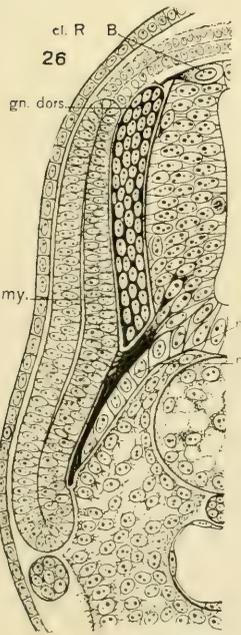
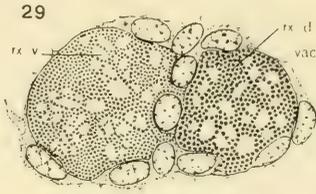
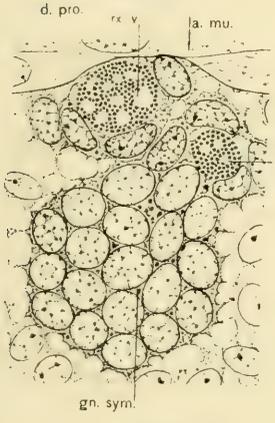
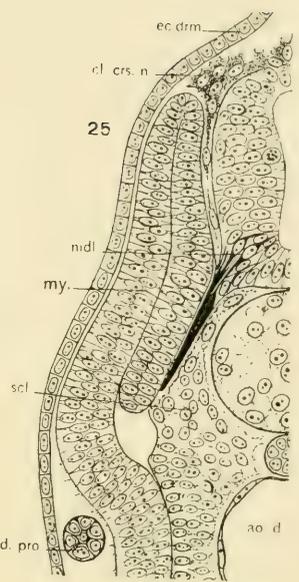
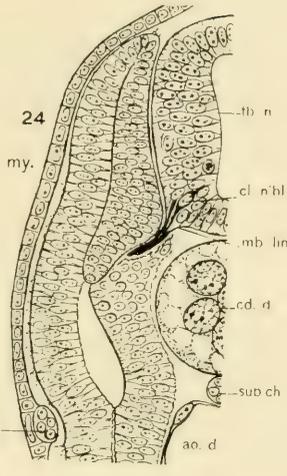
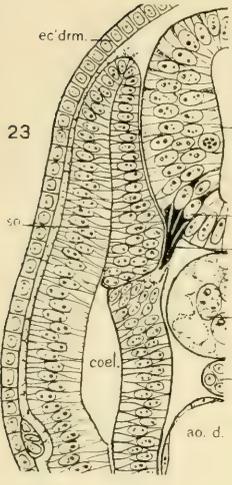
28 A semi-diagrammatic drawing of a cross-section (LAB 5-3-17) in the middle trunk region of a 17 mm. embryo, showing a stage in the development of a somatic motor nerve, when the anlage of a sympathetic ganglion has made its appearance at a point opposite the dorsal aorta and median to the trunk of the spinal nerve of the metamere. At this stage fibrous connection of the dorsal ganglion with the neural tube has been established and both dorsal and ventral rami have reached a considerable extent in their development. The migration of medullary cells into the somatic motor root has ceased. The cells that have

PLATE 5: EXPLANATION OF FIGURES (CONTINUED)

wandered into the nerve anlage form a sheath around the fibrillar part of the nerve. The section shows a Rohon-Beard cell with neuraxon entering the root of the dorsal nerve—a rather exceptional relation.

29 A cross-section of a spinal nerve (MM 21-2-4) of a 46 mm. *Squalus* embryo, showing the fibrillar portion of the nerve consisting of deeply stained fibrils and a surrounding layer of sheath cells, presumably of medullary origin. The neuraxon fibers of the sensory bundle are somewhat larger in diameter than those of the motor bundle. Both appear as highly refractive granules, separated by a somewhat vacuolated intercellular plasma.

30 A cross-section of a spinal nerve in a 17 mm. embryo, showing sensory and motor components at the level of a sympathetic ganglion. The motor bundle lies nearer the myotome than does the sensory bundle, and each is surrounded by a single layer of sheath cells. The sympathetic ganglion anlage lies median to and somewhat between the two.



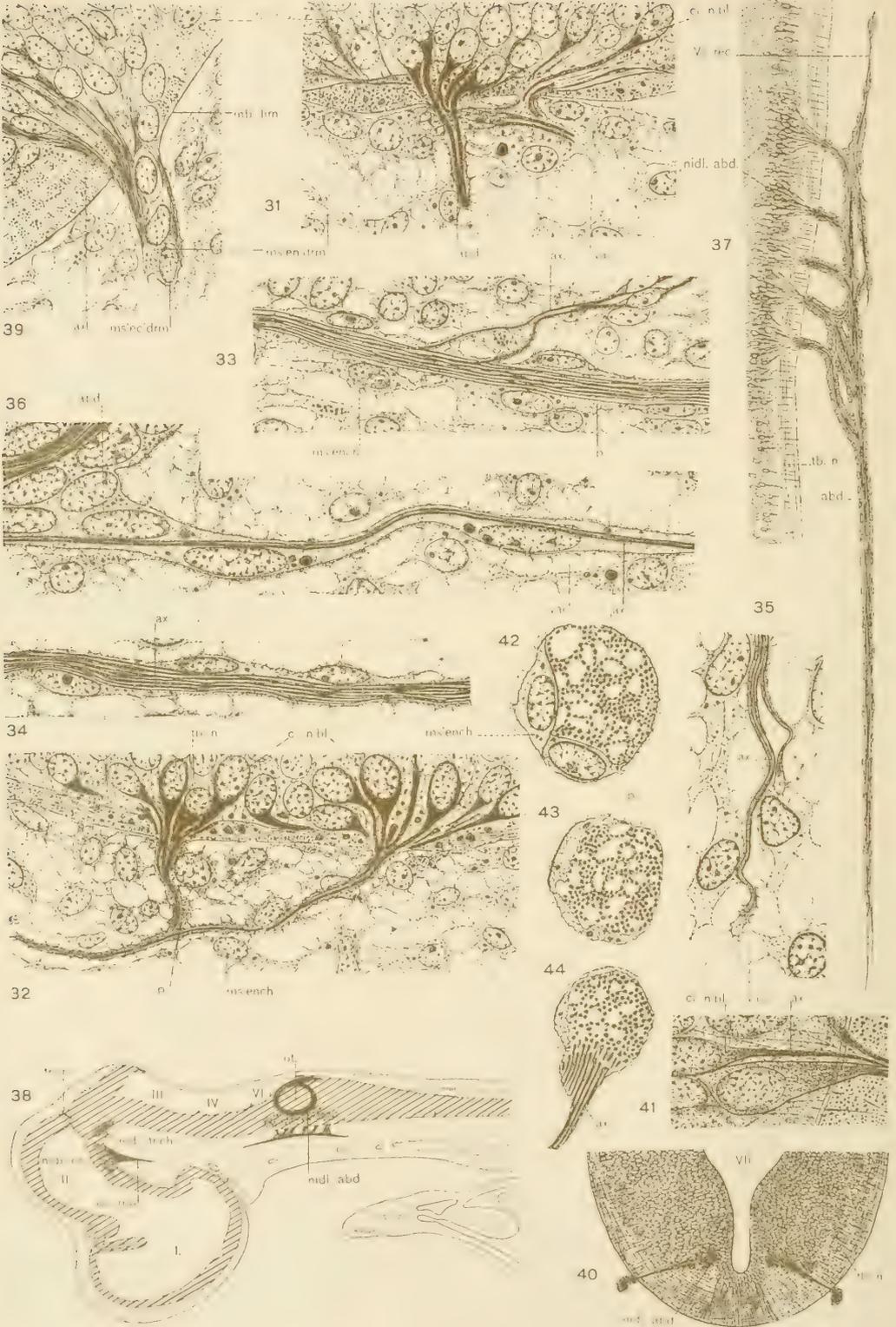


PLATE 6

EXPLANATION OF FIGURES

A series of camera drawings illustrating the histogenesis of the abducens nerve. With the exception of figures 37, 38 and 40, all were drawn with one-twelfth homogeneous oil-immersion objective and compensation ocular 6 or 8 of Zeiss.

31 A portion of a parasagittal section (KP 2-1-6) of a 10 mm. embryo, showing an early stage in the development of the abducens anlage, which makes its appearance as a product of the union of the processes of neuroblasts in the ventrolateral wall of the medulla immediately posterior to the otic capsule. The presence of loose mesenchyma in the region of the nerve anlage complicates the picture, but the processes of deeply stained neuroblasts may easily be traced into the nerve anlage. The mesenchyma cells are granular, much vacuolated, and stain lightly, showing in some cases protoplasmic connections with the abducens anlage. In every essential respect the picture resembles an early stage in the histogenesis of a spinal somatic motor nerve. The chief differences consist in the relatively early appearance of a loose mesenchyma and in the remoteness of the myotome from the point of emergence of the neuraxon processes of the nerve anlage. The presence of a lightly staining granular protoplasm surrounding the deeply stained processes of the neuroblasts is noteworthy, but it is difficult to determine whether this ensheathing protoplasm is of mesenchymatous or medullary in origin. Analogy with spinal nerves would favor the conclusion that it is an outflow from medullary cells.

32 A portion of a parasagittal section (KR 1-3-4, 5, 6)—strictly, a combination of three sections—of an 11 mm. embryo, showing the anlage of the abducens nerve, when it possesses two roots and when its growing tip extends for some distance anteriorly in the mesenchyma lying at the base of the medulla. Deeply stained processes of medullary neuroblasts may be traced into the nerve anlage with its sheath of granular protoplasm. The highest powers of the microscope fail to resolve the neuraxon processes into constituent neurofibrils. As no evidence of the migration of medullary elements into the nerve anlage appears in this or in preceding stages, the cells in the vicinity of the nerve anlage are presumably mesenchymatous.

33 A portion of a parasagittal section (KS 2-2-7) of an 13 mm. embryo, showing a portion of the abducens anlage in a later stage of development than in figure 32. The nerve anlage consists at this stage of a compact bundle of deeply stained neuraxons surrounded more or less completely by a sheath of mesenchymatous cells. The section is somewhat exceptional in showing two isolated neuraxons which join the nerve anlage and each of which consists of a deeply stained fibril surrounded by a very thin, lightly stained granular protoplasmic sheath. Each of the neuraxons is also associated with a nucleus presumably of mesenchymatous origin.

34 A portion of a parasagittal section (LAA 2-1-3) of a 19 mm. embryo, showing a peripheral portion of the abducens anlage, consisting of a bundle of highly refractive neuraxons surrounded by a sheath of mesenchymatous cells and granular protoplasm.

35 to 44 (See pp. 178 and 179).

PLATE 6: EXPLANATION OF FIGURES (CONTINUED)

35 A portion of a parasagittal section (LB 2-2-6) of a 17 mm. embryo, showing the peripheral termination and growing tip of the abducens nerve anlage at a stage when it has nearly reached the posterior rectus muscle. The amoeboid termination closely resembles those of somatic motor spinal nerves and of the Rohon-Beard cells, and the section thus strongly favors the outgrowth theory of nerve histogenesis and the opinion that the abducens in its histogenesis resembles the spinal somatic motor nerves.

36 A portion of a parasagittal section (LK 2-2-10) of a 19.25 mm. embryo showing a portion of the abducens anlage in the vicinity of its point of emergence from the medulla. The section is of interest chiefly because it shows a single deeply stained fibril surrounded by a sheath of spindle-shaped cells. It was such evidence as this that led Dohrn ('91) at one time to infer that the spindle-shaped cells were genetically related to the neuraxon fiber. The evidence presented in this paper, however, tends to show that there is no genetic relation between the fiber and its surrounding cellular sheath. At this stage of development of the nerve anlage the process of migration of medullary cells has already begun and the ensheathing cells shown in the figure may be of medullary origin.

37 A graphic reconstruction of the anlage of the abducens nerve as seen in parasagittal sections of a 19 mm. embryo. The nerve arises from the base of the seventh and eighth brain neuromere (Neal), four roots emerging from the seventh and one only from the eighth. Its connection with these two distinct segments of the brain suggests the inference that the abducens is the nerve of at least two metameres. The posteriorly directed ramus recurrens of the abducens also suggests that the nerve was once distributed to musculature posterior to that which it now innervates. This branch soon atrophies. The roots of the nerve show a tendency to form a network or plexus.

38 A semi-diagrammatic drawing based on a parasagittal section through the head of a 19 mm. embryo, intended to show the position of the niduli of the eye muscle nerves. The niduli of the oculomotorius and of the trochlearis belong to adjacent metameres, while the nidulus of the abducens is several neuromeres removed.

39 A portion of a cross-section (LF 3-5-19) in the region of the nidulus of the abducens, showing evidence of the migration of medullary cells into the anlage of the nerve. The phenomena are similar to those which appear in somewhat advanced stages of histogenesis of spinal somatic motor nerves. As in the latter, the nerve anlage contains both fibrillar and cellular components, the fibrillar portion showing genetic relations with neuroblast cells in the ventro-lateral wall of the neural tube. Evidence that the cellular portion of the nerve is medullary and not mesenchymatous in origin is found in the fact that the cells first appear in the proximal portion of the nerve anlage and that, in successive stages, there appear to be more and more nuclei in this portion of the nerve, and a larger number apparently in the process of migration.

40 A portion of a cross-section (LAD 1-7-17) in the region of the nidulus of the abducens of a 19 mm. embryo, showing the ventral half of the medulla as seen under the low power microscope. The section shows the position of the nidulus of the abducens and its relation to the bundle of deeply stained neuraxon fibers which traverse the marginal zone and enter the nerve root.

PLATE 6: EXPLANATION OF FIGURES (CONTINUED)

41 A portion of the same section (LAD 1-7-17) enlarged to show the structure of the neuroblasts. These are pear-shaped, deeply stained cells with protoplasm extending into the fibrillar neuraxon process.

42, 43, 44 A series of cross-sections of the anlage of the abducens nerve as seen in a 19 mm. embryo. Figure 42 is the most posterior of the sections. The nerve anlage at this stage consists chiefly of a bundle of deeply stained neuraxons irregularly grouped together among vacuolar spaces and partly enclosed by a layer of mesenchymatous cells. In figure 44, the relations of the neuraxons of the nerve as it subdivides into a branch are shown. The highly refractive granules seen in cross-section correspond with the deeply stained fibers appearing in longitudinal sections.

PLATE 7

EXPLANATION OF FIGURES

Most of the figures are camera drawings with one-twelfth homogeneous oil-immersion objective and No. 6 compensation ocular of Zeiss showing stages in the histogenesis of the trochlearis nerve. Figures 55 and 56 are graphic reconstructions from parasagittal sections as seen under low power objective.

45 and 46 Portions of cross-sections (LAG 1-6-1, 2) of a 19 mm. embryo through the region of constriction between midbrain and hindbrain vesicles, showing portions of the trochlearis anlage as it passes through the mesenchyma lateral to the wall of the brain. The growing tip of the nerve is not shown. At this stage the anlage is wholly fibrillar and its deeply staining fibrils contrast strikingly with the granular vacuolated protoplasm of the surrounding mesenchyma. A thin layer of granular protoplasm may be seen covering portions of the nerve anlage. The fibrils of the nerve may be traced dorsally into the chiasma at the anterior boundary of the cerebellar neuromere, but the nerve has not yet become connected with the myotome of VanWijhe's second somite.

47 and 48 Proximal and distal portions of the trochlearis anlage in cross-sections (MBB 1-4-17 and MBB 1-5-2) of a 21 mm. embryo in the region of the cerebellar neuromere. The nerve anlage differs from the previous stage only in the larger number of fibers composing the nerve bundle. No cells closely associated with the nerve anlage are to be found. The growing tip of the nerve is not shown.

49 A group of neuraxon fibers near the peripheral termination of the trochlearis nerve anlage as seen in a parasagittal section (ME 5-1-4) of a 21 mm. embryo, showing the nerve anlage as a loose brush of neuraxons without any closely associated cells. The highly refractive fibrils show a thin sheath of granular protoplasm.

50 and 51 Portions of the trochlearis nerve anlage as seen in parasagittal sections (MD 2-2-5 and MD 2-2-8) of a 22 mm. embryo, showing the fibrillar character of the nerve. Figure 51 shows a distal and figure 50 a proximal portion of the nerve anlage. In its proximal portion, the trochlearis consists of a compact bundle of neuraxon fibers, naked or with a thin coating of granular protoplasm and devoid of closely associated cells. Distally, however, where the nerve anlage breaks up into a brush of loose fibers, mesenchymatous cells are more closely associated with the individual cells, but there is no evidence of a genetic relation between these cells and the neuraxon fibers.

52 to 56 (See p. 180).

PLATE 7: EXPLANATION OF FIGURES (CONTINUED)

52 A cross-section of the two roots of the trochlearis anlage near the point of emergence from the neural tube as seen in a parasagittal section (MID 5-1-3) of a 22 mm. embryo. At this stage the nerve anlage consists exclusively of deeply stained neuraxon fibers devoid of nuclei. A sheath of granular protoplasm, however, may be distinguished and mesenchymatous cells show loose relationships with the distal portion of the nerve.

53 One of the roots of the trochlearis as seen in a parasagittal section OA 3-1-8) of a 45 mm. embryo, showing evidence of the migration of nuclei from the neural tube into the roots of the nerve. Proximally the nerve root is cut longitudinally, while distally it is cut transversely. Nuclei appear within the nerve anlage among the neuraxon fibers and also partly within and partly without the neural tube. No mesenchymatous cells are closely associated with the nerve anlage near its point of emergence from the neural tube, and there is no evidence that the nuclei lying within the roots of the nerve have any other than a medullary origin.

54 A portion of a cross-section (MAG 1-6-7) of a 25 mm. embryo in the region of the optic vesicle, showing a peripheral portion of the trochlearis anlage at a stage when it has reached its termination in the superior oblique muscle. The section shows the relation of the trochlearis to the median surface of the muscle and to the ramus ophthalmicus superficialis trigemini which extends along the dorsal surface of the muscle, appearing in cross-section in the figure. Between the two nerveanlagen lies a mass of cells (*gn.sym.*) presumably derived from the r. ophth. sup. trigemini (Miss Platt '91). Both of the distal branches of the trochlearis anlage show a similar aggregation of cells where they cross the ramus ophthalmicus superficialis trigemini. Their derivation and relations show that such groups of cells are to be regarded as sympathetic ganglia and not as primitive sensory ganglia of the trochlearis. They afford as little evidence of the sensory character of the trochlearis as do the sympathetic ganglia associated with somatic motor spinal nerves.

55 A graphic reconstruction of the trochlearis anlage and the structures associated with it made from a series of parasagittal sections (MAA 3, etc.) of a 24 to 25 mm. embryo as seen from the right lateral aspect under low power microscope. At its anterior extremity the ramus ophthalmicus superficialis trigemini terminates in a group of cells derived from the neural crest. The group of cells (*gn.sym.*) associated with the same nerve at the point of crossing of the trochlearis have a similar origin and their relations to the trochlearis anlage are secondary.

56 A graphic reconstruction of the head of a 25 mm. embryo as viewed from the left lateral aspect, based upon parasagittal sections (MAH 3, etc.), showing the relations of the cranial nerves at this stage. In order not to complicate the figure, only two of the eye muscles are shown.

H. V. NEAL

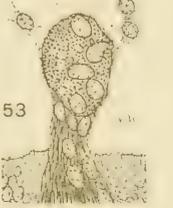
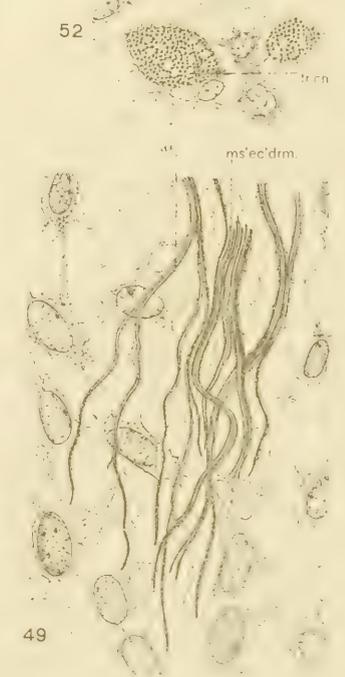
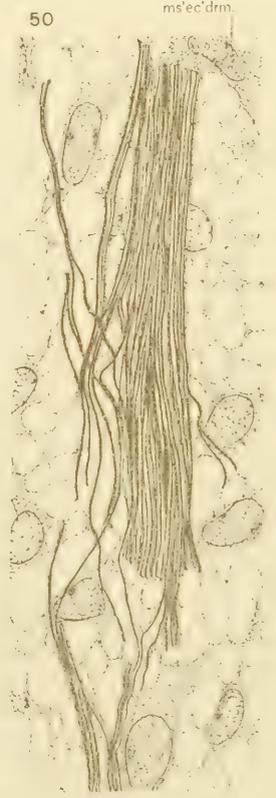
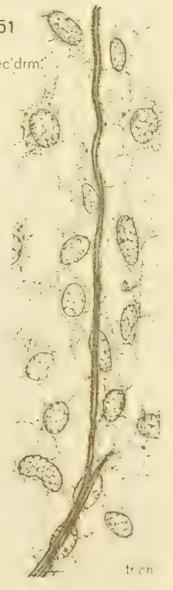
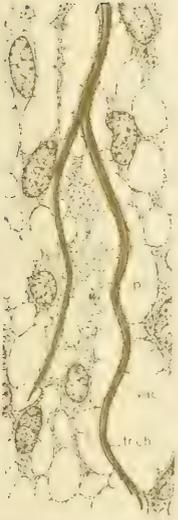
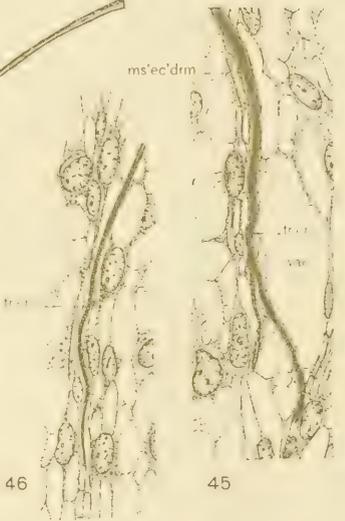
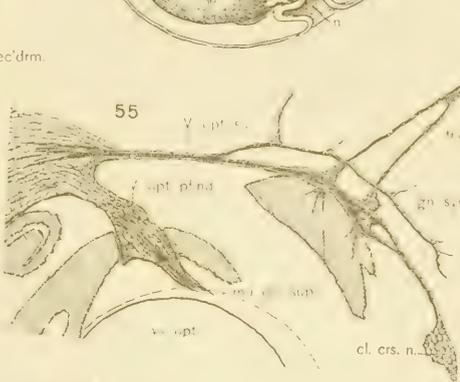
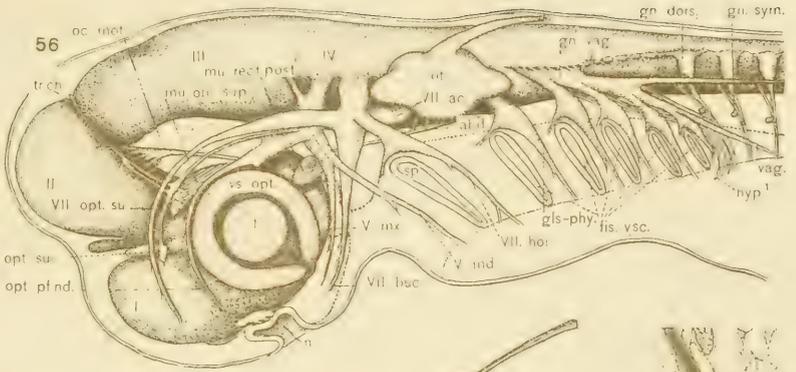


PLATE 8

EXPLANATION OF FIGURES

A series of camera drawings, showing the histogenesis of the oculomotorius nerve. Most of the drawings were made with one-twelfth homogeneous oil-immersion objective, No. 6 compensation ocular, and Abbe camera.

57 A parasagittal section (KQ 1-4-18) of a 9.5 mm. embryo viewed from the right side, showing the relations of the oculomotorius anlage at the time of its first appearance. The nerve anlage passes directly from the base of the mid-brain vesicle towards the first somite of VanWijhe, and closely associated with the pre-cardinal vein.

58 A portion of the same section as in figure 57 (KQ 1-4-18) as seen under a 4 mm. apochromatic objective and No. 6 compensation ocular, showing the oculomotorius anlage as a product of neuroblasts in the base of the midbrain vesicle. Shrinkage has resulted in breaking the roots of the nerve anlage, but there seems no reason to doubt the genetic relation of the nerve anlage with deeply stained neuroblasts lying in the ventral wall of the midbrain, the deeply stained processes of which may be traced to, and beyond the limiting membrane of the brain wall. The nerve anlage shows a deeply stained fibril surrounded by a sheath of granular protoplasm. At the point of union of the two roots of the nerve anlage is a mesenchymatous cell. There is no evidence that this cell has any genetic relation to the nerve anlage, nor is there any evidence of a migration of medullary elements from the neural tube at this stage.

59 A portion of a parasagittal section (KH 2-1-1) of a 9.5 mm. embryo, showing the oculomotorius anlage at a somewhat more advanced stage of histogenesis than that shown in the previous figure, as evinced by the greater number of neuroblasts and neuraxons. There is no evidence of migration of cells from the neural tube at this stage, but several mesenchymatous cells are somewhat closely associated with the nerve anlage. The highly refractive fibrils of the latter are in sharp contrast with the lightly staining vacuolated and granular protoplasm of the mesenchymatous cells.

60. A combination of four sections (KL 2-2-1, 2, 3, 4) of a 10 mm. *Squalus* embryo in the head region, showing the relations of the oculomotorius anlage, which extends from the midbrain to the first myotome, as seen under the low power microscope.

61 A combination of four sections (KL 2-2-1, 2, 3, 4) showing a part of the oculomotorius anlage in a 10 mm. *Squalus* embryo. The association of the fibrillar portion of the nerve anlage with medullary neuroblasts in the base of the midbrain is clearly seen. Distally, near the myotome, the nerve breaks up into a brush of separate fibrils, more or less closely associated with mesenchymatous cells. As there is no evidence up to this stage of the migration of medullary cells from the neural tube into the nerve anlage, and as the cells associated with the nerve anlage are more abundant in its peripheral portion near the myotome and the profundus ganglion, the evidence favors the conclusion that these cells are either mesenchymatous or that they are derived from the profundus ganglion, or from both sources.

62 to 71 (See pp. 184 and 185).

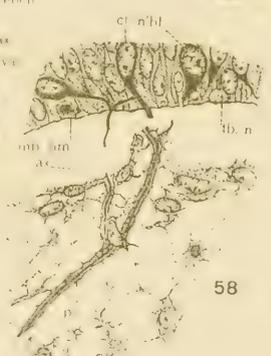
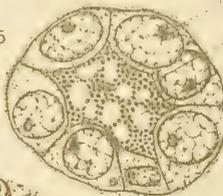
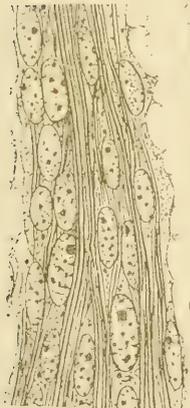
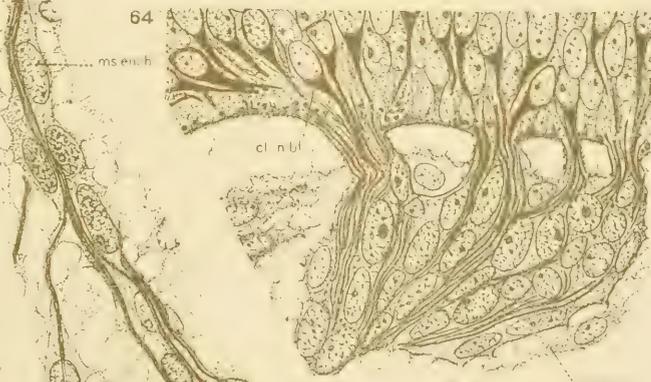
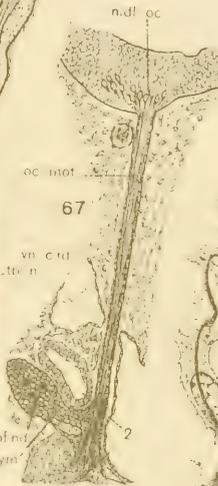
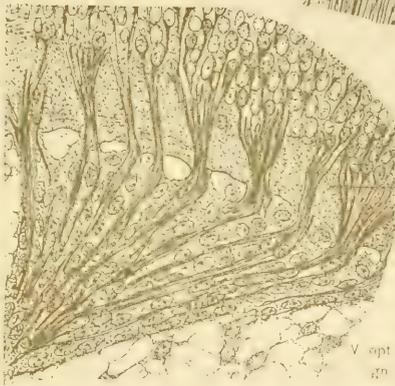
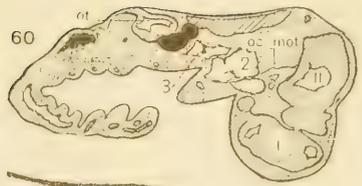
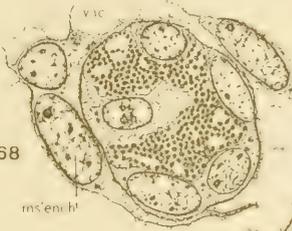
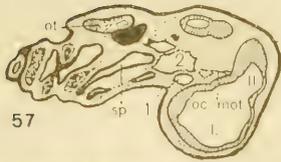


PLATE 8: EXPLANATION OF FIGURES (CONTINUED)

62 A single neuraxon of the oculomotorius anlage in a parasagittal section (KL 1-5-12) of a 10 mm. embryo, showing its relations with mesenchymatous cells at the base of the midbrain vesicle. The fibril shows the characteristic granular sheath and the evidence which has been interpreted by some investigators in favor of the theory that the neuraxons are differentiated from a protoplasmic reticulum. Such a conclusion, however, ignores the evidence of the peripheral growth of the neuraxons from medullary neuroblasts. The picture is strikingly similar to Gast's ('09) figure 3, plate 13.

63 A portion of the oculomotorius anlage in a parasagittal section (LA 4-2-8) of a 16 mm. embryo, showing the axial fibrillar bundle and the cellular sheath. The conditions are the same as those seen in spinal somatic motor nerves and in the other eye-muscle nerves at similar stages of development. There is no doubt as to the neuroblastic origin of the fibrillar portion of the nerve anlage, but it is not possible to determine positively whether the cellular sheath is ectodermal or mesodermal in origin.

64 A portion of a parasagittal section (LC 2-1-10) of a 16 mm. embryo in the region of the roots of the oculomotorius anlage, showing the relation of fibers of the nerve anlage to neuroblasts lying in the base of the midbrain vesicle. No evidence of cellular migration from the brain into the nerve anlage can be seen, and the source of the cells grouped at the roots of the nerve is doubtful.

65 A cross-section (LG 2-2-9) of the oculomotorius nerve anlage in its peripheral portion in a 15 mm. embryo, showing the bundle of neuraxon fibers with the sheath of mesenchymatous cells. The structure is essentially the same as that in similar sections of the anlagen of spinal ventral nerves. The nerve figured shows approximately two hundred highly refractive granules in the axial bundle, evincing approximately that number of neuraxons in the nerve anlage.

66 A longitudinal section of the oculomotorius anlage in the distal portion of the nerve of a 17 mm. embryo, showing the protoplasmic sheath—no nuclei appear in the portion figured—and the axial bundle of fibers.

67 The oculomotorius anlage as frontal sections (LAG 1-4-24 and 25) of a 16 mm. embryo, showing the relations of the nerve to the ganglion of the ramus ophthalmicus profundus trigemini and to the first somite of VanWijhe. The group of cells lying between the ganglion of the profundus and the oculomotorius may be traced in later stages into the ciliary ganglion of the adult and thus form the anlage of that sympathetic ganglion. The evidence in *Squalus* seems to favor the view that these cells are derived from the profundus nerve; the possibility, however, that they are in part derived by migration from the brain is not excluded. Of this, however, I am able to find no evidence.

68 A cross-section (LAB 8-13-20) of the oculomotorius anlage in a 17 mm. embryo, showing an early stage in the penetration of the bundle of neuraxon fibers by neurilemma cells. As development continues the number of such cells within the fibrillar bundle steadily increases, beginning at the proximal and distal ends of the nerve anlage.

69 A part of the proximal portion of the oculomotorius anlage in a parasagittal section (MC 4-1-4) of a 21 mm. embryo, showing the penetration of the neuraxon bundle by neurilemma cells.

PLATE 8: EXPLANATION OF FIGURES (CONTINUED)

70 The nidulus and roots of the oculomotorius anlage in a parasagittal section (ME 3-1-10) of a 21 mm. embryo, showing the increase in the number of roots and the relation of the fibers of the nerve to deeply staining neuroblasts in the ventral wall of the midbrain. With the growth of marginal zone of fibers, the neuroblasts have receded from the limiting membrane of the wall of the midbrain, but they still retain their deeply staining properties and their neuraxon processes may be easily traced through the marginal zone of fibers into the roots of the nerve.

71 A single neuroblast of the nidulus of the oculomotorius in a 46 mm. embryo, showing the beginning of multipolarity and the relation of the neuraxon process.

PLATE 9

EXPLANATION OF FIGURES

A series of diagrams designed to show the metameric relations of the eye muscle nerves and their comparability with spinal somatic motor nerves.

72 A diagram showing the topographic relations of dorsal and sympathetic ganglia to a somatic motor nerve in the trunk region of a *Squalus* embryo of 15 mm.

73 A diagram showing the relations of the oculomotorius nerve to a sympathetic (ciliary) ganglion and to the ganglion of the ramus ophthalmicus profundus trigemini in a *Squalus* embryo of 11 mm. A comparison of these relations with those of a spinal somatic motor nerve as shown in figure 72 shows that they are essentially the same. The differences are such as would be brought about, following a reduction of the myotome. The evidence favors the view of the similar mode of formation of the sympathetic ganglion. The reduction of the myotome of VanWijhe's first somite allows the precocious connection of the profundus nerve with the skin and brings about the slightly different topographic relations of nerve, ganglia and myotome shown in the diagram.

74 A diagram showing the relations of the abducens nerve in a *Squalus* embryo of 13 mm. Like the primitive somatic motor nerves of *Amphioxus*, the abducens does not become connected with a dorsal nerve and (therefore ?) is not associated with any sympathetic ganglion. Its nidulus, that is, its central relations, and its distribution to somatic musculature, however, sufficiently attest its serial homology with spinal somatic motor nerves. The nidulus of the abducens is elongated, extending through two brain neuromeres (VII and VIII), from both of which the cells which form the vagus ganglion are proliferated.

75 A diagram showing the relations of the trochlearis in a *Squalus* embryo of 25 mm. Except for the dorsal chiasma, the relations are similar to those of the oculomotorius (fig. 73). The nidulus of the trochlearis lies in the same zone as that of the oculomotorius and immediately behind it in the floor of the cerebellar anlage (third brain neuromere). In precisely the same way that the oculomotorius is associated with the profundus nerve and the ciliary ganglion, the trochlearis is associated with the ramus ophthalmicus superficialis trigemini and with a transient (?) sympathetic ganglion.

76 to 82 (See p. 186).

PLATE 9: EXPLANATION OF FIGURES (CONTINUED)

76 A diagram showing the greatly modified metameric relations of the eye muscle nerves, based upon the conditions found in *Squalus* embryos. The diagram is a composite of different stages of development. The relations of the oculomotorius nerve to the ramus ophthalmicus profundus trigemini are those which appear in a 12 mm. embryo, while those of the trochlearis to the ramus superficialis are such as appear in a 25 mm. embryo. The ramus recurrens of the abducens appears in a 19 mm. embryo. The neuromeres and somites are as they appear in much earlier stages of development. The diagram is not intended to show any hypothetical stage in the phylogeny of vertebrates, but merely to present graphically the highly modified metameric relations of the eye muscle nerves to the primary neuromeres and the somites of VanWijhe.

77 A diagram of the left lateral aspect of the head of a *Petromyzon* embryo of 50 mm., showing the relations of the post-otic myotomes and their associated ventral nerves. The innervation of the first three post-otic myotomes (4, 5, 6) by the ventral nerves of the ventral nerves of the fourth and fifth post-otic myotomes (7, 8) is especially to be noted, as is, also, the splitting of the first post-otic myotome (4) along the dorso-lateral line into a dorsal and ventral division. The homology of the first post-otic somite of *Petromyzon* with the fourth somite of *Squalus* is based on the evidence given by Koltzoff ('02).

78 A diagram based upon a reconstruction of a 3.5 mm. embryo of *Petromyzon* by Koltzoff ('02) showing the splitting of the anterior post-otic myotomes into median and lateral divisions. In order to explain the present relations of the eye-muscle nerves (fig. 76) to the pre-otic myotomes in *Squalus*, a similar subdivision of the myotomes of VanWijhe's first, second and third somites may be assumed.

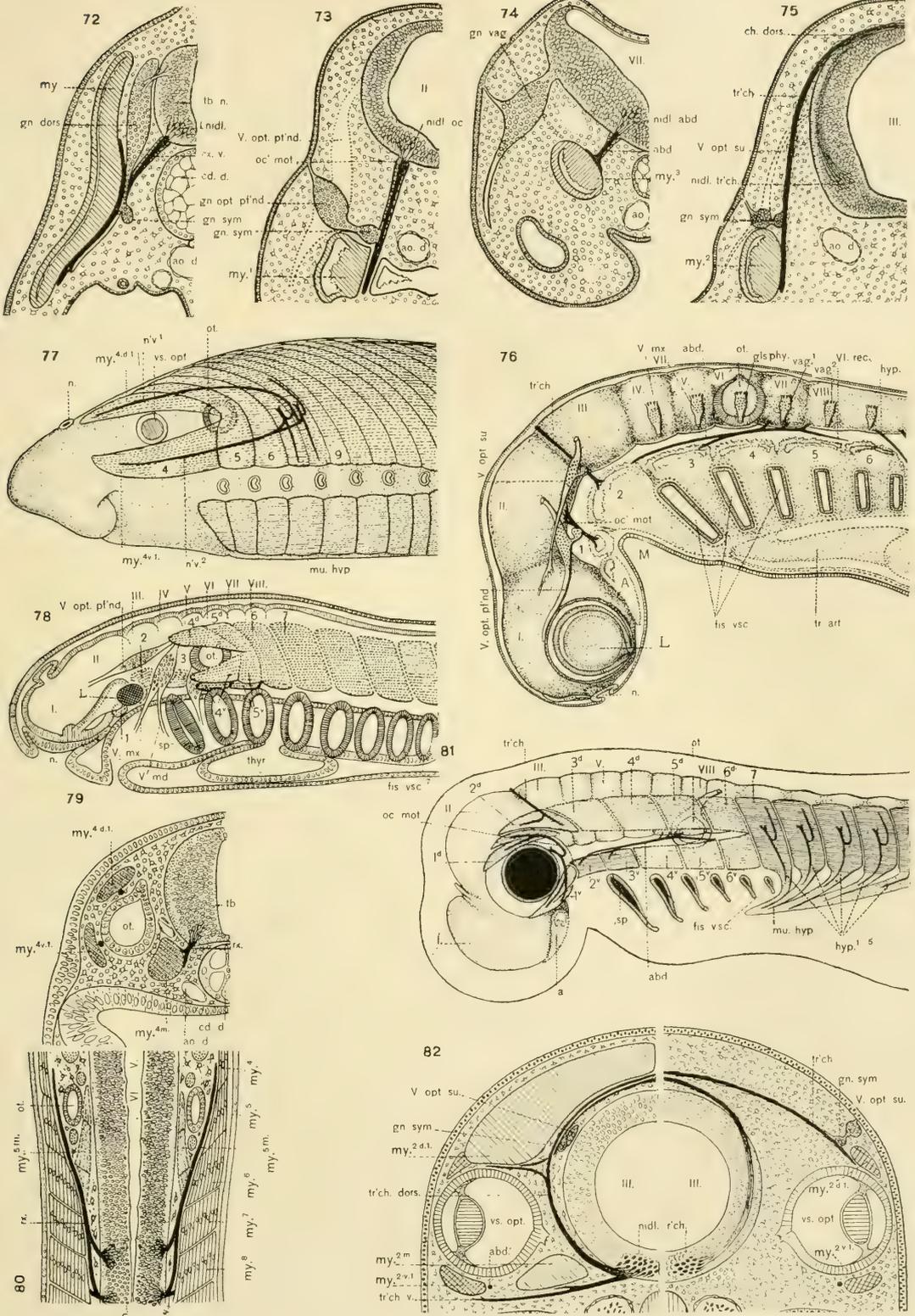
79 A diagram based upon a cross-section of a *Petromyzon* embryo of 3.5 mm., showing the three divisions (my. 4 m., my. 4 d. l., my. 4v. l.) of the first post-otic myotome, and their relations to the otic capsule. The hypothetical primitive connection of a somatic motor nerve with the median (transient) division of the myotome is shown. The two divisions of the myotome lateral to the otic capsule are permanent and become innervated by the ventral nerves of the fourth and fifth post-otic myotomes. By a similar substitution, it may be inferred, the rectus posterior muscle—a pre-otic muscle—has become innervated by the abducens—a post-otic nerve.

80 A diagram based upon a frontal section in the occipital region of a *Petromyzon* embryo of 4 mm., showing the relations of the anterior most post-otic ventral nerves to the myotomes. The division of these myotomes into a median portion and a portion lateral to the otic capsule is shown.

81 A diagram of the left lateral aspect of a *Squalus* embryo, showing the hypothetical primitive relations of the eye muscles to the lateral trunk musculature. Those myotomic divisions which are not functional in the adult *Squalus* are indicated by broken lines. Only those pre-otic myotomes which have acquired connection with the bulbus oculi and have become functional as eye muscles have persisted. The homologues of myotomes 4, 5 and 6, however, are functional in *Amphioxus* and *Petromyzonts*.

82 A diagram intended to show the conditions under which the dorsal chiasma of the trochlearis has been acquired. The left side of the diagram shows an hypothetical stage when the myotome of VanWijhe's second somite was split into three moieties. The right side of the diagram corresponds in all essential respects with the conditions found in a 25 mm. *Squalus* embryo. For an interpretation of the figure, see p. 114 of the text.

H. V. NEAL



THE GENESIS OF THE PLASMA-STRUCTURE IN THE EGG OF HYDRACTINIA ECHINATA

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SIXTY-SIX FIGURES (EIGHT PLATES)

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I. INTRODUCTION

This paper is offered as a contribution to the discussion concerning the origin and relationship of the protoplasmic¹ granules, especially the chromidia and mitochondria. Although this complex and difficult problem has received much attention, it is still far from a satisfactory solution. A considerable group of observers, including Goldschmidt and his followers, have endeavored to extend Richard Hertwig's conception of chromidia in Protozoa, to certain of the protoplasmic granules in the metazoan cell, attributing a nuclear origin to the latter (chromidia) and regarding them as only temporary elements of the protoplasm. A second group of observers, led especially by Meves and Duesberg, are highly skeptical in regard to this hypothesis and have reached the conclusion that the most constant and characteristic of the granules (mitochondria, plastochondria) are purely protoplasmic in origin. According to them, the granules in question form an essential constituent of the protoplasm and are permanent cell-elements which exhibit a genetic continuity, and are the bearers of protoplasmic heredity ('plastochondrial germ-plasm'). The present paper is concerned especially with the conclusions of Schaxel, which are more or less intermediate between the two foregoing conceptions. He finds both types of protoplasmic granules present, 'extra-nuclear chromatin-granules' (corresponding to chromidia) which control differentiation, and mitochondria of purely protoplasmic origin.

¹The term 'protoplasm' is used throughout the paper instead of 'cytoplasm', according to the usage of the earlier writers on the subject.

II. REVIEW OF SCHAXEL'S WORK

Since Schaxel's results are rather complicated, it may be well to review them briefly here. His interesting conception has been derived from observations on the eggs of animals from widely varying groups (Hydrozoa, '11b; Scyphozoa, '10; echinoderms, '11a; annelids, '12; ascidians, '09) in all of which he describes essentially the following conditions. After the last oogonial division the nucleus is reconstructed in the typical fashion, that is, the chromosomes elongate as smooth threads to form a bouquet-stage. These threads then lose their smooth contour, become granular, and, by further diffusion, soon form a typical granular reticulum, the threads of which as a rule center in the nucleolus. Only a small layer of protoplasm (which at this time takes an acid or 'plasma' stain) surrounds the nucleus. This is the 'pre-emission stage' and the protoplasm is said to be in a state of 'primary achromasie.'

This stage merges gradually into the next or 'emission' stage, characterised by the accumulation of chromatin-granules on the nuclear net, especially at those points where the threads of the net touch the nuclear membrane. At the same time, by filtration through the membrane, groups of granules collect on the outside of the nucleus directly against the wall, at the ends of linin-threads which are poor in granules. The stage of actual emission of material is of rather brief duration and at its close the protoplasm is in a state of 'chromasie.' This 'extra-nuclear chromatin' stains with basic dyes.

The 'post emission' stage follows rapidly and is characterised by further reconstruction of the nucleus, spreading of the extra-nuclear chromatin throughout the protoplasm ('complete chromasie') and rapid growth and differentiation of the yolk. The latter is formed indirectly from the extra-nuclear chromatin, as a small island of yolk in a 'nest' of granules, at the expense of which it increases. In his earliest paper on ascidians, Schaxel ('09) describes a 'secondary achromasie' of the protoplasm of the mature egg, any extra granules not used in the formation of the yolk being absorbed by the phagocytic action of the test-

cells. In the other forms described, Schaxel emphasizes the fact that that part of the extra-nuclear chromatin, not used in the formation of the yolk, remains between the yolk-spheres as 'intravitelline chromatin.' The latter has been traced in the echinoderms and annelids to the end of cleavage, when it has completely disappeared, having, presumably, been used in the process of differentiation. The cells of the blastula are again in a state of 'achromasie.' In *Aricia*, in which later stages of development were studied, a 'secondary chromatin-emission' occurs in the cells of the gastrula. The granules of this emission are used in the differentiation of cells into body tissues. In later papers² Schaxel describes mitochondria in the cell, *in addition* to the extra-nuclear granules. He considers the two elements distinct, since the mitochondria are present in the egg before the chromatin-emission occurs and also remain as constant cell-constituents from cell generation to generation, while the protoplasmic chromatin disappears from the cell in the process of differentiation.

Schaxel's conclusions regarding the chromatic nature of the extra-nuclear granules are based on both staining reactions and morphological evidence. He is well aware that not too great emphasis can be placed on the fact that the granules stain as chromatin, yet this fact combined with the morphological evidence, has some weight. The morphological argument seems stronger, since granules outside the nucleus at the end of threads having few or no granules, strongly suggests the passage of those granules through the membrane. His figures are most convincing.

My immediate purpose has been to obtain more accurate evidence for or against these interesting conclusions by testing the staining reaction of the extra-nuclear granules in order to determine if possible to what extent such reactions can be relied upon as an indication of their nature; but I have also endeavored to study the origin and ultimate fate of the granules, their relation to the yolk and mitochondria, and the origin and fate of the

² Mitochondria as such are not described in Schaxel's early papers. They are described carefully in his later ones on echinoderms and annelids to correct the mistaken conception that the nuclear granules represent the chromatic origin of the mitochondria.

latter. Since *Hydractinia echinata* shows both mitochondria and protoplasmic granules which take 'nuclear' stains, it forms a favorable object for study of the origin and relation of these elements, the results of which form the first portion of the paper. In a second part a few observations are briefly presented which are intended to supplement previous descriptions of maturation and fertilization in *Hydractinia echinata* and *Eudendrium ramosum*.

This investigation was undertaken at the suggestion of Prof. E. B. Wilson, to whom I wish to express sincere thanks for his kindly direction of the work. For suggestions concerning certain experimental aspects of the problem I am indebted to Prof. T. H. Morgan. I wish also to express my thanks to Prof. F. R. Lillie for his generosity in putting a room at my disposal during several summers at the Marine Biological Laboratory at Woods Hole.

III. PROTOPLASMIC GRANULES AND MITOCHONDRIA IN HYDRACTINIA

A. MATERIAL AND METHOD

The material for this work was obtained at Woods Hole during the summers of 1910, 1911 and 1912. The egg of *Hydractinia* is very favorable for staining tests, since all stages of development, from the early egg in the entoderm to the mature egg in the gonophore, are present on the same stalk, making it certain in staining tests that all stages receive the same treatment. Since a change in staining reaction occurs during development of the egg, this is of much importance. One possible difficulty with this material is the well-known fact that the nucleus early loses its affinity for basic stains and may take acid stains but slightly. If care be used in determining the staining reaction of the chromatin in the very early stages, no confusion need result from this condition.

Various killing fluids were used, the results of which for the sake of brevity and clearness have been tabulated (table 1). Material fixed in fluids containing chromic and osmic acids is best for the study of both nucleus and protoplasm, since the integrity of the elements is much better preserved than in other

TABLE 1
Young egg

STAIN	KILLING FLUID	NUCLEUS	NUCLEOLUS	PS. CHR. GRAN.	LARGE GLOBULES
No stain.....	Meves ²				brown
Benda ³	Meves	brownish yellow purplish red	violet or brown ⁴ clear red	brownish yellow greenish gray	brown or violet ⁴ clear red
Bensley ^{5b}	Bensley ⁵				light brown-bleached black
No stain ^{5c}	Bensley				
Bleached (no stain).....	Bensley				
Bleached (Iron-hem).....	Bensley	gray or black	black	gray	black
Iron-hematoxylin and Licht Grün.....	Flemming's	gray	brownish	dark ³ gray	black
	Meves'	gray	brownish	gray	black
	sub-acetic	blue-black		black	black
	pic-acetic formalin ⁶	blue-black black	black black	black black	black black
	100 per cent alc.	blue-black	black	black	black
	alc.-acetic hot water ⁷	blue-black black	black black	black black	black black
	Flemming's or Meves' sub-acetic	red, blue, young	blue	blue	blue
Thionin and eosin.....	sub-acetic	red, blue young	blue	blue	purplish blue purplish blue
Lithium carmine and Lyons blue.....	pic-acetic	red, blue, young	blue	blue	blue
	formalin	blue	blue	blue	blue
	Suba- { separate { together	red red	red red	blue red	blue red
	pic-acetic sub-acetic	red, green, young	blue	greenish blue	greenish blue
Auerbach ¹²	pic-acetic	red, green	blue	greenish blue	greenish blue
	alc. 100 per cent	red, green	blue	green	greenish blue
Saffranin and Licht Grün.....	Flemming's	purplish or red	red	purplish or red	red
	Meves'	purplish or red	red	purplish or red	red
Saffranin and Methyl-violet.....	Flemming's	violet	red	violet	red

¹ The color of the globules is given if they take a different stain from the ground-substance of the sphere. In that case the ground-substance always takes the 'plasma' stain.

² Meves' killing fluid for mitochondria is a modification of Flemming's fluid, i.e., the acetic acid content is much reduced (Erg. d. Anat. u. Entw., Bd. 12; Lee, last ed.).

³ Benda's stain for mitochondria is a double stain of sulphalizarinate of soda and crystal-violet. (Erg. d. Anat. u. Entw., Bd. 12, or Lee, last ed.)

⁴ The color of the oil depends on the degree of extraction. With long extraction the violet is removed, leaving the blackening caused by the osmic acid in the fixative.

⁵ Bensley's mitochondrial methods (modification of Altman's methods):

a. Killing fluid:

Osmic acid 4%.....	2 cc.	} 24 hours
Potassium bichromate 2.5%.....	3 cc.	
Acetic acid.....	2 drops	

b. Stains:

Altman's anilin fuchsin.....	6 min.
1% methyl green 00.....	dip
Wash in 95% alcohol	

c. Bleach to be used after the above killing fluid:

30 sec.....	1% potassium permanganate
30 sec.....	.5% oxalic acid
Wash in water	
Dip in.....	2.5 % potassium bichromate

TABLE 1—Continued

Old egg

NUCLEUS	NUCLEOLUS ¹¹	PROTOPLASM	MITOCHON- DRIA	COMPOUND YOLK	FINE YOLK	OIL
brownish yellow reddish	violet or brown ⁴ red (clear)	brownish yellow reddish purple	light brown deep violet red (pur- plish)	violet red (clear)	brown and violet purplish red	brown violet or brown ⁴ greenish or brown dark brown light brown black
gray	black	gray	black	black	black	black
green	black	green	black	black	black	brownish black
green	black	green	black	brownish black	brownish black	brownish black
blue-black	black	green	⁹	green and black	green and black	black
blue-black	black	green	⁹	green	green	black
green	black	green	black	black	green	black
blue-black	black	green	¹⁰	black	black	black
blue-black	black	green	¹⁰	black	green	black
green	black	green	black	black	green	black
red	blue	reddish	reddish	bright blue	pale blue	brownish blue
red	blue	red	⁹	red	red	red
red	blue	red	⁹	red	red	red
red	blue	red	¹¹	blue	pale blue	blue
red	red	blue	¹¹	blue	blue	blue
red	red	red	¹¹	bluish	bluish	bluish
red	red	red	¹¹	bluish	bluish	bluish
bluish red	blue	red	⁹	red	red	red
bluish red	blue	red	⁹	red	red	red
bluish red	blue	red	¹⁰	red	red	red
green	red	green	bright red	bright red	light red	brownish red
green	red	green	bright red	bright red	light red	brownish red
light violet	red	violet	violet	red	reddish violet	deep purple

⁶ Five per cent formalin was made in normal salt solution and the formic acid neutralized with sodium carbonate (Mann, '02).

⁷ Hot water proves an excellent fixation both for nuclear and protoplasmic structures.

⁸ The pseudochromatin-granules lie in a protoplasmic net taking the 'plasma' stain.

⁹ The mitochondria are absent, probably dissolved by the acetic acid of the fixative.

¹⁰ The mitochondria cannot be identified because of the poor fixation given by alcohol.

¹¹ The mitochondria cannot be differentiated because they stain exactly like the other protoplasmic elements.

¹² Auerbach's stain, used as a chemical test, is a mixture of acidulated methyl-green and acid fuchsin. *Jenaische Zeitschrift*, Bd. 30, 1896.

fluids. Chemical tests were made on material fixed in indifferent killing fluids, such as alcohol, hot water and formalin in which the formic acid is neutralized. A large number of stains were also used, the results of which are again shown in table 1. Some experiments with 'intra-vitam' stains gave most useful results, which are recorded in table 2.

Other methods to determine the chromatic nature of the protoplasmic granules have been tried, such as artificial digestion of fresh or alcoholic tissue, and tests for nucleo-histone and phosphorus (Mann '02). The possible proteid nature of the granules was also investigated by the use of Millon's reagent. Experiments on the staining reaction of egg-albumen which had been fixed with various ones of the same killing fluids were also made.

B. PROTOPLASMIC STRUCTURES OF THE MATURE EGG

It is essential to differentiate the protoplasmic structures of the mature egg in order to make clear their relation to the extra-nuclear granules which I prefer to call 'pseudochromatin-granules.' The nucleus will therefore not be considered at present. The egg of *Hydractinia* is large and filled with various granules and spheres which fall into four groups:

1. *Small simple yolk-spheres* which vary greatly in size and occur throughout the egg.

2. *Compound yolk-spheres*, the largest elements in the egg. They form in general a crescentic layer around the egg, a little below the surface and are found to some extent throughout the center of the egg.

3. *Oily bodies*, about the size of the small yolk-spheres, which darken in osmic acid and are often irregular in shape. These pervade the whole egg.

4. *Mitochondria*, small bacillus-like rods, slightly more abundant at the surface of the egg, but rather evenly distributed throughout the protoplasm.

These elements of the mature egg lie in a finely granular protoplasm, which stains entirely with plasma stain, that is, there are

at this stage no basic-staining granules that correspond to Schaxel's intra-vitelline chromatin³ (fig. 14).

No one staining method differentiates these various types of structure, since, as a rule, all stain the same way with certain dyes used after some killing fluids. For example, as shown in table 1, if iron-hematoxylin is used on material in Flemming's or Meves' killing fluids, all the elements take the hematoxylin stain in slightly varying degrees of intensity (fig. 16). By the use of various killing fluids and stains, however, they can be shown to be distinct elements in the following way (table 1).

The *oily bodies* are most easily distinguished from the yolk since the former darken somewhat in osmic acid. After staining in saffranin and methyl-violet they take a deep violet color, while the yolk is red. Also, the oil can be completely separated from the other cell-elements by centrifuging the egg.

The *two kinds of yolk*, simple and compound (often indistinguishable from each other when iron-hematoxylin is used after Meves' or Flemming's fixation) show their individuality when stained either with Benda's stain or with saffranin and methyl-violet. These stains reveal, in certain of the spheres, drops or globules which stain differently from the ground-substance of the sphere. The geometrical regularity of these is shown in figure 15, in which young and old compound spheres are represented. After the use of Benda's stain the mature simple yolk is uniformly violet in color, while the compound spheres show brilliant violet globules in a yellow ground-substance. When stained in saffranin and methyl-violet, the globules of the compound sphere are brilliant red and the ground-substance light lavender, while the simple yolk is reddish violet. The most striking differentiation appears in material fixed in picro-acetic killing fluid and stained in iron-hematoxylin and light green, for the simple

³ These elements are diagrammatically represented in figure 14 in the following manner: Young yolk is represented by solid gray circles; mature simple yolk is represented by hollow circles; mature compound yolk is represented by hollow circles containing small circles; oil is represented by hollow circles containing dots; mitochondria are represented as black rods; the protoplasm is uniformly gray.

yolk takes the plasma stain, while the globules of the compound spheres are the only bodies in the egg which take the hematoxylin.

Since the globules of the compound yolk-spheres are of similar size and may take the same stain as the mitochondria (the violet of Benda's stain) they at first suggest nests of dividing mitochondria. This is an impossibility however, since the compound yolk-spheres are the most conspicuous structures in eggs fixed in killing fluids which dissolve the mitochondria (picro-acetic).

The *mitochondria* are so identified because of their rod-like shape and their typical mitochondrial behavior; that is, they dissolve in fixatives containing acetic acid, do not dissolve in alcohol, are darkened by osmic acid, and give the typical response to the so-called mitochondrial stains (Benda, Bensley or Altman, and iron-hematoxylin). In living material they are highly refractive bodies which take Janus green as a vital stain.

C. HISTORY OF THE YOUNG EGG, BOUQUET-STAGE

The youngest eggs studied were found in the proliferating area of the stalk of the gonophore. Here the entoderm cells are smaller than those of other regions, their protoplasm is not vacuolated, and their nuclei are more deeply staining. The eggs, when differentiated from the surrounding entoderm cells, are somewhat larger and have nuclei in the bouquet-state; a nucleolus is present in which the chromatin-loops center (figs. 1 b, 2,). A slightly later stage shows the nucleolus in which the chromosomes center, pressed against one end of the nucleus (fig. 3). The origin of these cells was not determined. Since they are evidently the result of a recent division (they are usually found in pairs) it seems probable that they are the product of an oogonial division (fig. 1). The egg cells are conspicuous in this stage, since the chromatin and nucleolus take the basic stains intensely. The protoplasm, which is a very thin homogeneous layer around the nucleus, stains but lightly with plasma stains and thus contrasts strongly with the nucleus. This stage corresponds to Schaxel's 'pre-emission stage' The structure is the same, whatever fixative is used. No attempt to study synapsis was made, although many cells showed some evidence of double threads (fig. 1 a).

D. EARLY GROWTH PERIOD

1. *Nuclear reconstruction*

The growth-period begins directly after the preceding stage, both nucleus and protoplasm increasing in size. Nuclear changes appear, which result in the reconstruction of the nucleus; that is, the densely staining smooth chromatin-loops break up into a coarse, open, granular net, the threads of which center in the nucleolus and radiate to the periphery of the nucleus (figs. 4, 5, 6). The granules of chromatin are conspicuous in this period at the nodes of the net, while a few fine granules lie against the inner wall of the membrane. By the time this stage is reached the chromatin takes most basic stains somewhat less intensely than previously, and may even fail to stain with most basic dyes or take the plasma stain instead. The latter condition is striking when eggs fixed in either sublimate-acetic or picro-acetic killing fluids are stained with thionin and eosine or Auerbach's fluid. As the egg grows, the radial arrangement of the nuclear net is lost (figs. 7, 8, 18) until by the time the egg has reached the gonophore the chromatin is coarsely granular, with little evidence of the net-like arrangement left. At the same time, its affinity for basic dyes has continued to diminish (figs. 19, 20, 22, 25, 26, 27, 28). A more complete account of the disappearance of the net is given later.

2. *Pseudochromatin-granules*

When but a slight increase in the size of the egg has occurred and when the above nuclear changes are occurring, there appears throughout the protoplasm a fine granular precipitate ('Emission stage' of Schaxel) which, as a rule, takes the basic stains (figs. 4, 5, 6). The granules of which this substance consists I will call 'pseudochromatin-granules.' Striking differences appear in the form and staining reactions of these granules as a result of different modes of fixation. After Meves' method of fixation, they are fine and evenly distributed throughout the egg. If stained with iron-hematoxylin they appear gray in color while the chromatin is deep gray or black. If stained according to

Benda's method, the protoplasmic granules and the chromatin both take the yellow color of the alizarine. Flemming's killing fluid produces a slightly coarser but still evenly distributed precipitate which stains somewhat more intensely with iron-hematoxylin than that killed in Meves' fluid. Several other fixatives (sublimate-acetic, picro-acetic) give very striking pictures, since they produce a strong coarse precipitate, which is not evenly distributed, but more or less massed in the region of the nucleus, and which has a great affinity for basic stains. If stained with iron-hematoxylin, the pseudochromatin-granules are intensely black. If stained with Auerbach's fluid or double stained with thionin and eosin, a striking contrast is produced since the nucleus here takes the 'plasma' stain (fuchsin or eosin), thus emphasizing the fact that the protoplasmic granules stain with the basic dyes (methyl-green or thionin).

In my material I can discover no such striking picture as Schaxel finds of groups of granules on the outside of the nuclear wall at the ends of nuclear threads, regarded by him as centers of distribution and diffusion into the protoplasm. Poor fixation, resulting from the use of sublimate-acetic and picro-acetic fluids, may give rise to some such appearance, but in good fixations I find a uniform distribution of the granules from the beginning (figs. 4, 5, 6, 7, 17). Furthermore, the egg is constantly increasing in size and yet the granular mass which increases with it, retains at all times its uniform distribution. By the time the egg has reached the gonophore, it is of considerable size and is completely filled with these densely staining granules (figs. 8, 9). In the figures they are always represented by gray granules.

3. Basic staining globules

In the young egg, in addition to the pseudochromatin-granules, a second element, consisting of basic-staining globules, appears against the nuclear membrane (fig. 17). Since these globules ordinarily stain as the granules do (iron-hematoxylin), and since they appear very much like similar bodies figured by Schaxel in *Pelagia*, which he interprets as centers of dispersal of the 'extra-

nuclear chromatin,' it is important to determine their nature. They are differentiated from other cell elements diagrammatically in figures 4, 5, 7, 8 and 10 by the use of circles containing parallel lines. Although they are so closely applied to the nuclear membrane, again I find no evidence of a direct nuclear origin. Their distinction from both pseudochromatin-granules and chromatin is apparent for several reasons. (1) These globules darken in osmic acid while the chromatin and granules as seen in unstained preparations, do not. (2) After Benda's stain, they take a deep violet color, while the chromatin and granules stain yellow. (3) Also in material stained with saffranin and methyl-violet the pseudochromatin-granules are violet, while the globules stain a brilliant red. It is evident therefore that the globules are identical, neither with the nuclear chromatin, nor with the pseudochromatin-granules. Since I find, as shown in the figures, no grouping of the granules around the globules, they can hardly be dispersion-centers. The time when globules first appear in the egg is variable, for they may be present as soon as the pseudochromatin-granules appear, or not until later. They are found up to the time the yolk begins to appear, sometimes lying a short distance from the nucleus, but never far away (figs. 7-10).

To sum up: There are in the early growth-period two protoplasmic elements, one a fine granular precipitate (pseudochromatin-granules) which is scattered throughout the protoplasm and takes basic stains, the other, large drop-like masses which appear near the nuclear wall and which are also probably not chromatin. Neither of these elements appear as such in the mature egg, both being completely used during development.

During this period the egg is migrating from its original position in the entoderm of the stalk toward the gonophore, where it becomes established in the ectoderm. The increase in the size of the egg is considerable during this process, but still further growth takes place after the egg has reached the gonophore before any change in the condition described occurs, or any evidence of the structures characteristic of the adult egg appear.

E. DIFFERENTIATION OF THE EGG

1. *Nucleus*

The nuclear structure will be described in detail in a later section, but may be considered briefly here. After certain killing fluids (Flemming, Meves, neutral formalin, hot water) the nucleus early loses its affinity for all basic stains and shows a very fine, nearly homogeneous structure, in which little or no evidence of a fine net or chromosomes appears. It now takes even the plasma stains very slightly (figs. 21, 28). After some killing fluids (sublimate-acetic, picro-acetic, alcohol), a heavy net-like precipitate, which takes basic stains strongly, is formed (fig. 22). The nucleus, which reaches a relatively enormous size, lies at first in the center of the egg (fig. 19) and moves to the periphery only a short time before growth of the egg is completed (fig. 23). A basic staining nucleolus, which contains one or several vacuoles staining with acid dyes, is constantly present.

2. *Protoplasmic changes*

a. Development of the simple yolk. The first elements to appear in the protoplasm are small yolk-spheres which develop into the simple yolk and possibly the compound yolk-spheres of the mature egg. It seems evident that these spheres develop directly from the pseudochromatin-granules as described by Smallwood ('09). Material fixed in Flemmings or Meves' fluid or formalin is best for these observations, since the individual yolk-spheres are kept distinct. In eggs about one-fourth grown, which have been stained according to Benda's method, the extra-nuclear granules can be seen to have enlarged slightly and uniformly (fig. 9). They still take the yellow-brown color which Benda's stain gives the granules. In a slightly older egg (about one-third grown) some of the spheres are seen to have grown more rapidly than the others, so that the uniformity in size is lost (fig. 10). A few now increase in size very rapidly, giving yolk-spheres of very unequal sizes (fig. 11). It is important to notice that the spheres still stain like the pseudochromatin-granules (yellow-

brown). In my material, as shown in the figures, there are no nests of 'extra-nuclear' granules within which yolk-material with a different staining reaction is secreted, as described by Schaxel. There is, rather, a graded series from the smallest spherical granules to the largest spheres; and these latter do not as yet differ in staining reaction from the pseudochromatin-granules, a condition subsequently seen.

A little before the egg is half grown, however, a change of staining reaction begins. A few of the largest yolk-spheres change their reaction in the Benda stain, the brown of the alizarin being replaced by violet. Figure 19 shows such an egg under low magnification, while figure 12 represents this change diagrammatically. As the egg grows, more and more of the yolk-spheres take the violet color until the whole egg is dominated by violet, a few small yellow ones remaining among the large violet spheres (figs. 13, 14, diagrammatic). This change in staining reaction is very striking on a slide in which all stages in the growth of the egg are present, when obviously they have all received the same treatment in the staining process.

These points are less strikingly but well shown when some of the same material is stained in iron-hematoxylin, since the pseudochromatin-granules are grayish, the young yolk gray, the nature yolk black (figs. 16). Although micro-actic and sublimate-acetic fixations are poor for determining the development of the yolk, the change in staining reaction is even more strikingly shown when iron-hematoxylin counterstained with light green is used, since the granules of the young egg take an intense black, while the yolk-spheres of the mature egg are green. This fixation also makes it evident that the pseudochromatin-granules as such are not present in the mature egg between the yolk-spheres ('intra-vitelline chromatin' of Schaxel) for there are no deeply staining granules present in such eggs, while young eggs after the same treatment are dominated by black granules.

b. Development of the compound yolk. Since the young stages of the simple yolk-spheres and the compound spheres stain alike, they cannot be distinguished from one another until they are nearly grown, when internal globules appear in the compound

spheres. These are differentiated rather suddenly about the time that the staining reaction of the simple yolk begins to change (fig. 13). The Benda stain shows one or several small globules staining with crystal-violet, within a large compound yolk-sphere, the ground-substance of which is yellow brown (figs. 14, 15). The size of these spheres varies considerably, some of them forming the largest elements in the egg. The globules are arranged symmetrically, and increase in size until, in the mature egg, they may merge and practically fill the sphere. Usually a notched edge indicates such an origin (fig. 15). Certain stains always differentiate the compound from the simple yolk. Thus, saffranin followed by methyl-violet gives the inner globules a bright red color and the external ground-substance a pale violet color. Some of the spheres never reach this state of development but appear in the mature egg with small distinct globules within. It seems probable from staining reactions that certain of the simple yolk-spheres are utilized for the storing of a different material in the form of globules.

c. The appearance of the mitochondria. The mitochondria are not seen in the egg until after the yolk is well formed, but before the staining reaction of the latter has changed. As is usual for mitochondria, special fixatives (Meves' fluid, Flemming's fluid, Bensley's fluid, formalin, hot water) are essential. When Benda's staining method is used after fixation with Meves' killing fluid, a few small rounded bodies, violet in color, appear here and there among the yellow spheres, uniformly distributed throughout the egg (fig. 12). These are larger than the pseudochromatin-granules, about the size of some of the smaller yolk-spheres, and of uniform size. They are comparatively few at first, but gradually increase in number and size until they are so numerous in the mature egg that they fill all the spaces between the yolk-spheres. From their first appearance they take the typical mitochondrial stains, that is, violet in Benda's stain; intense red in Bensley's stain; and deep black in iron-hematoxylin. Since the question of the origin of the mitochondria has been variously answered, it is of interest to determine that point in *Hydractinia*. As they are scattered throughout the

egg from the beginning, they are certainly not of direct nuclear origin. Again I find no evidence for their origin from the pseudochromatin-granules; for, when the mitochondria first come into view, they are larger than the granules and also stain entirely differently if the Benda method be used. Also they can hardly originate from certain of the yolk-spheres which are of similar size, since again the staining reaction is different and yolk-spheres much larger than the mitochondria are yellow in this stain. These facts all point to their formation *de novo* in the protoplasm.

d. Oil globules. These appear very early while the egg is still in the entoderm of the stalk; that is, before the yolk formation has begun and even before all the pseudochromatin-granules are formed (figs. 7, 8, 9, 10). They lie scattered among the granules, the number varying much in different individuals. They are in some cases few in number, but in others are frequently very numerous even in this early stage (fig. 8). At this time they are easily confused with the globules which appear against the nuclear wall, since they usually take the same stain. That they are distinct from the nuclear globules is apparent by their greater blackening in osmic acid. Also, in double staining with saffranin and methyl-violet the oil is violet while the globules are red. Again, after Benda's stain, the oil is brownish and the globules violet. Auerbach's stain distinguishes the oil from the pseudochromatin-granules, for the latter are green while the former are red. In later stages the oil globules increase greatly in number until the egg is profusely dotted with them (figs. 13, 14). The oil is also formed *de novo* throughout the growth-period in the protoplasm of the egg.

Since none of the elements of the mature egg correspond to the large globular masses which appear against the nuclear wall in the early growth-period, it is possible that they form a source of elaborated food which is used during the process of growth.

F. THE NATURE OF THE PROTOPLASMIC GRANULES

Because of the theoretical interest connected with this question, it is essential to determine, if possible, whether the extra-nuclear granules are chromatin, extruded from the nucleus as such, or whether they are of protoplasmic origin. Certainly, the first impression given by these granules, staining intensely in basic dyes, favors the conclusion urged by Schaxel, that they are chromatin sent into the protoplasm. The picture is so striking that after many staining tests I was still convinced that such was the case; for all ordinary dyes show identical staining reactions for the granules and the chromatin. It was only after an extended study of the effect of many dyes, both on fixed and living material, that I finally reached a different conclusion, based on the following facts:

1. The uniform distribution of the basic-staining granules, as described above, makes their direct nuclear origin doubtful. In my material no accumulation of the granules against the nuclear wall, no corresponding accumulation of chromatin-granules within the nucleus, is to be seen at any time. This is especially noticeable in the nucleus of late stages when the basic granules are rapidly increasing, for the nucleus is now uniformly homogeneous with an affinity for plasma-stains only.

2. The amount of granular material in *Hydractinia* can hardly be accounted for if it is all given off in the early growth-period as Schaxel finds in his forms, since this period is of too short duration. An enormous increase in amount takes place after the stage corresponding to Schaxel's 'emission' stage which cannot be explained by simple separation and distribution of pre-existing granules. And since the granules are of the same size in all stages, they cannot be formed by repeated separation without the addition of further material.

3. If further material is added, there is no evidence that it arises from the nucleus, since at the time when the pseudochromatin-granules are increasing rapidly, the nucleus is changing into the so-called resting state, during which time it takes the plasmatic stains while the granules are colored by the nuclear stains. It

seems no more probable that an emission should occur with the chromatin in a diffuse state than when it is in the condensed condition of the early growth-period, at which time no emission could be proved.

4. It is true that globular masses appear against the nuclear wall which after certain stains may be colored like both the pseudochromatin-granules and the chromatin. That they are not the same was shown by Benda's stain and double staining with safranin and methyl-violet (table 1). This similarity of staining reactions in some cases makes confusion of the three elements easy. The globules also, as stated above, do not form dispersion centers.

5. Although in the majority of cases the pseudochromatin-granules take the basic stains, there is still strong evidence from staining reactions against their chromatin character. Paradoxical as it may seem, the most convincing evidence against their being extruded chromatin is, in fact, from staining tests.

As can be seen from table 1, which presents the results of staining tests on sections after many fixations, the granules usually stain with the basic stains like the chromatin. For example, the result is the same when iron-hematoxylin is used after any killing fluid, whether it contains osmic acid, a heavy metal, or is an indifferent fluid such as neutral formalin, alcohol, or hot water. In fact, in these indifferent fluids, the granules stain as intensely as in material fixed in acid fluids, such as sublimate-acetic and picro-acetic fixatives, while they stain much more strongly than in eggs killed in the usual osmic acid containing fixatives. Again, in material fixed in Meves' fluid and stained according to Benda's method, the granules and the chromatin stain alike (i.e., yellow). Also, staining with thionin or Auerbach's fluid gives the same results, the granules take the basic stain. This is the result with the large majority of stains.

There are, however, some exceptions to this condition. The first fact to come to my notice was the variable results obtained with safranin and light green when used after Meves' and Fleming's killing fluids. In the early stages the nucleus stains with

the saffranin very intensely while the protoplasm is colored green. The color of the granules differs, however, according to the length of time the slide is left in the solution of light green, and consequently to the degree of extraction of the saffranin, while the chromatin stains in the same way under all circumstances. For example, if the saffranin is but slightly extracted, the granules are bright red like the chromatin, the protoplasm green. If the saffranin is somewhat further extracted, the granules appear purplish (i.e., a combination of the two colors), while the chromatin remains bright red. If the extraction is more complete, the granules become entirely green while the chromatin still appears bright red.

Since the above experiments suggested that the granules may not respond to all chromatin-stains, further tests were made. Several investigators (Crampton '96, Foot '96) have used solutions of lithium carmine and Lyons blue to distinguish the yolk-nucleus from the chromatin; the yolk-nucleus is stained blue, the chromatin stains with the carmine. Obviously, if the protoplasmic granules in *Hydractinia* are chromatin, they should stain with carmine. I find, however, that here again the granules stain differently from the chromatin, since they take the blue stain and the nucleus the red, this last even in late stages when the nucleus has usually lost its affinity for basic dyes. Further differences between the granules and the chromatin are shown by slight variations in their staining reactions when iron-hematoxylin is used after different killing fluids. As previously indicated, the granules are gray after Meves' killing fluid, slightly darker after Flemming's fluid, and intensely black after fixation in sublimate-acetic, formalin, alcohol, and hot water. The staining tests on preserved material indicate then, that the granules are not necessarily the same in their reaction as chromatin.

More striking and decisive results are given by experiments with stains on fresh material (table 2). I first tried a dilute solution of methyl-green slightly acidulated with acetic acid, as recommended by Lee ('03) for a chromatin stain. Here the results are as in the majority of experiments on fixed material, i.e., the granules take the basic stain (methyl-green) strongly

while the nucleus is unstained. Auerbach's stain, which combines acidulated methyl-green and acid fuchsin, also gives similar results since the granules take the green basic stain and the nucleus stains with the red plasma stain.

Since the above stains contain an acid, several of the more usual basic *intra vitam* stains were now tried (neutral red, methylene-blue and dahlia). Different results appeared immediately, since both the granules and the chromatin are stained intensely with the neutral red or the methylene-blue. A still different result was obtained with dahlia, for while the nucleus is stained intensely purple, the granules were but slightly tinged.

Since Lee states that these *intra vitam* stains are harmful to the cell and the results not trustworthy, some further tests were made with some comparatively new stains (footnote 4, table 2), which have been found by other workers to be perfectly harmless to the cell and to stain chromatin (Kite '13). These give the most striking results. Three stains were used; new methylene-blue G.G., new methylene-blue R., and diamond-fuchsin. A very dilute solution was made by adding a small amount of the stain to sea-water containing *Hydractinia* eggs. After a few hours the eggs were mounted in glycerine. Methylene-blue G.G. and R. gave the most striking results, although diamond-fuchsin gives convincing preparations. *The nucleus in every case takes the basic stain strongly while the pseudochromatin-granules are left colorless.* A noticeable difference in staining reaction occurs between fixed and living material, for in living material the nucleus stains in all stages with these basic stains while in fixed material the nucleus in late stages stains only in acid stains. Since in certain cases the granules in living material do not take the chromatin-stains, while the nucleus does, they cannot be the same as the chromatin in the nucleus and are therefore not formed chromatin extruded from the nucleus. And since with a stain that is acid, the staining reaction is reversed, i.e., the granules stain with the basic dye and the nucleus is either non-staining or stains lightly with acid stains, it seems probable that the presence of the acid is the determining factor in this reaction. The same explanation would hold for the behavior after many killing fluids.

In indifferent killing fluids (alcohol, neutral formalin, hot water) the reversed staining reaction of the granules must be due to some other cause than the presence of the acid. It is possible that mere precipitation changes the chemical composition of the granules, giving it an affinity for basic dyes.

Similarity in staining reaction for the identifying of materials has long be questioned. The physical and chemical nature of staining processes has been ably discussed by many writers (Fisher '09, Hardy '99, Lilienfeld '93, Heidenhain '11, Némec '10, Mathews

TABLE 2
Vital stains and digestion tests
Young eggs

KILLING FLUID		STAIN	NUCLEUS	NUCLEOLUS	PS. CHR.-GR.	LARGE GLOBULES
95 per cent or 100 per cent alcohol	Control ¹	iron-hem. and	blue black	black	black	black
	Digested	light green	black	black	black	black
Hot water	Control ¹	Auerbach	green(young)	blue	greenish blue	greenish blue
	Digested	iron-hem. and	green	green	green	green
	Control ¹	light green	black	black	black	black
	Digested	acidulated	no stain	5	intense	5
	Control ¹	meth-green	no stain	5	green	5
	Digested	Auerbach's	no stain	5	intense	5
	Control ¹	fluid	no stain	5	green	5
	Digested					
	Control ¹	New methylene blue	intense blue	5	no stain	5
	Digested	G. G. (4)	no stain	5	green	5
Fresh Material	Control ¹	New meth- ylene blue	intense blue	5	no stain	5
	Digested	R. (4)	no stain	5	green	5
	Control ¹	Diamond fuchsin	intense pink	5	no stain	5
	Digested		no stain	5	pink	5
	Control ¹	Millon's reagent	no stain	5	brick red	5
	Digested		no stain	5	no stain	5
	Control ¹	Neutral red	deep pink	deep pink	deep pink	5
	Digested	Methylene blue	deep green	deep green	deep green	5
	Control ¹	Dahlia	deep purple	deep purple	deep laven- der	5
	Digested					

¹ Sections of fresh material were digested in artificial gastric fluid (1 per cent pepsin in 0.2 per cent HCl) at body temperature from two to three hours. In the adult egg the yolk and mitochondria are digested, leaving a framework of protoplasm. Millon's reagent shows that some proteid from the protoplasm of the young egg is removed while the pseudochromatin-granules are not affected.

² The fixation is so poor that the mitochondria cannot be identified.

'98, Mann '02, Prenant '10 a, Lundegård '12, et al.). The consensus of opinion on the subject is that even though staining may be chemical it cannot be relied upon as indicative of chemical similarity of cell materials. This is clearly shown by Mathews ('98) and Zacharias ('97) who have demonstrated that previous acid or alkaline treatment determines what stain is selected by any cell element. The above experiments confirm the conclusion that similarly staining elements can hardly be considered identical. Again, experiments with egg albumen fixed in the various killing fluids according to Mann's method, confirm his results in that an

TABLE 2—Continued
Mature eggs

NUCLEUS	NUCLEOLUS	PROTOPLASM	MITOCHON- DRIA	COMPOUND YOLK	SIMPLE YOLK	OIL
blue black greenish gray	black black	green greenish gray	2	black	black	black 3
bluish red	blue	red	purplish	red	red	red
greenish red green green blue green	green black black 5	red green green light gray green	black no stain	black no stain	green no stain	black 5
blue green purplish pink	5 5	gray grayish pink	no stain	no stain	no stain	5
purplish pink	5	grayish pink	no stain	no stain	no stain	5
intense blue	5	pale gray	no stain	no stain	no stain	5
no stain intense blue	5 5	pale gray pale gray	no stain no stain	no stain no stain	no stain no stain	5 5
no stain intense pink	5 5	pale gray pinkish gray	no stain no stain	no stain no stain	no stain no stain	5 5
no stain	5	pinkish gray	no stain	no stain	no stain	5
no stain no stain pink pale green	5 5 pink pale green	no stain no stain pale pink very pale green	no stain no stain no stain no stain	no stain no stain no stain no stain	no stain no stain no stain no stain	5 5 5 5
deep purple	deep purple	pale purple	no stain	pale purple	pale purple	5

³ The oil globules were not identified in any of these tests.

⁴ These stains furnished by the Casella Color Company, New York, were found to be perfectly harmless used in dilute solution (Kite '13).

⁵ The nucleolus, large globules and oil have not been identified in fresh material.

apparently differential stain may be obtained on coagulated egg-albumen which may depend on purely physical differences or differences in density. Experiments were also made to test Heidenhain's contention that selective staining is most successful when the stains are applied simultaneously and progressively. Since different results are obtained when stains are used successively, simultaneously, progressively, or regressively, a true selective value can hardly be maintained. The evidence from all sides indicates that staining reactions are unreliable as chemical tests.

6. Artificial peptic digestion tests were tried to determine if possible, whether the granules are chromatin (table 2). If one accept the non-digestion of any material in the cell as proof of its chromatic character, then this test supports the nuclear origin of the granules. Material killed in alcohol and in boiling water as well as fresh material, was used in these tests. The fixed material was sectioned and digested on the slide in a 1 per cent solution of pepsin in a $\frac{1}{2}$ per cent solution of hydrochloric acid. Such digested sections together with an undigested control were stained with iron-hematoxylin and light green or with Auerbach's fluid. The fresh material was stained both before and after digestion with acidulated methyl-green, Auerbach's fluid, new methylene-blue G.G., new methylene-blue R., and diamond fuchsin, and mounted in glycerine. In all cases, after two to three hours of digestion at body temperature, the yolk, mitochondria and the bulk of the protoplasm were digested in the adult egg, leaving a slight framework or net which stained only with plasma stains. In the young eggs the protoplasmic granules remain undigested and take the basic stains, while the nucleus is non-staining. This reversal in staining of digested material as compared with fresh material when stained with the same neutral vital stains may well be due to the acid in the digestive medium, a point which supports the view that staining depends on previous treatment.

Even though the granules in fixed material, whether digested or undigested, stain with basic dyes, a slight difference between the two occurs. If two slides, one containing digested, the other undi-

gested sections, are run back to back through staining jars to ensure similar treatment, the granules in the digested sections after staining with iron-hematoxylin, take the stain much less intensely than those of the control slide. Also after Auerbach's stain the granules are grayish in the digested material, rather than green as in the undigested sections.

Still another point suggests that there may be other materials than chromatin in the cell which may not be digested by peptic digestion. In sections placed in a digestive medium in which the acid content is strong 0.5 HCl and digested either a long time (18 to 20 hours) at room temperature, or a short time (2 to 3 hours) at body temperature, all the material in the nucleus is digested while the granules remain undigested. This confirms the impression that the granules are of a different nature from chromatin. Again, the failure of a certain material to be digested by peptic digestion is not necessarily a proof of its chromatic character, since peptic digestion depends on the degree to which a substance is penetrated and therefore on the density of the material. In the above case it is possible that the acid aids in the penetration of the nuclear material but is unsuccessful in penetrating the granules. On this basis peptic digestion is no test for chromatin. Since pancreatic digestion digests the whole cell, it is evident that an alkaline medium is essential for the digestion of the pseudochromatin-granules.

7. Millon's proteid test also is in harmony with the possible chromatin nature of the granules. Before digestion, young eggs containing granules (as well as mature eggs) give the typical brick red reaction very strongly, while no proteid test is obtained after digestion, although the granules are still present as stated above. The proteid reactions in the undigested egg must then be given either by the protoplasm or some proteid associated with the granules, rather than by the granules themselves.

8. Attempts to locate chromatin by testing for histone as described by Mann ('02) were only partially successful. The results, however, support the view that the granules are not the same as the chromatin. The strongest color reaction indicating the presence of histone occurred in the nucleus, while the proto-

plasm of the young eggs containing the granules, although responding to the test slightly, did so no more strongly than the adult egg in which no granules are present.

9. Attempts to locate chromatin derivatives in the protoplasm by tests for phosphorus as described by Mann were also tried. These again are useless since Bensley ('06) has recently shown that the standard tests for phosphorus are unreliable.

To sum up: The balance of the evidence in *Hydractinia* decidedly indicates the nonchromatic nature of the granules in question. In all cases which seem to indicate the contrary conclusion (some staining and digestive tests and tests for proteid) the result can be interpreted in some other way. When to this are added the definite results from staining reactions in both fixed and living material and the morphological evidence that has been given above, we are, I think, forced to the conclusion that the granules are not chromatin extruded as such from the nucleus.

The above conclusions differ decidedly from Schaxel's, which appear to be based on very careful and detailed observations. Whether these conflicting views are due to the different forms studied, or to the fact that the large number of methods of fixation and staining used on *Hydractinia* has made the nature of the granules more evident, it is impossible to say. Since *Hydractinia* behaves, when Schaxel's methods are employed, in the same way that his material does, it seems probable that if the above methods were used on the forms studied by Schaxel they would yield similar results.

G. OTHER ACCOUNTS OF CHROMATIN-EMISSION IN HYDROIDS

Chromatin in the protoplasm has recently been described by several observers in a number of Hydroids. C. T. Hargitt ('13) finds a chromatin-emission in *Campanularia* brought about by the fragmentation of the nucleolus. The yolk develops from this extruded chromatin. Stschelkanowzew ('06) has described in *Cunina* a similar chromatin-emission through chromatin-nucleoli (secondary nucleoli). This condition is not found in *Hydractinia*, as the description of the nucleolus in a later section indicates. A

still different method of chromatin-emission is described by Smallwood ('09) in *Hydractinia*. Soon before maturation small particles of chromatin leave the nucleus and wander into the protoplasm. Smallwood says, "The reason for regarding them as chromatin is because they give the same color reactions as similar shaped bodies in the nucleus, and for the further reason which is obvious in figure 1, namely, the actual migration of the chromatin from the nucleus." An exactly similar chromatin-emission is described by Smallwood ('07) for *Pennaria*, taking place here, however, after maturation rather than before. Also in this form the chromidia are stated to arise from both male and female germ-nuclei. In these two Hydroids the protoplasmic chromatin is connected with neither yolk formation nor differentiation. As will be shown in the next section, I have traced the nucleus in *Hydractinia* through the growth-period, the reappearance of the chromosomes and maturation stages, and in no case are bodies of the type described to be found. From Smallwood's figures, it is evident that the fixation is defective, since a very strong coagulation net is present which does not appear with good fixation. It seems to me the bodies in question may well be artifacts.

Trinci ('07) has described basophilic granules in the oocytes of a number of Hydroids, which he considers as a differentiation of the protoplasm brought about by the influence of the nucleus and as belonging in the same category as chromidia, mitochondria or plastosomes. The granules disappear during the development of the egg.

Van Herwerden ('13) has recently tested Schaxel's hypothesis of the nuclear origin of the basophilic granules by the use of nuclease. After treating the mature echinoderm egg (in which mitochondria are visible in life) with a preparation of nuclease, he finds that the mitochondria (basophilic granules) have disappeared. He concludes that the mitochondria are a nucleic acid compound and therefore properly chromidia. Since in the young, living egg he can see none of the basophilic granules which appear in fixed material, he believes the latter are artifacts. Unlike Schaxel, however, he holds the mitochondria to be developed from this basophilic substance, since it is also a nucleic acid

compound as shown by its digestion with nuclease. Direct observation on young, living eggs failed to show a direct migration of material from the nucleus into the protoplasm. Diffusion currents present in the egg, coincident with a slight nuclear shrinkage, he feels, favors the diffusion of a soluble substance through the nuclear wall. That he is dealing with a substance that differs from the granules of *Hydractinia* is apparent, since vital stains give different results in the two cases. His granules are stained with dahlia, those of *Hydractinia* are not. With methylene-blue and neutral red his granules do not stain, while those of *Hydractinia* do. Also the fate of the granules differs in the two cases. They can hardly then be homologized. Although he has been unable to furnish any more definite proof of a direct migration of nuclear material into the cytoplasm, the nuclease digestion indicates nucleic acid present in the granules and mitochondria.

H. MITOCHONDRIA

1. *Experimental*

The presence and behavior of the mitochondria as seen in sections have been sufficiently described above. Further investigation of the function and behavior of these bodies was carried on by means of some centrifuging experiments. In previous experiments of this sort, no attempt has been made to locate the mitochondria after centrifuging the egg and to determine their further behavior in development. *Hydractinia* eggs within 5 minutes after fertilization were placed in a water-centrifuge and revolved at a moderate speed for $1\frac{1}{4}$ to $1\frac{1}{2}$ hours, or until the control eggs had divided once. The cell-materials are separated into three layers (fig. 61, a, b). Sections of eggs killed as soon as removed show the oil at the small end of the pear-shaped egg, forming an oil-cap. A clear protoplasmic layer lies below this, while the broad end of the egg is filled with a mingled mass of yolk and mitochondria (fig. 62). If such eggs were removed to sea water and allowed to develop it was found that the first cleavage-plane cuts the egg without reference to the stratification

(fig. 63). The majority of the eggs cleave so that the different materials are equally distributed in the two blastomeres; often, however, the distribution is unequal. *Complete separation of the kinds of material may occur, as when the cleavage plane comes in through the protoplasmic layer between the ends* (figs. 63, b; 65, g; 66, a, b, e, f). Sections show this to be due to the position of the nucleus in regard to the stratification. Since the eggs are not centrifuged sufficiently to move the nucleus, it may lie in any relation whatever to the egg-materials. It is, however, usually found in the protoplasmic layer (fig. 64, a, d) in which case the egg divides so as to distribute the materials equally (fig. 63, f). The nucleus may also lie in the yolk-end of the egg (fig. 64, c) in which case the cleavage-plane comes in through the yolk (fig. 63, a). The nucleus not infrequently appears at one side of the protoplasmic layer (fig. 64, d), in which case the first cleavage-plane separates the two kinds of material, one blastomere receiving in addition to part of the protoplasm, yolk and mitochondria and the other the oil mass.

Individuals showing various distributions of the materials were isolated and their development was followed, apparently normal swimming larvae resulting (fig. 65). The yolk and mitochondria are confined to one region of the resulting planula, the oil to another. Also eggs which cleaved so that the different materials segregated in the two blastomeres were cut apart, separating the blastomere containing yolk and mitochondria from the one containing oil (fig. 66). These were isolated and the development followed, and although many died, I succeeded in getting a considerable number of these half larvae. If the protoplasmic area were nearly evenly divided between the two blastomeres, both were apt to live. So far as I could tell those that lived were perfectly normal, except in size, the one being small and white from its oil content, the other large and greenish from the yolk content. Both were ciliated planulae. Sections of these planulae which were killed in Meves' killing fluid and stained with Benda's method, show the mitochondria apparently unchanged in one, while the other contains none. It is apparent, then, that up to this point of development the mitochondria are not essential for

development. Hydroid planulae are hard to carry beyond this point of development in the laboratory, since any disturbance prevents the planula from attaching, which is essential for further development. Even so slight a disturbance as changing the water in the dish is sufficient to prevent attachment, so that further development after so great a disturbance as separating the blastomeres is impossible.

The above experiments are of value only in indicating that the mitochondria are not essential for differentiation as far as the planula-stage and that they can hardly be vital constituents of the protoplasm since they may be centrifuged out of the protoplasm like any metaplasmic body, such as yolk.

2. Discussion

Mitochondria in *Hydractinia* do not agree in a number of points with descriptions of these bodies in other forms. An extensive review of the subject will not be attempted, however, since it has been so well discussed and reviewed by many recent investigators (Fauré-Fremiet '10, Prenant '10 b, Montgomery '11, Duesberg '11). Duesberg presents a monumental review of the literature on the subject in which he brings together in classified form and in the most exhaustive way, all papers concerned with mitochondria and chromidia. The opposing views as to the nuclear or protoplasmic nature of these bodies have already been stated. The above writers assume the identity of mitochondria and chromidia. Schaxel, on the other hand, considers them quite distinct, as has been sufficiently indicated above. It is also apparent from the foregoing description that *Hydractinia* corresponds with Schaxel's observations in this respect, that is, that two distinct elements are present, mitochondria of undoubted protoplasmic origin and basophilic granules in the protoplasm. The latter, however, are not chromatic in *Hydractinia* and therefore not chromidia (extra-nuclear chromatin of Schaxel).

I also agree with Schaxel in finding that the pseudochromatin-granules first appear in the early growth-period of the egg, thus giving no evidence of continuity from cell generation to generation.

Again, Schaxel finds mitochondria already present in the early growth-period of the egg before his 'chromatin-emission' occurs. In this respect results with *Hydractinia* are different, since the mitochondria appear only after the egg is a third grown. *Hydractinia* is an exception to the rule in this, since in most forms the mitochondria are either already present or appear in the early growth-period. The possibility of direct nuclear origin of the mitochondria in *Hydractinia* is also excluded, since they arise *de novo* throughout the egg and are not collected in a definite body against the nucleus (yolk-nucleus) as described in many forms.⁴ The mitochondria in *Hydractinia* do not contribute to the formation of the yolk, as described for other forms by a number of workers.⁵ I also find no grouping of the mitochondria around an idiozome, indicating their origin through the retrogressive development of the 'centroplasm' as suggested by Vedjovsky. Again, *Hydractinia* gives no evidence of the mitochondria forming a part of the architecture of the protoplasm (Fauré-Fremiet and others), since in centrifuged eggs they are carried to one pole of the egg together with the yolk, leaving a free layer of protoplasm which has the usual protoplasmic structure. Since the blastomere of a centrifuged egg containing no mitochondria develops into a swimming larva, they can hardly be vital units of the protoplasm. I find no indication of their multiplication by division

⁴ A yolk nucleus of mitochondria is described in the eggs of the stint (Lams '04, Arch. Anat. micr., T. 6); *Rana* (Lams '07, Arch. Anat. micr., T. 9); *Proteus* (Schmidt '04, Anat. Hefte, Bd. 27, and M. Jörgensen '10, Festschr., R. Hertwig); *Testudo* (Loyez '05, '06, Arch. Anat. micr., Bd. 8); chick (D'Hollander '04, Arch. Anat. Micr., T. 7); some birds (Loyez '05, '06, Arch. Anat. micr., T. 8); human egg (Van der Stricht, '05, Bull. Acad. Belgique); cat, (Russo, '09, '10, Arch. f. Zellfor., Bd. 4-5); bat and guinea-pig (Van der Stricht '05, Compt. Rend. Assoc. Anat., Geneve); *Ascaris* (Schoonjans '09., Bull. Soc. Roy. Sci. Med., Brussels); *Ciona intestinalis* (Loyez '09, Assoc. Anat., Nancy).

⁵ Yolk is described as being formed directly from the mitochondria by Russo ('09, '10) in cat; Loyez ('09), ascidians and human egg, Compt. rend. Assoc. Anat., Paris; Fauré Fremiet ('10, Arch. Anat. micr., T. 11); in Lithobius; Zoja ('91, Mem. del R. Inst. Lomb. di Sci., vol. 16) etc. Yolk is described as formed indirectly under the influence of mitochondria by Van der Stricht ('05) in the bat; Lams et Devorene ('08, Arch. de Biol., T. 23) in some mammals; Van Durne ('07, Ann. Soc. Med. de Gand, T. 88); Schoonjans ('09) *Ascaris*; Bluntzschli ('04, Morph. Jahrb., Bd. 32) etc.

at any time during the growth-period or cleavage, as suggested by Duesberg ('10) and Fauré-Fremiet ('10 a). Their origin and behavior in *Hydractinia* indicate that they may be either precociously differentiated portions of the protoplasm (Vedjovsky '07) or metaplasmic bodies.

Scepticism as to the identity of the bodies described as plasmosomes, chondriosomes, chromidia, ergastroplasm, etc., has been expressed by a number of observers (Veratti '09, Penas '11, Lundegård '10, Gurwitsch '10 and others) who believe that they have nothing in common but their name. Such an impression is certainly gained in reviewing the literature. The experiments with staining tests lead the writer to join these investigators in the belief that structures which have the same staining reactions may have been confused. If the standard tests for mitochondria are to be relied on for identifying them, then the mitochondria of *Hydractinia*, which respond to these tests, do not conform in many respects to the conditions found in other forms.

IV. MATURATION PHENOMENA AND AMITOSIS IN HYDRACTINIA AND EUDENDRIUM

When this work was begun it was my purpose to re-examine the eggs of several Hydroids which had been described as showing no mitotic figures during maturation, a nuclear disintegration or fragmentation occurring at the time of the disappearance of the germinal vesicle. The suggestion was made "that reduction phenomena of maturation may well be accomplished without any of the complex and spectacular processes of mitosis" (Hargitt '06). Nuclear reconstruction was described as occurring later through the collection of these fragments in several 'nuclear nests' throughout the egg (C. W. Hargitt, *Pennaria*, '04, *Eudendrium* '04, *Clava* '06; Allen, *Tubularia crocea*, '00); and the cleavage of the egg as frequently amitotic. Smallwood ('09) and G. T. Hargitt ('09), have since established the occurrence of typical maturation phenomena and mitotic cleavage in *Pennaria* and *Tubularia*, while independent studies by the writer ('09) gave the same result both in *Pennaria* and in *Clava*. Since

Eudendrium, among the above forms, has not been re-examined, a brief account of the maturation stages occurring in this form will be given here. This section is concerned chiefly with a brief account of the maturation stages in *Hydractinia* to supplement Smallwood's account which as he states is incomplete because of lack of material. I have been fortunate in finding the stages lacking in his description.

A. HYDRACTINIA

1. *Nucleus of the growth-period*

As described in the previous section, in the early growth-period the nuclear net is centered in the nucleolus from which it radiates (figs. 2, 3, 4). As the egg grows this radial arrangement is lost, the chromatin assuming a reticular form (figs. 7, 8, 25). While these early stages show the same nuclear structure after preservation with any killing fluid, later stages are profoundly modified. When preserved in sublimate-acetic or picro-acetic killing fluids, a coarse, deeply staining, granular reticulum appears in a colorless ground-substance (fig. 12). That this reticulum is a coagulation phenomenon is suggested by comparing this nucleus with those shown in figures 19, 20, 21 and 23, which are sections of eggs killed in Meves' killing fluid. Here the nuclear net, which has changed into a fine net, stains more lightly in basic dyes than after the former fixatives, and lies in a finely granular, homogeneous ground substance which stains lightly with plasma stains (figs. 18, 19, 25). Figures 20 and 28 show a slightly older stage in which a process of diffusion of the chromatin net, previously begun, has proceeded until there is just a suggestion of the net in the homogeneous ground-substance. This leads directly to the condition shown in figure 21, in which all trace of the net has disappeared and only the ground-substance is left. The question of the method of disappearance of the chromatin will be taken up in detail a little later. The net completely disappears before the egg is one-third grown. The nucleus lies at the center of the egg until near the end of the growth-period when it moves to the free surface of the egg.

2. *Maturation stages*

The maturation phenomena take place while the egg is still in the gonophore as Smallwood ('09) states, and not after leaving it (Bunting '94). The lightly staining, homogeneous condition of the nucleus, which has long been recognized as a characteristic of the hydroid egg, and which has led to much confusion concerning the maturation stages, persists until the nucleus breaks down to form the chromosomes. The first indication of reappearing chromosomes occurs in gonophores killed from 20 to 30 minutes before eggs from the same colony are deposited. Since the reformation of the chromosomes is best seen in material preserved in fluids which do not cause a heavy precipitate, the following description is based on material fixed either in Meves' or Flemming's fluid, neutral formalin, or hot water. The deeply staining nucleolus is usually still present at the inner border of the nucleus; its history will be described more fully later.

Out of the apparently homogeneous ground-substance of the resting nucleus, there appear very lightly staining threads on which are groups of granules staining a little more intensely than the threads. These threads appear in pairs, either parallel or X like in form (figs. 30, 31). In either case many fine branches merge from the main threads into the general homogeneous ground-substance. The number of these groups of threads in a single nucleus corresponds to the haploid number of chromosomes (12 or 14). The exact haploid number has not been determined but since 14 such pairs of threads is the number most frequently found in a nucleus and since 14 tetrads is the usual number found in the later stages (figs. 4, 46), it seems certain that this represents the haploid number and that they are bivalent chromosomes. The chromosomes now condense rapidly into tetrads which are very much smaller than the crosses. The initial stage in this process consists in a shorting of the arms of the X and the collection of the granules in a mass at the center, the latter taking a slightly deeper stain than before (fig. 32). In a later stage (fig. 33) the ends of the arms of the X are still visible, although the bulk of the granules appear at the center. The condensation

consists apparently in the migration of the granules along the linin-threads toward the central point, leaving the lightly staining net merging into the ground-substance. The chromosomes now condense rapidly into small compact tetrads which stain intensely. A number of stages in the formation of a tetrad are shown in figure 24. The nuclear membrane breaks down about this time and the chromosomes which have been scattered through the nucleus collect at the center (fig. 24).

Up to this point there has been no evidence of a spindle. When it does appear, the chromosomes are already collected in the center of the nuclear area (fig. 35) and the spindle apparently arises in connection with them from the achromatic portion of the nucleus. The spindle, which is many times smaller than the nucleus, differs entirely in structure according to the fixation. The general topography of such a spindle in the center of the nuclear area after Meves' fixation is shown in figure 37. The much enlarged spindle shown in figure 38 makes it clear that no centrosomes or astral radiations are present, the blunt spindle lying free in the nuclear area. If material is fixed in sublimate-acetic solution, in addition to the spindle, small asters appear, which are continuous with the coarse net present in the nuclei of such eggs (fig. 36). They give every appearance of being part of the coagulation phenomena caused by the killing fluid. In some cases a small centrosome-like body occurs at the center of the aster. But since it is not constant and may be asymmetrically placed in regard to the spindle, it also seems to be a result of the coagulation. In fact, I have not been able to find a true division-center, either outside or within the nucleus. The spindle fibers arise, apparently independently of an aster or centrosome, directly out of the nuclear ground-substance, for the spindle appears directly in the center of the large nuclear area, a considerable layer of the nuclear plasma surrounding it. The tetrads are now drawn on to the spindle and become arranged in an equatorial plate (figs. 37, 38). As stated above, the number of tetrads in the equatorial plate has not been definitely determined, the number being between 12 and 15. Since 14 was more commonly present and it is the number appearing in the polar body, it seems probable

that this is the haploid number (figs. 39, 40, 46). A small element near the center of the plate is characteristic.

The spindle, still without asters or centrosomes, now rotates 95° until it is perpendicular to the surface, and then moves out of the nuclear area toward the surface, where the first polar body is formed (figs. 43, 44, 45). As seen in some of the figures, a single or double granular mass—the remains of the nucleus—is left behind in the protoplasm, where it is absorbed. Since the manner in which the tetrads are formed is not determined, it is impossible to interpret this division in terms of reduction. The second polar spindle is formed immediately, as shown in figure 46. The egg is now shed from the gonophore, a small female germ nucleus being very rapidly reconstructed from the remaining chromatin (fig. 47). The very great difference in size between this nucleus and the germinal vesicle has been sufficiently emphasized by previous writers on Hydroids. The egg, which has been somewhat flattened in the gonophore, rounds up when shed.

This description of the formation of the first polar spindle differs from that given by Smallwood, who finds it appearing with asters in the protoplasm in connection with a very small nucleus which is many times smaller than the typical germinal vesicle of *Hydractinia*. The small size of the nucleus and the position of the spindle outside of the nucleus makes it probable that he has figured the first cleavage-nucleus and spindle. Since I have traced consecutive stages in the breaking down of the germinal vesicle and the formation of the chromosomes and spindle, (stages which Smallwood lacked) it seems conclusive that a blunt spindle minus asters and centrosomes lying in the center of the large nuclear area, is typical for *Hydractinia*. This conclusion is supported by the condition found in other forms, since a blunt spindle minus asters has been described in a number of Hydroids (*Gonothyrea*, Wulfert, '02; *Clava squamata*, Harin '02; *Clava leptostyla*, Beckwith '09; *Eudendrium*, present paper; *Linerages*, Conklin '08; *Cordylophora*, Morningstein '01; *Cunina*, Stschelkanowzew '06). Further the formation of the spindle within the nuclear area itself also find support in the condition described

for *Clava* by Harm ('02) who finds the first polar spindle very small and formed within the germinal vesicle from the achromatic portion, and the same condition in *Cordylophora*, as described by Morningstein ('01).

3. Fertilization

The egg is fertilized as soon as it is shed, the spermatozoon entering at any point on the surface. A fertilization-membrane is formed (fig. 42). The method of union of the two germ-nuclei depends on the point at which the spermatozoon enters. If, as often happens, it enters near the female germ-nucleus (fig. 47) the sperm head may enter the egg-nucleus bodily, without expanding (fig. 49). Here the chromatin of the egg-nucleus is already collected in masses to form the chromosomes of the first cleavage-spindle. An aster accompanies the sperm head in this case as in others, no spindle as yet having formed, however. If the sperm enter at some distance from the female germ-nucleus, it enlarges as usual before union and a spindle develops in connection with it. The degree of enlargement varies, as shown in figures 48 and 50. Complete fusion of the two nuclei may take place before the breaking up into chromosomes but this is not essential.

The entrance of the sperm head directly into the egg nucleus without expansion is described in some other Hydroids (Wulfert, '02, *Gonothyrea*; Harm, '02, *Clava squamata*; I have also observed it in *Pennaria*). Also as the figures show, I do not find the sperm head in *Hydractinia* forming a group of vesicles as figured by Smallwood ('09) in *Hydractinia* and Smallwood ('09) and Hargitt ('09) for *Pennaria*. It expands directly into a single vesicle.

4. Fertilization membrane and mitochondria

The hydroid egg is usually spoken of as naked (Wilson '00, Hargitt '04). Smallwood ('09), however, has described a membrane for *Pennaria*, formed at fertilization. I have found this membrane easy to demonstrate in fresh material if *intra vitam* staining methods described by Kite ('12) are used. In *Hydrac-*

tinia a membrane is also present. Sections of an unfertilized egg show a yolk-free area at the surface of the egg in which mitochondria are scattered irregularly (fig. 41). No distinct membrane can be seen at this time. Sections of fertilized eggs show the mitochondria arranged in a distinct layer directly at the surface of the egg, a more or less free space being left between them and the yolk-spheres. Outside the layer of mitochondria a very thin, transparent layer or membrane appears, which as a rule clings closely to the surface of the egg (fig. 42). The method of formation of the membrane was not determined.

5. *Nucleolus*

As stated earlier, a nucleolus is already present in the egg after the last oogonial division, when the chromatin is in the bouquet-stage (figs. 1, 2, 3). Its origin was not determined and it stains intensely in basic dyes. As the chromatin breaks up into a net, it is still pressed against the nuclear wall and may already show vacuoles (fig. 4). When the radial arrangement of the net is lost, the nucleolus is no longer flattened against the nuclear wall, but may be well toward the center of the nucleus (figs. 7, 18). In general, however, it retains its excentric position (figs. 19, 21, 22). During the growth-period the nucleolus increases in size and becomes vacuolated, the skeleton retaining its intense staining capacity for basic dyes while the vacuoles stain with acid dyes. One large vacuole may occupy the center, leaving a rim of basic staining material (fig. 29 b), or many fine vacuoles may appear, making the nucleus more or less spongy (figs. 27, 28, 29 a). Both conditions are typical for Hydroids. The increase in size continues throughout the greater part of the growth-period, a point in which *Hydractinia* differs from *Pennaria*, as described by Hargitt, since in the latter form growth stops and nucleolar disintegration begins as soon as the spireme is completely broken up. About the time of maturation—usually before the breaking down of the nuclear membrane—the nucleolus dwindles and disappears in the substance of the nucleus by a process of dissolution and not by fragmentation as described by Hargitt for *Tubularia*. It may have

disappeared before the reappearance of the chromosomes (fig. 23), or, as is more common, it may be disappearing as the chromosomes are reforming (fig. 29, a).

Because of its staining reaction, the nucleolus was at first thought to be chromatic. Since the nucleus, throughout the greater part of the growth-period, is non-staining with basic dyes while the nucleolus takes these stains intensely, it at first seemed evident that the chromatin which is to form the chromosomes is stored in the nucleolus during this stage, a condition described for a number of forms.⁶ This conclusion proves to be unfounded after further staining tests, since the nucleolus does not always take the characteristic chromatin-stain (table 1.) For example, in Hermann's saffranin methyl-violet stain, the nucleolus is red while the chromatin of the 'resting' nucleus is violet. Also, in Bensley's acid fuchsin methyl-green stain the nucleolus stains with the fuchsin or plasma stain. Again, in Auerbach's stain, which ordinarily stains the chromatin green, the nucleolus is stained blue. The strongest evidence that the nucleolus is not chromatin is given by Benda's stain, after which the chromatin is yellow-brown and the nucleolus violet. This point is confirmed by Dublin ('05) who finds decisive proof with Auerbach's stain that the basic staining-nucleoli of *Pedicellina* are not chromatin. The nucleolus in *Hydractinia* has at no time any direct connection with the chromosomes as described by Guenther ('03) and Dublin ('05). There is no evidence then that the disappearance of the nucleolus at the time of the reappearance of the chromosomes bears any relation to the same. From the above description of the nucleolus in *Hydractinia*, it is evident that no fragmentation of the nucleolus contributes to the formation of basic granules (pseudochromatin-granules) which lie in the protoplasm, as Hargitt finds in *Campanularia*.

⁶ Chromatic nucleoli have been described among hydroids in *Forskalia* and *Agalma* by Schaxel ('11); *Gonionemus*, Bigelow ('07); *Campanularia*, Hargitt ('13); *Cubomedusa*, Conant ('98) *Hydra*, Downing ('09); *Gonothyrea*, Wulfert ('02). Günther ('03) finds the nucleolus in the echinoderm egg forming out of the nuclear net, and the chromosomes reappearing from the nucleolus.

6. Chromosomes and continuity

My results contribute little to the solution of this question, but since some evidence in its favor appears in the nucleus of *Hydractinia* eggs, it can hardly be passed by without comment. As previously stated, when the chromatin becomes diffuse, the net-like arrangement of the chromatin is lost. This is brought about by the threads becoming gradually arranged in groups of two either parallel or cross-like threads (figs. 25, 26). These crosses or parallel threads gradually fade out completely, leaving the characteristic lightly staining nucleus (figs. 21, 27, 28). That the chromosomes reappear in exactly the same way in which they disappear, that is, as X's and parallel threads, is evident by comparing the figures just described with those of reappearing chromosomes (figs. 29, 1, 30, 31). In fact, they are so exactly alike in form, in many cases, that whether they are disappearing or reappearing can only be decided by other conditions in the egg and nucleus. It is hard to resist the impression that this identity in their method of disappearance and reappearance is significant of some sort of continuity.

B. *EUDENDRIUM RAMOSUM**Maturation and fertilization*

The complete history of the chromatin in the maturation of *Eudendrium* has not been worked out, but such stages as I have establish its regular character. Maturation, fertilization and development of the egg up to the planula stage take place in the gonophore. The position of the nucleus after it leaves the center of the egg differs from most other hydroids, since it does not lie at the free surface of the egg as it ordinarily does, but at the inner end which leads to the cavity of the gonophore (fig. 51). The maturation and fertilization stages take place at this point. I have sections only of material fixed in sublimate-acetic killing fluid, so that there is always present in the resting nucleus the heavy net-like structure caused by this fixation.

During the growth-period the nucleus consists of a fine net with a deeply staining nucleolus present (fig. 52), the whole very similar to a corresponding stage of *Hydractinia* after the same killing fluid (fig. 22). The nucleus grows enormously as is shown by comparing figures 52 and 53, the latter being ready for maturation. Because of the coagulation phenomena, I have been unable to trace the reappearance of the chromosomes in this form. Clumps of chromatin lying in the nuclear net give the first indication of reappearing chromosomes (fig. 53). The nucleolus here, as in *Hydractinia*, increases much in size throughout the growth-period, becomes vacuolated, and disappears before the polar spindle is formed. The origin of the spindle was not determined since the first spindles seen were completely formed and, in the equatorial plate state (figs. 52, 55), perpendicular to the surface. The spindle is of much smaller size than the nucleus and is also without asters and centrosomes. The chromosomes are not in the form of tetrads and, since their origin is not known, nothing can be said of their quadripartite condition (fig. 55). I have also too few sections of the equatorial plate to establish definitely the haploid number. Since 13 is the most constant number appearing, it is undoubtedly near the reduced number (fig. 56). Two stages in the formation of the second polar spindle are shown in figures 57 and 58. A female germ-nucleus (fig. 59), characteristically smaller than the germinal vesicle (fig. 53), is reconstructed after the last polar division. The only cases of fertilization which I have observed show the two germ-nuclei of equal size (fig. 60), indicating that the sperm enters the egg early and so expands before meeting the egg nucleus. I have found no spindle in connection with the fusion nucleus, but the fact that the protoplasm killed at this period is not well fixed, may account for this. Development was carried no farther, the question of cleavage and amitosis not being studied in this form. The establishment of a single cleavage-nucleus makes nuclear fragmentation and subsequent reorganization in 'nuclear nests' impossible and amitotic cleavage improbable.

V. SUMMARY

1. The bulk of the evidence from staining reactions and morphological conditions indicates that those protoplasmic granules in *Hydractinia* which often take the chromatic stains are not extruded from the nucleus as such, and points to their formation *de novo* throughout the protoplasm. The granules are therefore not comparable to the chromidia of Hertwig and the term 'pseudochromatin-granules' is justified.

2. There is no evidence of formed material passing through the nuclear membrane into the protoplasm either early (Schaxel) or late (Smallwood) in the growth-period. Globules, which may be mistaken for such material, are formed during the growth-period, flattened against the nuclear wall, but their staining reactions under certain conditions differ from those of chromatin.

3. We are still unable to differentiate with certainty by any of the above methods, the nuclear derivatives in the protoplasm, i.e., staining tests cannot be relied on as tests for chromatin.

4. The yolk is formed in *Hydractinia* directly from the scattered pseudochromatin-granules and not from nests of granules.

5. The pseudochromatin-granules correspond in a general way to the yolk-nucleus of many other forms. Here the granules are never gathered into a distinct body.

6. The pseudochromatin-granules are completely used up in the formation of the yolk; that is, none, as such, are left in the protoplasm between the yolk-spheres (intravitelline chromatin of Schaxel) to determine further differentiation.

7. The yolk is not formed from mitochondria in *Hydractinia* and there is no yolk-nucleus consisting of mitochondria.

8. The mitochondria are not of nuclear origin in *Hydractinia*, but arise *de novo* in the protoplasm after the formation of the yolk has begun. I find no evidence of multiplication of mitochondria by transverse division, or of their genetic continuity from one cell-generation to another.

9. The mitochondria and chromidia (extra-nuclear granules) are not identical in Hydractinia.

10. The mitochondria are not a vital part of the protoplasm in Hydractinia but are a highly differentiated product.

11. Maturation and fertilization are typical in Hydractinia and Eudendrium and cleavage is mitotic.

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PLATES

PLATE 17

EXPLANATION OF FIGURES

*Hydractinia echinata*⁸ × 2625

1 Two egg cells resulting from the last oogonial division; *a*, diplofene stage of spireme-thread; *b*, early bouquet-stage.

2 Early bouquet-stage, showing loops centered in the nucleolus.

3 Late bouquet-stage, showing the nucleolus pressed against one end of the nucleus.

4 to 8 Five stages in the early growth-period, showing the accumulation of 'pseudochromatin-granules' throughout the protoplasm, the appearance of globules against the nuclear wall and oil in the protoplasm; semidiagrammatic. Granules are represented by gray, oil by circles containing dots, nuclear globules by circles containing parallel lines.

5 to 11 Three later stages of the growth-period, showing the direct development of the yolk-spheres from the pseudochromatin granules; semidiagrammatic. Young yolk-spheres shown in gray.

12 Egg about one-third grown, showing the appearance of mitochondria between the simple yolk-spheres, some of which are now mature and possess a different staining reaction; semidiagrammatic. Mature simple yolk is represented by circles; mitochondria by black rods, young yolk and oil as above.

13 A nearly mature egg showing compound yolk-spheres in addition to the above elements, as well as more mature simple spheres; diagrammatic. Compound spheres are represented by circles containing small circles.

⁷ All figures except those of plate 8 and figure 61 were drawn with a camera.

⁸ All drawings were made from material fixed in Meves' or Flemming's killing fluid unless otherwise stated.

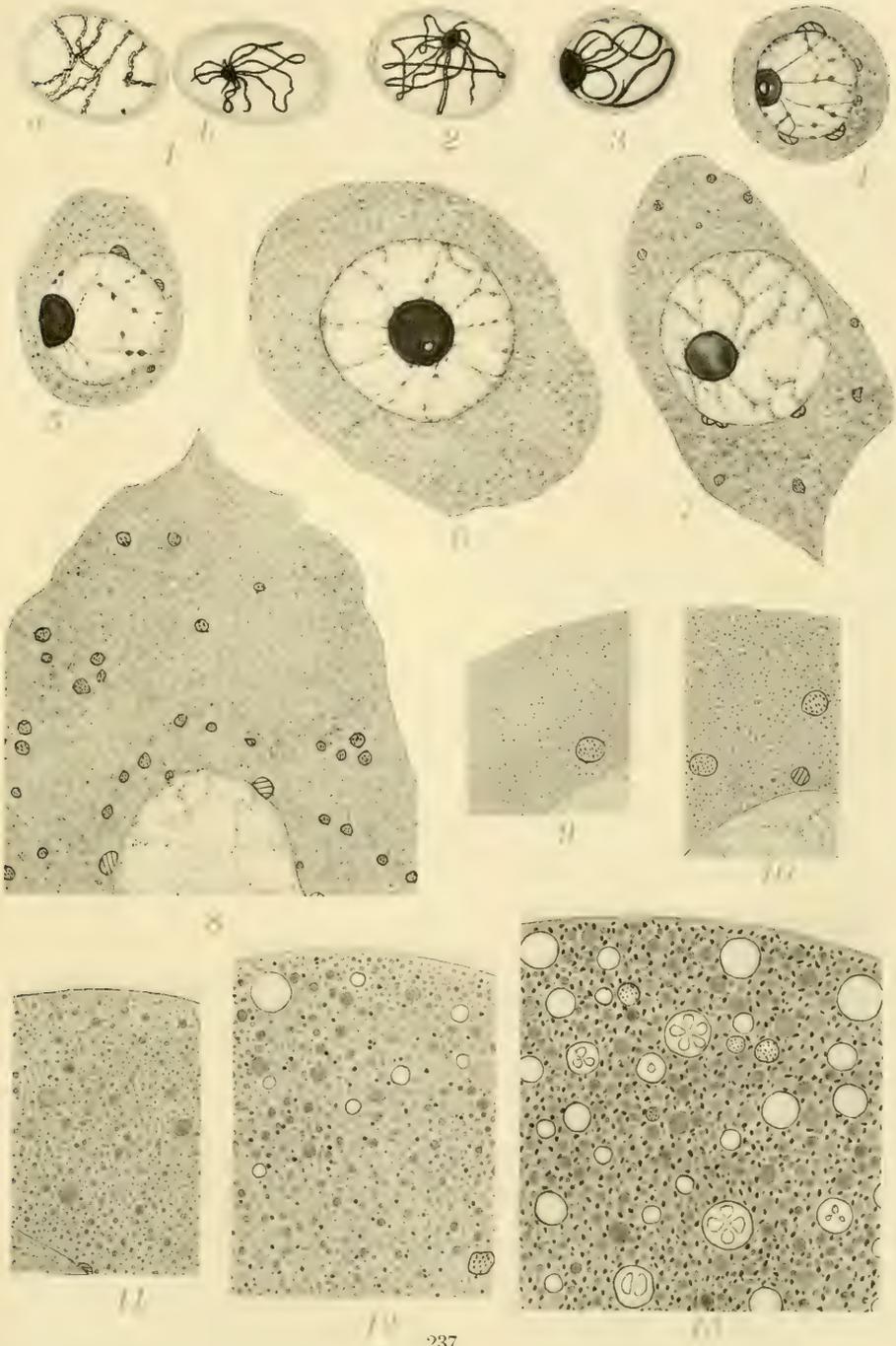
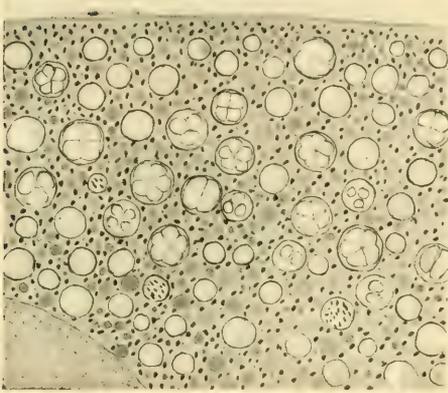


PLATE 2

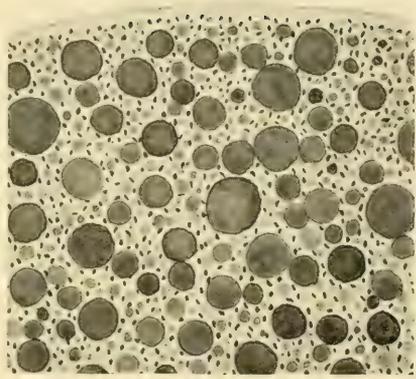
EXPLANATION OF FIGURES

Hydractinia echinata

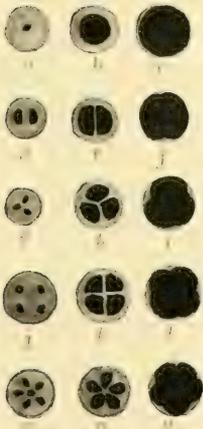
- 14 Mature egg, showing the arrangement of mitochondria, simple and compound yolk, and oil; diagrammatic. Symbols as in plate 1. \times 2625.
- 15 Types and development of compound yolk-spheres. \times 2625.
- 16 Mature egg as shown after staining with iron-hematoxylin. \times 2625
- 17 Young oocyte, showing radial arrangement of nuclear net. Meves' killing fluid. \times 825.
- 18 to 20 Older eggs showing the gradual disappearance of the nuclear net in a homogeneous ground-substance. Meves' killing fluid. \times 600.
- 21 Nucleus showing the complete disappearance of the nuclear net, a granular ground-substance only remaining. Meves' killing fluid. \times 600.
- 22 The same, after sublimate-acetic killing fluid, showing a strong coagulation net in a colorless ground-substance. \times 600.



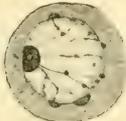
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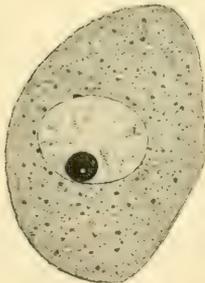
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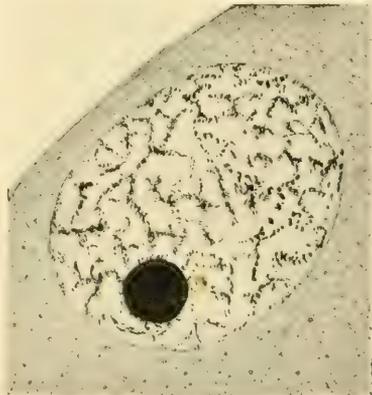
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PLATE 3

EXPLANATION OF FIGURES

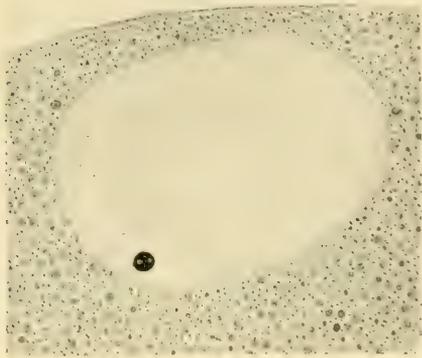
Hydractinia echinata

23 Nucleus just before the reappearance of the chromosomes, showing the homogeneous ground-substance devoid of chromatin net and the nucleolus already much reduced in size. $\times 600$.

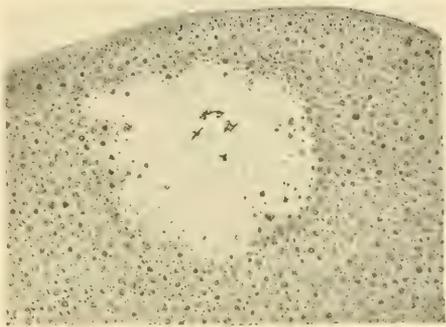
24 Chromosomes reappearing in the nuclear area, the nuclear wall having broken down. $\times 600$.

25 to 28 Four stages of nuclei in the early growth-period, showing the disappearance of the nuclear net by the chromatin arranging itself in crosses and parallel threads which become gradually fainter. $\times 1225$.

29 a The reappearance of a chromosome, just before maturation, in the form of a cross, the nucleolus with many vacuoles. *b*, nucleolus with one large vacuole. $\times 1225$.



23



24



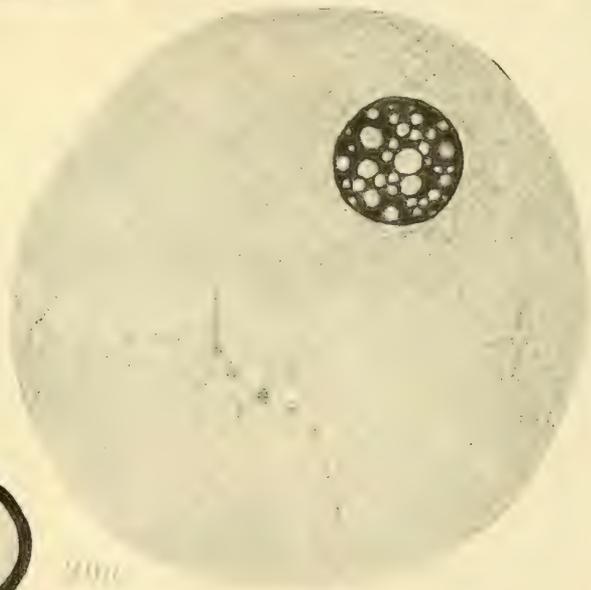
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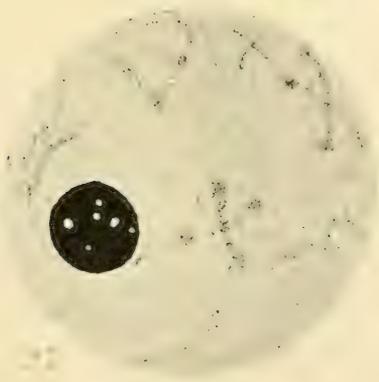
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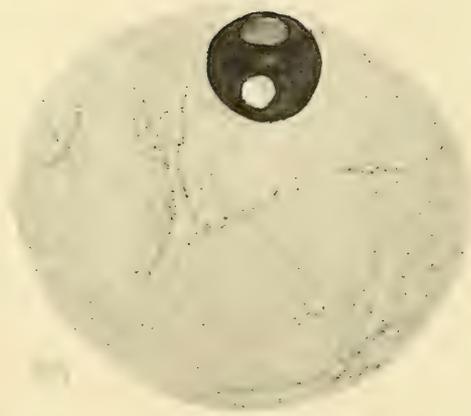
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PLATE 4

EXPLANATION OF FIGURES

Hydractinia echinata

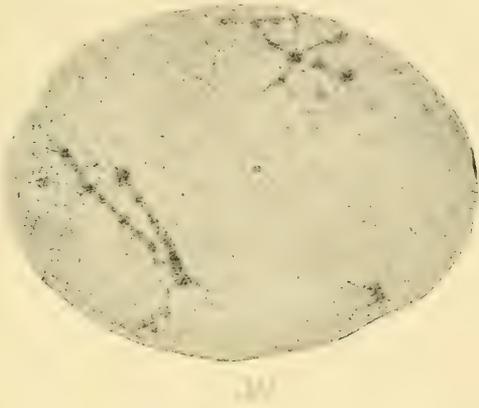
30 and 31 Two drawings from the same nucleus, showing the reappearance of the chromosomes in the form of crosses or parallel threads. $\times 1225$.

32 and 33 Two stages in the condensation of a cross-shaped chromosome. $\times 1225$.

34 Various stages in the late condensation of the crosses and parallel threads into tetrads, all found in the nucleus of figure 24. $\times 1818$.

35 Partially condensed tetrads arranged in an equatorial plate. $\times 1818$.

36 First polar spindle in the center of the nuclear area, showing astral radiations which form a part of the heavy nuclear net caused by sublimate-acetic fixation. $\times 600$.



10



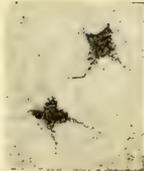
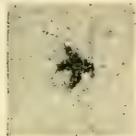
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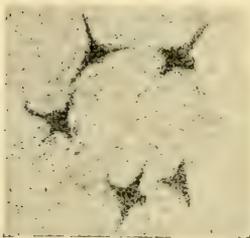
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20

PLATE 5

EXPLANATION OF FIGURES

Hydraetinia echinata

37 The first polar spindle (having no asters) in the center of the homogeneous nuclear area, which is characteristic after Meves' fixation. $\times 600$.

38 The first polar spindle, showing the tetrads becoming arranged at the equator. $\times 2625$

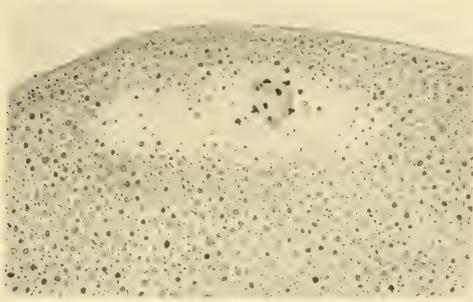
39 and 40 Two equatorial plates of the first polar spindle, showing 15 and 14 chromosomes respectively. $\times 2625$

41 Section to show the arrangement of cell-elements before fertilization. $\times 2625$

42 Section to show rearrangement of mitochondria and fertilization membrane after entrance of the sperm. $\times 2625$.

43 to 45 Three stages in the formation of the first polar body, the spindle having moved out of the nuclear area which remains as two homogeneous areas in the protoplasm. $\times 2625$

46 The formation of the second polar body. The first polar body shows 14 chromosomes. $\times 2625$



33

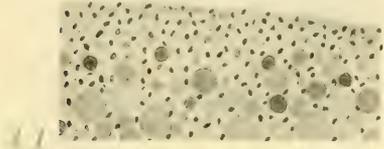


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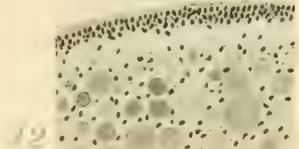


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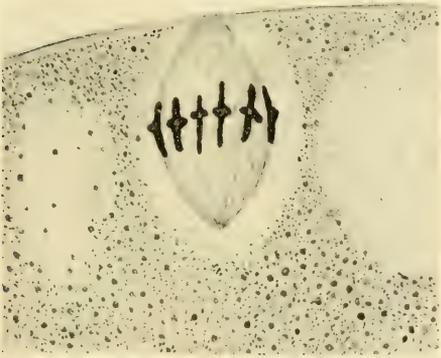
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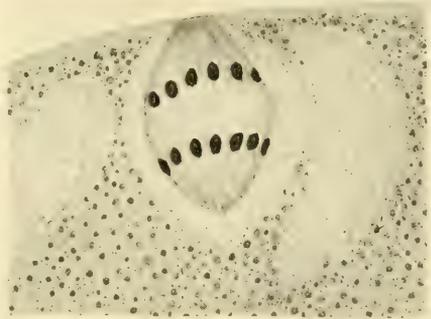
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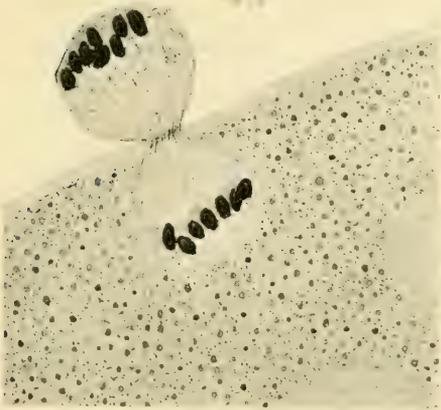
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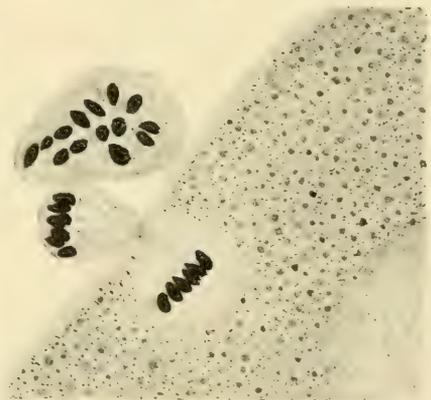
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PLATE 6

EXPLANATION OF FIGURES

Hydractinia echinata

47 Early fertilization stage in which the sperm has entered near the female germ-nucleus. \times 2625.

48 Later fertilization stage showing the male germ nucleus somewhat expanded. \times 2625

49 Fertilization stage in which the sperm head has entered the female germ-nucleus before expanding, the chromosomes of the egg nucleus being already formed. \times 2625.

50 Fertilization stage in which the two germ nuclei are of more nearly equal size before union. \times 2625.

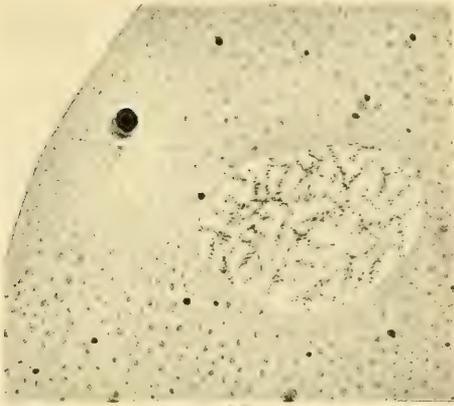
*Eudendrium ramosum*⁹

51 Gonophore containing an egg showing the position of the nucleus at the inner end. \times 75

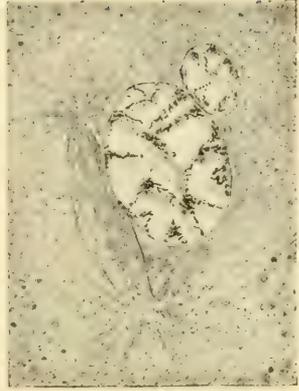
52 Same nucleus much enlarged, growth period. \times 2625.

53 Nucleus ready for maturation, showing the chromosomes forming. \times 2625.

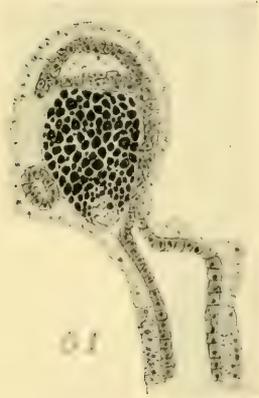
⁹ All *Eudendrium* sections are of material fixed with sublimate acetic.



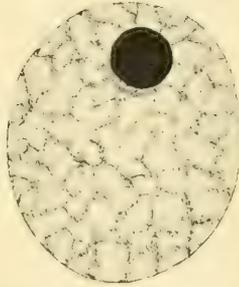
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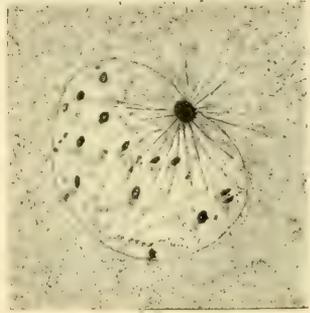
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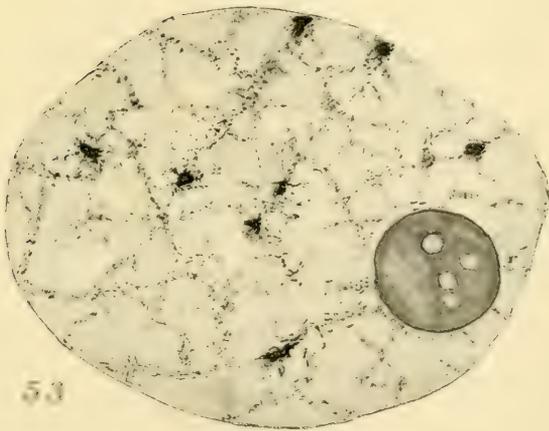
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PLATE 7

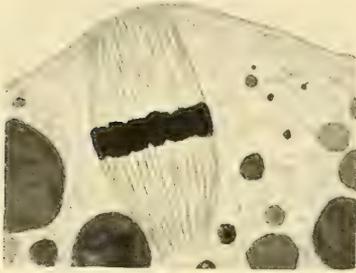
EXPLANATION OF FIGURES

Eudendrium ramosum

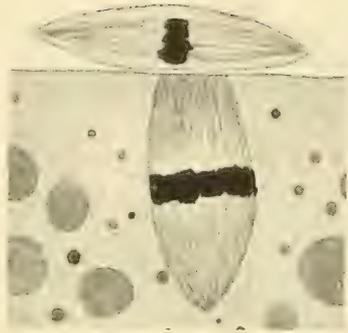
- 54 and 55 Two stages of the first polar spindle. $\times 2625$.
56 Equatorial plate of first polar spindle, showing 3 chromosomes. $\times 2625$.
57 and 58 Two stages in the formation of the second polar body. $\times 2625$
59 The female germ nucleus. $\times 2625$.
60 Fertilization stages, showing the two germ-nuclei of equal size. $\times 2625$.

Hydractinia echinata

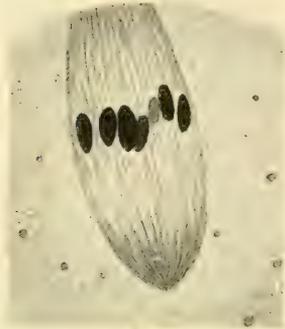
- 61 Two centrifuged eggs showing the egg materials separated into three layers. $\times 85$.
62 Section of a centrifuged egg showing the distribution of the cell-elements, oil at the narrow end, a protoplasmic layer below, yolk and mitochondria at the broad end. $\times 338$.



59



61



62



60



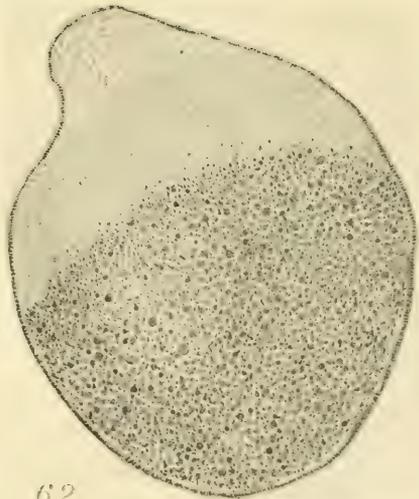
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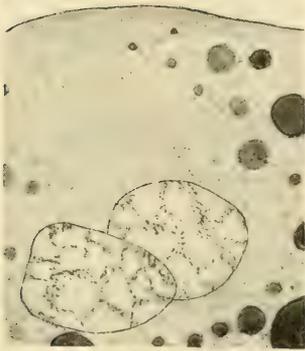
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PLATE 8

EXPLANATION OF FIGURES

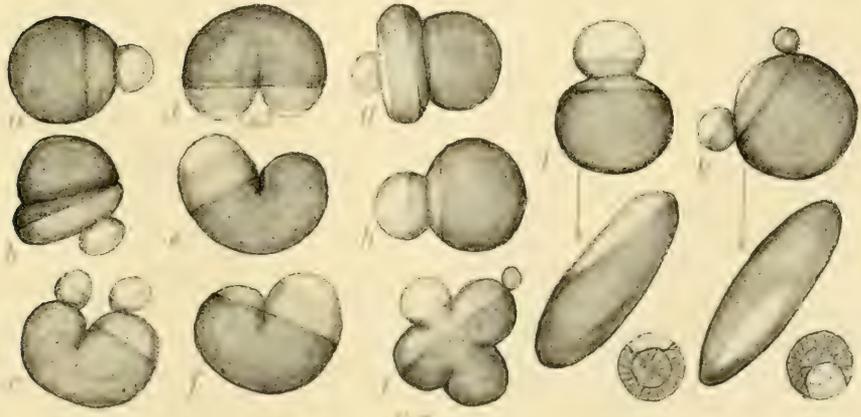
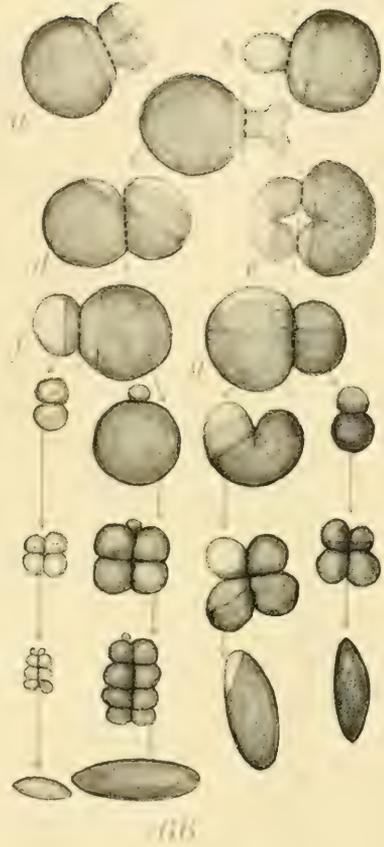
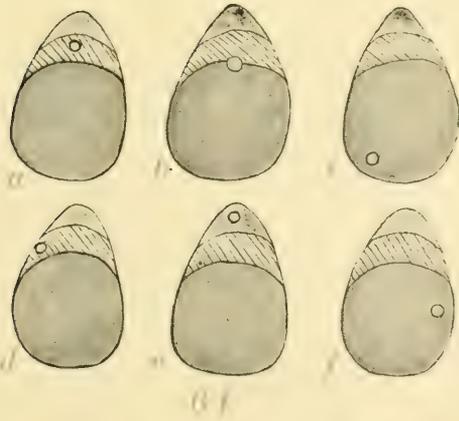
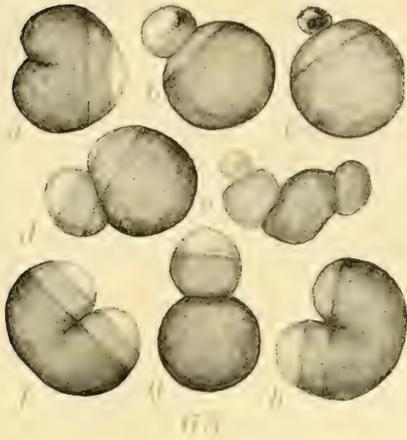
Hydractinia echinata. × 85

63 Centrifuged eggs, showing the direction of the first cleavage plane in reference to the stratification.

64 Sections of centrifuged eggs, showing the position of the nucleus in reference to stratification.

65 Centrifuged eggs which were isolated and development to planula followed: *J* and *K* show the resulting distribution of the materials in the planula.

66 Centrifuged eggs in which the blastomeres were separated in the two-cell stage, isolated, and followed to the formation of the planula. *F* and *G* show several stages in this development.



STUDIES ON CELL CONSTANCY IN THE GENUS
EORHYNCHUS¹

H. J. VAN CLEAVE

From the Zoological Laboratory of the University of Illinois

FORTY-SIX FIGURES

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¹ Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 28.

INTRODUCTION

1. Materials

During the fall of 1909 the writer began a study of the morphology of the Acanthocephala under the direction of Prof. Henry B. Ward. It happened that the form first taken up for study, *Eorhynchus*²*gracilisentis* (Van Cleave), displayed a marked uniformity in the number and arrangement of the nuclei in certain regions of the body. These observations opened the question of how far these phenomena of constancy existed in the other tissues and organs of the body. As additional species in the same genus came to the writer's attention, the analysis was carried on in a comparative manner until at length the present paper deals with the results obtained from the study of five species, namely; *Eo. gracilisentis* (Van C. 1913), *Eo. longirostris* (Van C. 1913), *Eo. emydis* (Leidy 1852), *Eo. cylindratus* (Van C. 1913), and *Eo. tenellus* (Van C. 1913). Camera drawings of the probosces (figs. 1, 21, 29, 32 and 35), individual hooks (figs. 2, 30, 33 and 36) and embryos (figs. 3, 24, 34 and 37) of these species, are given in order to show what extreme differences of diagnostic characters may occur even though this study brings out the fact that in many regions of the body each individual nucleus of one species may be homologized directly with a nucleus in a member of a distinctly different species.

2. Cell constancy and its relation to cell lineage

While studies in cell lineage have added invaluable support to the idea of orderliness in vital processes they have failed to carry this idea to its legitimate conclusion. The development of the embryo through the cleavage stages of the ovum to the formation of the germ layers and even up to the beginning of differentiation in the anlagen of the organs of the adult body has been worked out within various species of animals, but most investigators have dropped the problem here. The more or less reg-

² The name *Eorhynchus* has been substituted for *Neorhynchus*, preoccupied, in a paper now in press.

ular procedure of cleavage and development up to this point has been very generally demonstrated in the forms whose cell lineage has been determined, but it was left for such men as Weismann, Goldschmidt, and Martini to raise the question of a probable further continuation of an orderly process. It was already known that in many instances certain cells are very early set apart for the formation of definite groups of organs or tissues, but no one had actually determined how these organs arise, whether by mere indeterminate continuation of the process of cell division, or in an orderly, specifically predetermined manner. Might not the same forces at work upon and within the embryo during its cleavage stages continue to direct an accurately determined continuation of the cleavage throughout the course of development until the adult form is gained? The works of Goldschmidt and of Martini go a great way toward the confirmation of this hypothesis, at least within certain groups of animals. Their results indicate that in the forms studied mitosis has continued along very definite lines, resulting in a predetermined arrangement of the cellular elements of the adult organs and tissues. In his definition of cell constancy Martini institutes a comparison, not alone of the numbers of nuclei and their relative positions, but also of the size, shape, and finer structure of the cells, and especially of the staining reactions of the nuclei of the cells.

The writer is surprised to find no reference in the more recent literature on cell constancy to Weismann's exceptionally clear ideas as regards the phenomena of constancy. He writes on this point ('93, p. 59):

In smaller and simpler organisms each individual cell may well be determined from the germ onward, and not only with the result that the number of cells is a definite one, and the position of each definitely localized: the determination may also have caused individual peculiarities of each cell, in so far as they depend on changes in the germ plasma at all—i.e., are 'blastogenic,'—to appear in the corresponding cell in the next generation.

Such a statement of the fundamental principles underlying the theory of cell constancy coming from such a clear thinker as Weismann, even though at the time unsupported by any mass of

reliable data, is of extreme value in that it forshadowes a field of investigation from the viewpoint of the problem of the cytology of heredity before the exact facts in the case have been established.

3. The field of cell constancy

Martini has pointed out concisely the obstacles in the path of an investigation on cell constancy, in his comment upon the difficulty in determining either for or against any such position when extremely complicated relations exist between the component parts of a highly differentiated organ. It would be little less than folly to attempt a study of the cell constancy of an organ in one of the higher animals before the possibilities and the limitations of such a study in some of the simpler forms of life had been determined. As a similar instance, who would have considered the possibility of a regularity in the segmentation of the ovum of a vertebrate, had not the fundamental principles been marked out previously by the pioneers in the field of cell lineage, who chose for investigation such objects as displayed the phenomena of cleavage most clearly and with the least complication? Before going further it may be well to consider the nature of the limitations in the realm of cell constancy. Martini has very justly eliminated the cleavage stages of the ovum from consideration here, for the cells in these stages have not as yet arrived at a condition to which the term cell constancy could be applied properly, for in reality the field is limited to a study of such tissues and organs as have passed through the embryonic stage and are definitely differentiated as physiologically functioning organs. It must be kept in mind that though an organism may under normal conditions have portions displaying absolute constancy, yet in those regions where katabolic and secretory processes are proceeding at such a rate as actually to destroy the tissues, constancy, such as is found in the more stable regions which are practically free from radical metabolic changes, cannot exist. Likewise injury and mutilation of parts of the body with the accompanying excitation to renewed multiplication of cells, may profoundly alter the conditions even though the normal organism might display a

high degree of constancy in the number and the arrangement of its component cells.

Any tissue, the cells of which retain the power of continued multiplication after they have become differentiated, is said to possess the power of physiological regeneration. On the basis of the presence or of the absence of this property, Bizzozzero has distinguished three different types of tissues within the human body. Morgan ('01, p. 128) quotes him as follows:

1. Tissues made up of cells which multiply throughout life, as the parenchyma cells of those glands which form secretions of a definite morphological nature; the tissues of the testes, marrow; lymph glands, ovaries; the epithelium of certain tubular glands of the digestive tract and the uterus; and the wax glands;

2. Tissues which increase in number of their cells till birth, and only for a short time afterward, as the parenchyma of the glands with fluid secretions, the tissues of the liver, kidney, pancreas, thyroid, connective tissue, and cartilage;

3. Tissues in which multiplication of cells takes place only at an early embryonic stage, as striated muscle and nerve tissues. In these there is no physiological regeneration.

There is no need of limiting this classification to the tissues of the human body for the general principle applies equally well to the tissues of any animal. The direct dependence of the idea of cell constancy upon the conditions set forth in the second and third groups of this scheme is strikingly evident without the need of further discussion. Those tissues which in most animals possess the power of physiological regeneration have been eliminated or profoundly modified in all forms displaying cell constancy. Two of the most obvious factors involved in accomplishing this reduction of inconstant tissues are modification of organs through degeneration and complete elimination of parts through adaptation to parasitism. Thus in the *Acanthocephala* adaptation to the parasitic habit has been so complete that all traces of the glands usually associated with the processes of metabolism are wanting. With the elimination of this great group of organs there is presented a condition most favorable for the development of a permanent, fixed, relation of the component parts of the body. In the genus *Eorhynchus* this finds expression in a surprisingly

marked cell constancy, the evidences of which will be brought out in this paper.

How strict an interpretation is to be placed upon this term 'constancy?' Is the term to be applied with absolute precision, utterly excluding the possibility of variation of any kind, or may some value be placed upon instances in which there is clearly a normal number and arrangement of the cells but in which a few individuals depart from this condition? The former of these two interpretations means the elimination of the possibility of variability and thereby precludes all possibility of evolution, for an absolute constancy could result in nothing else than an absolute fixity of species, since variability is one of the necessary or primary factors in evolution. The question of real importance is how far these phenomena may vary from the normal without entirely invalidating conclusions based upon the varying character in question. It is a very generally accepted fact that the application of stimuli or the action of internal enzymes may give rise to either an acceleration or a suppression of mitosis, thus producing abnormal numbers of cells and resulting in a confusing arrangement of the products. Since endoparasitism leads to a practical elimination of those varying environmental conditions which so profoundly influence the development of the free-living forms, it would be quite natural to look among the endoparasites for materials adapted to the study of cell constancy. Such conditions have been found to exist in members of the genus *Eorhynchus*.

4. *Methods*

A method frequently employed in comparing the approximate number of cells in a given organ or region of the body of two individuals consists in making a count of the number of cells present in sections of the two animals at approximately the same plane. Such a method may be sufficient when the problem of relation of cell size to body size alone is being considered, but at best it can form the basis of but the crudest sort of a comparison and is valueless in determining the presence or absence of cell constancy. In order to avoid the possibility of misunderstanding regarding

the accuracy of the results set forth in the following study on cell constancy it should be understood that where a definite number of nuclei is recorded for a given organ that number has been obtained either by careful reconstructions of the part in question or by direct counts of all the nuclei in well cleared toto mounts. In no case has the writer based a strong claim of constancy upon the finding of a similar condition in as small a number as two individuals. In many instances even as high as two hundred individuals of the same species have been studied with the greatest care in order to establish the correctness of a finding.

For general results, killing and fixing in saturated aqueous solution of corrosive sublimate with about one per cent glacial acetic acid were by far the most satisfactory. Dilute aqueous solution of Ehrlich's acid hematoxylin proved best adapted for staining toto mounts. The chief advantage of this technic over all others consists in the remarkable translucence of the object after mounting in balsam. By this method the writer was enabled to study the cytology of even some of the larger forms in toto under a one-twelfth inch oil immersion lens. In the preparation of serial sections the same stain countered with eosin gave good differentiation, but for the finest work iron-hematoxylin was found most reliable.

5. Cell constancy vs. nuclear constancy

Since most of the cell walls in the Acanthocephala have disappeared, the question arises, whether the consideration of such syncytial structures may be included within the bounds of cell constancy or must be considered under an entirely separate heading which might be called nuclear constancy. To the writer the problem finds its solution in the known facts in the embryology of the group. Kaiser has recorded valuable information upon this question of the early development of the Acanthocephala. Regarding the determinate nature of the cleavage in the group he states ('13, p. 5): "Die Entwicklung der Echinorhynchen ist von der ersten Furchung bis zur Ausbildung des definitiven Wurmes völlig determiniert." Most writers have agreed that

the point wherein the Eorhynchi differ most essentially from other Acanthocephala lies in the fact that the nuclei of the mature worms in this genus are derived directly by the differentiation of the embryonic nuclei, while in the other genera there is introduced a period of indeterminate fragmentation of the nuclei at the time of the formation of the adult organs. In view of these facts the writer maintains that the term cell constancy is fully justifiable when dealing with the structure of the Eorhynchi, for each nucleus with the cytoplasm surrounding it represents a cell. Moreover the introduction of a wholly different term in the consideration of phenomena identical with those grouped under the head of cell constancy would but add confusion to the subject, for after all the wall is not the essential part of the cell.

PROOFS OF CELL CONSTANCY IN THE GENUS EORHYNCHUS

1. *Cuticula*

The cuticula of the Eorhynchi is a thin, homogeneous, layer covering the entire body. Even under a magnification of nine hundred diameters no specialized structure is visible. By virtue of its noncellular structure this body covering is readily eliminated from consideration in this connection.

2. *Subcuticula*

The subcuticula, which comprises by far the greater part of the body wall (figs. 4, 5 and 23 s), lies directly under the cuticula. While the general structure of this tissue in the Eorhynchi closely resembles that of other genera of Acanthocephala, the arrangement of the nuclei is decidedly distinctive of this genus. Another feature wherein these forms vary from their near relatives is that the component layers of the subcuticula are not as clearly defined as in many other genera. O. F. Müller in 1780 (plate 61) figured *Eo. rutili* (Müll.) and in the body wall showed five 'oscula,' as he called them later (1784), four of them on the dorsal side of the body and a single one on the ventral side near the anterior end. There can be no doubt that these structures are the giant nuclei

of the subcuticula, even though the actual discovery of the nucleus by Brown did not come until half a century later. Even as late a worker as Dujardin ('45) observed the swellings on the exterior of the body caused by these nuclei, though he did not understand their structure and significance. In regard to these same structures in the body of *Eorhynchus agilis* (Rud.), Dujardin ('45, p. 538) writes: “. . . strié transversalement et présentant du cote convexe une serie longitudinale de cinq a six grands pores (?) ou disques orbitulaires, et un seule du cote concave; . . . ” In speaking of the same species, Saeffigen ('84, p. 9) refers to four or five subcuticular nuclei in the dorsal canal and two in the ventral canal:

Bei *Ech. clavaceps* haben die Subcuticulakerne einen mächtigen Umfang, ihr grösster Durchmesser übersteigt oft 0.2 mm. . . . Sie sind bei dieser Species nur in beschränkter Anzahl, ausschliesslich in den Kanälen, und zwar in den Hauptkanälen, deren ganzes Lumen sie einnehmen, vorhanden. Im Dorsalgefäss finden sich ihrer vier bis fünf, im Ventralgefäss zwei, dergleichen ein bis zwei in jedem Lemniscus.

Linton ('89, p. 490), in describing what he called *Echinorhynchus agilis* Rud., but was in all probability *Eorhynchus cylindratulus*, notes “large circular spaces in the vascular layer clearly defined by a circular thickened ring of connective tissue.” Without further delay he accepts the name ‘pores’ or ‘orbicular discs’ introduced by Dujardin, to whose work reference has already been made. However, Linton’s data differ from those of Dujardin, as indicated in the following: “In the specimens which I have examined,” says Linton, “there does not appear to be either this regularity or proportion in the arrangement, e.g., one specimen had four nuclei on the concave side and two on the convex. In others they could not be made out definitely, but enough could be made out to show they were irregularly placed.” However, in justification of modern development of histological methods it would scarcely be proper to leave this statement of Linton’s position without noting the fact that his studies were upon living material under a compressor. While it is true that a good preparation of a stained toto mount does display the subcuticular nuclei with remarkable distinctness, yet observations of such nature must

ordinarily be subordinated to those upon carefully prepared serial sections if points of structure are to receive a final analysis.

Hamann ('91, p. 28) refers to the condition of the subcuticular nuclei of *Echinorhynchus clavaceps*, now *Eorhynchus agilis*, in the following terms:

Das Larvenstadium, in dem die Haut ein Syncytium mit wenigen Riesenkernen bildet, ist bei *Echinorhynchus clavaceps* dauernd fixiert. Wie ich in dem systematischen Teil zeigen werde, ist diese Art auf dem Larvenstadium stehen geblieben, was ihre Haut, Muskulatur, Lemnischen anlangt, wir haben einen Fall von Phylo-Paedogenesis vor uns.

Bereits am lebenden Thiere sieht man die 0.2 mm. grossen, eiförmigen bis kugeligen Rieskerne in der Haut. Ich zählte bei dem in Fig. 1 auf Taf. IX abgebildeten männlichen Tiere acht Kerne in der Epidermis und je zwei in den Lemnischen.

It is interesting to observe that the foregoing statement as to the number of nuclei is not put in general form but only indicates that one individual possessed eight subcuticular nuclei. As the result of the examination of several hundred individuals of the genus *Eorhynchus* the writer has found but a single one in which the arrangement and number of nuclei varied from the typical condition of one ventral and five dorsal subcuticular nuclei. This, in itself, would seem to indicate the specimen which Hamann was describing was abnormal. Lühe ('04, p. 294), commenting upon the works of Müller and Hamann regarding the nuclei of the subcuticula, writes: "In der Figur Hamann's sind freilich 6 Kerne in der Haut gezeichnet. Aber wenn die Zahl dieser Kerne auch innerhalb gewisser Grenzen schwankt, so habe ich doch gerade die von O. F. Müller gezeichnete Fünffzahl verhältnismässig häufig beobachtet und alsdann auch stets in der von Müller gezeichneten Anordnung."

These records, while of importance historically, cannot be regarded as infallible, primarily because in each case they were made in the form of incidental observations rather than as accurate determinations. The following paragraphs present the results of my studies on subcuticular nuclei of five American species.

Eorhynchus gracilisentis. In this species the nuclei of the subcuticula conform to so distinct a pattern, as regards numbers and

arrangement, that variation beyond that which is capable of explanation on the basis of the degree of contraction is limited to a single instance. Each one of more than two hundred individuals had six nuclei in the subcuticula, arranged according to the following system: Five large nuclei occupy positions in the mid-dorsal line of the body. Three of these constitute a group toward the posterior end of the body while the remaining two are separated slightly farther from the rest and lie anterior to the group of three, but are included in the same sagittal plane as the posterior nuclei. In sexually mature individuals these nuclei averaged 0.078 mm. by 0.045 mm. The sixth nucleus of the subcuticula is located in the mid-ventral line, near the anterior end of the body, usually at a point between the anterior and posterior groups of the dorsal series. It is more elongated and larger than the other nuclei of this tissue, having a long axis of 0.125 mm. and a smaller diameter of 0.050 mm. These nuclei are irregularly ovoid in shape, though occasionally they are reniform as shown in figure 4, *sn.5*. The chromatin usually has a closely compacted arrangement, but sometimes takes the form of an irregularly branching network within the nucleus. The shape of the nuclei in this tissue of the Eorhynchi is in decided contrast to that described by Graybill ('02) for the nuclei of the subcuticula of *Echinorhynchus thecatus* Linton. According to that writer the nuclei of this last named species are of a wonderfully dendritic nature, each having a broad expanse of finely branching processes. The contrast is none the less striking if a comparison be made with the subcuticular nuclei of any other type of Acanthocephala. Three types of subcuticular nuclei are thus distinguishable in the Acanthocephala; the giant nuclei of the Eorhynchi, the dendritic nuclei of *E. thecatus*, and the numerous small nuclei scattered throughout the subcuticula as in most other Echinorhynchi and some of the other genera.

Only one specimen of all the Eorhynchi examined showed variation in the number and arrangement of these nuclei such as might be expected, judging from the statement of previous investigators. This individual was a small, immature female. Even here the variation was purely one of arrangement of the com-

ponent nuclei, for numerical constancy remained undisturbed. In this case one nucleus normally in the anterior dorsal group had taken a position on the ventral side of the body. Before trying to explain this departure from the normal, attention must be called to the following points:

1. The nuclei in the dorsal line lie within the dorsal longitudinal canal, and the one nucleus on the ventral side of the body is similarly situated in the ventral canal. These two canals communicate directly through the circular canals of the lacunar system.

2. Hamann ('91, p. 21) ascribes to the nuclei of an *Echinorhynchus* in the embryonic state the power of amoeboid movement.

3. Since it is a well-known fact that some nuclei may wander to that part of the cell where they can best perform their functions, and may even penetrate heavy cell walls in order to reach a distant point of injury; may not the displacement of the nucleus in the abnormal specimen of *Eorhynchus gracilisentis* just cited be explained as the result of either active amoeboid movements which carried it along the lacunae to the opposite side of the body, or as a migration of the nucleus brought about by some local irritation or injury of the body on the ventral side?

In no other instance has the writer seen the parallel of Lühe's figure of *Eo. rutili* (copied as fig. 31) in which he portrays four dorsal and two ventral subcuticular nuclei (Lühe '11, p. 12, fig. 1).

Eorhynchus longirostris. The nuclei of the subcuticula of this species differ but little from those of the preceding species. In the first place the median dorsal row of five nuclei is not so clearly divided into an anterior group of two and a posterior group of three nuclei. The nuclei of this region are almost equidistant one from another. Moreover the terminal member of this series approaches nearer to the posterior tip of the body than does the terminal nucleus in *Eo. gracilisentis*. The swellings upon the surface of the body caused by the presence of these nuclei are more distinct in toto mounts of *Eo. longirostris* than in mounts of the preceding species. The dorsal nuclei have as an average, a long

diameter of 0.132 mm. and a short axis of 0.048 mm. while the ventral nucleus ranges in size near 0.250×0.057 mm. Instead of occupying a position posterior to the second dorsal nucleus, as in *Eo. gracilientis*, the ventral nucleus of this species is typically opposite the foremost of the dorsal series. A slight variation does occur with regard to the relative location of the ventral nucleus but this probably is due to difference in degree of contraction of various individuals.

Eorhynchus emydis. Though the individuals of this species are very much larger than those of either of the preceding species, being from 3 to 32 mm. long, yet the number of nuclei in the subcuticula is the same. Using the arrangement of the nuclei of the subcuticula as a basis for argument it appears that the excessive size of the species is brought about by an elongation of the posterior region of the body rather than by a uniformly distributed elongation. The evidence for this lies in the fact that, while the two anterior dorsal nuclei remain relatively close together, the three of the remaining group are separated from each other by a marked interval. The ventral nucleus occupies a position opposite that of the foremost of the dorsal row, or, occasionally, a little anterior to it. No variations of number or of arrangement were observed in the numerous individuals of this species which were studied.

Eorhynchus cylindratus. Here, again, is found the typical number of subcuticular nuclei. The distribution of the dorsal nuclei closely approaches that found in *Eorhynchus emydis* (Leidy), but the ventral nucleus more often takes a position midway between the two anterior dorsals, approaching nearer to the posterior of these than to the anterior as in *Eo. emydis*. The distribution of the dorsals strongly suggests that found in the preceding species.

Eorhynchus tenellus. In this species, likewise, the five dorsal nuclei and one ventral nucleus in the subcuticula have an arrangement similar to that of *Eo. emydis*.

3. Lemnisci

These unique structures, while extremely simple in organization, have been the subject of considerable speculation among writers dealing with the genus *Eorhynchus*. Saeftigen ('84, p. 9) recorded two nuclei in each lemniscus of *Eo. rutili* (Müll.). Hamann ('91, p. 140) corroborated this for the same species and later ('95, p. 30), described the same condition for the only other then known species of the genus, *Eo. agilis* (Rud.). He mentioned an instance of an immature form of *Eo. rutili* in which only one nucleus was present in each lemniscus, while the second nucleus was just in the process of formation. He says, "Ein junger *Echinorhynchus clavaceps*, der mir vorliegt, zeigt gering entwickelte Lemniscen, die aber bereits den einen Kern enthalten, während der andere noch an der Grenze ihrer Entstehung liegt." This condition finds ready explanation in the light of facts brought out later in this article. Still more recently Lühe ('11, p. 12) recorded two nuclei in each lemniscus of *Eo. rutili*. His figure 1, copied here as figure 31, shows but a single nucleus in each lemniscus.

It seems hardly necessary to mention that these observations as given by various writers on the *Acanthocephala* add nothing to the direct evidence for or against the problem of cell constancy. However, they do serve to indicate the ease with which one may be led to accept the assertion of an earlier worker without fully following out the evidence upon which his statements are founded. To one working on a purely anatomical problem it is extremely plausible to decide that a form is immature when only a single nucleus appears where others say two should occur. Such seems to have been the condition when Hamann reported this abnormality, but, in the light of the present study of the structure of the lemnisci of the numerous examples of *Eorhynchi*, it is difficult to see the source of this evidence of a second nucleus just at the beginning of its formation. All the facts gained from the study of five species of this genus point to a condition mentioned by none of these previous investigators, namely, while one lemniscus possesses the two nuclei so generally ascribed to each, the other

in no case has more than a single one. Figures 22 and 43 indicate clearly this relation of the nuclei in the lemnisci.

An obvious explanation of the cause of so many investigators going astray on the question of the number of nuclei in the lemnisci is found in the generally accepted conclusion that these are paired lateral organs. Therefore as soon as two nuclei were demonstrated in one of them it was the natural thing to expect a duplication of this condition in the organ of the other side of the body. Failure to recognize both nuclei in each of the two lemnisci could be accounted for by the interference of overlying parts. Or even in case serial sections were examined in this connection the lemnisci are usually so much twisted and coiled about one another that, unless reconstruction drawings are made, one is not sure of the interpretation of the numbers and relationships of the nuclei.

Eorhynchus gracilientis. The small size of the lemnisci in this species, and the fact that their usual condition is a mass of twists and turns in the anterior end of the body, together serve to render an accurate analysis of the exact structure very perplexing. The careful study of serial sections of numerous individuals fully demonstrated the inequality of the two organs. Invariably one lemniscus was found to be supplied with two large nuclei lying in the axis of the canal, while the other contained but a single nucleus in its canal. These nuclei so closely resemble those of the subcuticula that no special description seems necessary for them. In shape they are more elongated and display more of a tendency toward a pointed condition at either end.

Eorhynchus longirostris. In this species the lemnisci demonstrate the point in question most admirably (fig. 22). Here reconstructions from serial sections were needless, since the nature of the lemnisci was such that each stood out as a distinct, practically straight tube extending backward into the body cavity. An examination of toto mounts was sufficient to demonstrate that one of these organs possessed two nuclei of the subcuticular type while the other had but a single nucleus.

Eorhynchus emydis. It was from the study of the whole mounts of immature forms of this species that the writer first

gained the correct conception of structure of these organs, which has been carried in a comparative way to the other members of the genus. In these sexually immature stages the lemnisci appear as a pair of small sac-like projections into the anterior end of the body cavity, and in these sacs the nuclei are readily distinguished. It might be argued that as sexual maturity is reached changes occur in the structure of the body, resulting in either an increase in the number of the nuclei or a redistribution of them. Hence the study of these juvenile forms was supplemented by careful observations on the condition in fully mature individuals. These entirely confirm the results obtained for the much smaller, sexually immature members of the species. Figure 43 indicates the absolute clearness with which the nuclei appear in these organs.

Eorhynchus cylindratus from the black bass, *Micropterus salmoides* (Lacépède) has much longer lemnisci than the preceding species but the arrangement and number of its nuclei are identical with the conditions described for each of the preceding species of *Eorhynchus*.

Eorhynchus tenellus. Here again the same condition is met in the nuclei of these organs.

Hamann ('95, p. 9) said: "Die Lemniscen sind als Fortsetzung der Haut der Körperwand anzusehen." In all the foregoing species the nuclei of the lemnisci so closely resemble those of the subcuticula in structure that this fact might well be used as an argument in support of the view that these organs arise as in-pocketings of the body-wall. Moreover, the fact that their position in the canal of each lemniscus corresponds to the condition found in the subcuticula where the nuclei also occur in the longitudinal canals of the body, indicates a possible connection or relationship between these two sets of canals. No direct connection, however, has been made out by the writer in members of this genus, although such a connection is rather easily demonstrated in members of other genera of *Acanthocephala*.

One rather common variation in the form of the nuclei of this region of the body consists in the tendency to assume a rounded outline in immature forms as contrasted with the more elon-

gated and less regular outline of each nucleus in the fully mature animal. At the same time this variation in shape is accompanied by just as striking a difference in the manner in which the chromatin material is distributed. While in the adult the chromatic substance is arranged in a more or less irregular branching figure in the midst of the nucleus, in the immature condition the chromatin is more evenly distributed throughout the nuclear mass.

4. *Proboscis and proboscis receptacle*

While ordinarily the proboscis and its receptacle are considered as two separate organs or regions in the Acanthocephala, in the following it seems better to consider the two together, since certain structures are not limited to either but extend through both. Even though the proboscis shows practically no individual variation within the species, yet the different species may vary to a remarkable degree. On the other hand, several species of this genus present such slight variation in this organ of attachment that the only evident point of contrast is in the relative size of the various rows of hooks. In spite of the considerable range of shapes and structures in different species, the following analysis will show that in all probability the same nuclei contributed to the formation of structures which are distinctly different in one group from those found in closely allied species of the same genus. In other words, it seems that during the processes of differentiation variations may have occurred in the resulting structures but that the cellular elements entering into the differentiation were the same in number in each species.

At the tip of the proboscis (figs. 6 and 38 *po.*) is a structure of questionable function and of variable form within the genus. This structure, which occupies the central region of the proboscis, is, in some species, an elongated cylindrical sac while in others it more nearly approaches a globular form. Various functions have been ascribed to this peculiar mass of tissue. Lespes ('64) considered a similar structure in *Echinorhynchus clavaceps* as the indication of an alimentary canal. Of a similarly located structure made up of two cells in *E. proteus* Baltzer ('80, p. 26)

made the statement "Wahrscheinlich stehen diese Zellen mit einem, hier vorhandenen Tastvermögen in Beziehung." Saefftingen ('84) thought this condition in *E. clavaiceps* resulted from the four retractors of the proboscis forming a space between them and in this the nuclei had come to lie.

Hamann ('91, p. 51) has shown that in the ontogeny of *E. proteus* a group of three cells is laid down at the anterior end of the proboscis early in development. "An der Spitze sind auf dem Schnitt drei der grossen Kerne mit ihrem kugeligen Kernkörperchen getroffen, deren Zellsubstanz den centralen Teil der Rüsselanlage bildet," He has found such cells in all species of *Echinorhynchus* which he has investigated. As to their location and relations he adds ('91, p. 57): "Bei *Echinorhynchus proteus* und *angustus* liegen sie nebeneinander, bei *Ech. clavaiceps* hintereinander Seine Abbildungen lassen das Verhältnis dieser Zellen zur Haut (Subcuticula) nicht deutlich erkennen." Hamann considers the evidence insufficient to warrant the naming of this structure an alimentary tract. On the other hand he has offered the suggestion that the staining reaction of this tissue in *E. clavaiceps* is such that the granular structure with hematoxylin staining indicates a glandular function. For this reason he called the cells gland-cells. I do not consider the evidence sufficient to warrant ascribing a definite glandular function to this structure, but since there does not seem to be any close relationship between it and the other organs, might it not be the vestige of some organ which through disuse has degenerated since the *Acanthocephala* have acquired the parasitic habit?

Eorhynchus gracilientis. This terminal organ of the proboscis in *Eo. gracilientis* (fig. 6). contains three large oval nuclei lying in its long axis. It will be recalled that this arrangement of the nuclei is what Hamann ('91, p. 57) has described for *Eo. rutili*. The relative position of these is apparently influenced by the state of contraction of the proboscis muscles. In case the proboscis is fully extended these nuclei assume a regular oval form, the long axis of each coinciding with the long axis of the body, but in various stages of contraction of the proboscis these nuclei become slightly modified in their relations. When forced closer together, they, at times, come to lie with their long axes at right angles to the main

axis of the body. However it does not seem necessary to do more than mention that this abnormal or unnatural position is simply the result of the action of an external physical force and for this reason would not come under the consideration of abnormalities of location such as were discussed under the nuclei of the subcuticula.

A dense chromatin mass lies in the center of each of these nuclei. This mass at times is distinctly oval, again it may be irregularly branched, and finally it frequently appears as several smaller rounded masses scattered throughout the nucleus. In this same species a pair of small nuclei occurs in a clearly demarked area at the base of the terminal organ of the proboscis. These are usually almost perfectly spherical, but at times they assume a slightly ovoid shape (fig. 7).

Eorhynchus longirostris. In this species (figs. 22 and 26, *po*) the nuclei of the terminal proboscis organ have the same number and arrangement as just described for *Eo. gracilientis*.

Eorhynchus emydis (Leidy). This form possesses a terminal proboscis organ of the type described by Hamann for *E. proteus* and *E. angustatus*. It is supplied with two large oval nuclei in the central enlarged portion and with two small spherical nuclei in a region at their posterior margin. The general relations of these parts are well shown in figure 45, a copy of Hamann's figure 28, plate 3, representing the condition found in *Eo. agilis* (Rud.).

Eorhynchus cylindratus. The terminal organ of the proboscis has the same structure and arrangement of nuclei as *emydis*. The two large oval nuclei lie side by side, while the two smaller ones occur posterior to them.

Eorhynchus tenellus. Here the organ at the end of the proboscis (fig. 38, *po*) is of the type just described for the last two species, and the constancy of the cellular elements is identical.

5. *Proboscis wall and receptacle wall*

Eorhynchus gracilientis. The nuclei of the walls of the proboscis and its receptacle in this species have the following arrangement: The anterior end of the proboscis bears a circle of twelve nuclei, located in the region near the bases of the terminal hooks,

yet no direct connection could be traced between nuclei and hooks. Figure 10 shows the relations of these nuclei in a cross section of the proboscis. The nuclei of this group are small spheres of chromatin 0.005 mm. in diameter, each lying in the center of a 0.007 mm. clear space. Two pairs of nuclei are connected with the muscle sheath which effects the retraction of the hooks. These lie, one on the dorsal side of the proboscis and one on the ventral side, in the region between the second and basal rows of hooks. The location of these nuclei is shown in figure 6, which is a single sagittal section including but one nucleus of each of the two pairs.

The muscular wall of the proboscis receptacle proper has three pairs of nuclei. The most conspicuous of these is a pair of oval nuclei connected with the sheath musculature directly at the base of the brain (fig. 8). The other two pairs are located, one pair dorsally and the other ventrally, on the wall of the receptacle just anterior to the brain.

Eorhynchus longirostris. Due to the crowded condition of the receptacle in this species, brought about by the usual partial inversion of the proboscis, very little could be made out regarding the cellular structure of the proboscis receptacle. The two nuclei associated with the muscular wall at the base of the brain are very distinct.

Eorhynchus emydis. The muscular wall of the proboscis receptacle is supplied with three pairs of oval nuclei. One pair is located on the inner wall of the receptacle at the base of the brain. The other two pairs are located on the inner side of the dorsal and ventral walls, a short distance cephalad from the brain. In the proboscis two pairs occur, one on the dorsal side of the proboscis wall and the other pair on the ventral, in the region of the bases of the middle row of hooks.

Eorhynchus cylindratus presents a nuclear constitution of the proboscis and its receptacle identical with that of *Eorhynchus emydis*.

Eorhynchus tenellus has the same arrangement of the muscular nuclei in the proboscis sheath and proboscis as the two preceding

species. This arrangement is shown in figure 38, from which one nucleus of each pair of dorsal and ventral receptacle wall nuclei has been omitted in the reconstruction.

6. *Invertors of the proboscis*

Eorhynchus gracilisentis. The proboscis invertors consist of two strips of tissue which extend backward along the dorsal and ventral surfaces of the terminal proboscis organ and pass through the base of the proboscis sheath. In the region anterior to the brain each invertor bears a pair of elongated or spindle shaped nuclei. In this species the invertors are so highly developed that they almost entirely fill the space within the walls of the proboscis and its sheath.

Eorhynchus longirostris. In this species the same structures are found. The invertors are somewhat reduced in size so that they stand out more clearly as separate structures. As in the preceding case each of them is supplied with two nuclei. Figure 26 shows these invertors cut in a slightly diagonal plane so that but a single nucleus shows in each.

Eorhynchus emydis. Figure 44 is a drawing from a toto mount showing the pair of nuclei associated with each invertor.

Eorhynchus cylindratus. The nuclei in the invertors are identical with those found in *Eo. emydis*. The invertors are in about the same stage of development.

Eorhynchus tenellus presents the same distribution of one pair of nuclei to each invertor, as shown in figure 38.

7. *Retractors of the proboscis receptacle*

In the genus *Eorhynchus* the retraction of the proboscis receptacle is accomplished through the contraction of two long fibrous bands which proceed from the posterior end of the proboscis receptacle and find attachment, one on the dorsal wall of the body and the other on the ventral body-wall. These retractors are direct continuations of the proboscis invertors.

Eorhynchus gracilisentis. The dorsal retractor of the proboscis receptacle in this species is supplied with two oval or spindle

shaped nuclei whose arrangement is shown in figure 16. The ventral retractor of this same species is similarly furnished with two nuclei. These are of the same type as those already described for the dorsal retractor. In no case was any evidence discovered indicating the presence of other nuclei of any type in these structures.

Eorhynchus longirostris. In members of this species the arrangement of the proboscis receptacle retractors is identical with that just set forth for *Eo. gracilisentis*. The nuclei are of the same number, the same appearance, and have identical relations within the retractors. Figure 28 shows the nuclei of the dorsal retractor of the proboscis receptacle.

Eorhynchus emydis (Leidy), *Eorhynchus cylindratus*, and *Eorhynchus tenellus* show the identical condition of the retractors of the receptacle as described for the other species of the genus.

8. Cement gland

In members of the genus *Eorhynchus* the cement gland presents a condition at wide variance from the type most commonly found in the other genera of *Acanthocephala*. Instead of consisting of a series of independent sac-like glands in the posterior region of the male, as in many genera, the cement gland is a compact mass. Bieler ('13) has suggested that this deviation in structure, together with the fact that a single covering encases the mass, is of value in differentiating the *Eorhynchi* from their near relatives. This characteristic was not mentioned by Hamann in his definition of the genus. In all of the species examined the nuclei of the cement gland are oval with a compact central chromatin mass.

Eorhynchus gracilisentis. In this species eight large oval nuclei lie imbedded in the mass of the gland (fig. 9, *cg.*) These are the only nuclei present in the entire structure. The study of toto mounts and of sagittal sections demonstrated that, though these nuclei are uniformly distributed through the gland, there is an evident arrangement in two lateral groups each of four nuclei.

Eorhynchus longirostris. The gland in this species stands in marked contrast to the type preceding. While the nuclei are clearly of the same character as those of *Eo. gracilisentis* their number is just doubled. Instead of the eight large oval nuclei there are sixteen of the same general character (fig. 20, *cg*).

Eorhynchus emydis. This species contains a long cylindrical cement gland bearing eight nuclei. The size of the gland is indicative of the degree of maturity of the individual. Figure 42 shows this gland in an immature male. The eight nuclei within this small mass are arranged in two distinct groups. The same gland in a fully matured worm not only has increased considerably in size (fig. 41) but at the same time has undergone a complete rearrangement of the component nuclei, resulting in a single line of nuclei running the length of the gland.

Eorhynchus cylindratus and *Eorhynchus tenellus* both have cement glands of the type just described. In each case the eight large nuclei occur in an elongated cylindrical organ. The arrangement of the nuclei in each of these species is the same as that described for *Eo. emydis*.

9. *The male genital apparatus*

Eorhynchus gracilisentis. The cirrus in this form is supplied with nuclei of two types, and in some instances a clear distinction could be drawn between the two kinds of tissues connected with the respective types of nuclei. The portion of the cirrus lying nearest to the sperm duct has a pair of large lateral nuclei in the dorsal region (fig. 18, *ci*) in which the chromatin has a strikingly characteristic arrangement in small masses scattered through the nucleus, with a tendency toward collecting on the nuclear wall. These spheroidal nuclei are 0.007 mm. in diameter. Slightly anterior to this pair of nuclei and in the region of the cirrus farthest removed from the duct are two slightly larger spherical nuclei (fig. 18, *ci*) measuring about 0.009 mm. In these the chromatin tends toward the formation of closely compacted masses lying in the center of the nuclei.

The walls forming the vas deferens have two nuclei just ventral to the cement gland. These nuclei have a diameter of 0.007 mm., and each contains a single chromatin mass 0.003 mm. in diameter. A slight ventral dilation of the vas deferens in that part of its course just opposite the posterior edge of the cement gland where it enters the sperm reservoir, is supplied with two small nuclei. These are bilateral in arrangement. Each has a long axis of 0.005 mm., and a short axis of 0.003 mm.

The muscular sac posterior to the reservoir of the cement gland contains two star-shaped nuclei which lie close to the anterior end of this structure. These have the appearance of lying in a very loose mass of tissue, sending out small projections to the more solid surrounding portions of the sac wall. The muscular wall of this sac contains a small pair of nuclei on its dorsal surface near the posterior end. These are of the type of small oval nuclei found in the body musculature of this genus. Figure 17, *ms*, shows the relations of this structure as presented in a reconstruction from sagittal sections. Very similar conditions have been found in the other species of *Eorhynchus* but the study has not been carried out in detail. Figure 25 indicates the appearance of the pair of nuclei within the muscular sac of *Eo. longirostris*.

The inturned portion of the posterior region of the male, extending from the exterior to the cirrus, is curiously modified in the region nearest the genital opening when the copulatory apparatus is retracted. At this place there are formed a series of five closely fitted wedge-shaped structures, each of which bears three nuclei, as shown in figure 18, which is a reconstruction drawing from serial sagittal sections. The same condition has been demonstrated in well cleared toto mounts of this species. Connecting this structure surrounding the genital orifice with the arms of the copulatory bursa there is an extremely irregular, inconstant portion, the walls of which vary so in appearance with the various states of retraction or of protrusion of the genital apparatus that the writer has not been able to establish a definite number or arrangement of the nuclei contained in it (fig. 18).

10. The female genital apparatus

Eorhynchus gracilisentis. The vagina presents a clear example of constancy of number and arrangement of the nuclei. Four large nuclei (figs. 11, 12 and 13, *v*) are found in its inner muscular layer between the genital orifice and the sphincter vaginae. Two nuclei are associated with the sphincter. The outer non-muscular layer of the vagina in the region of the sphincter contains three nuclei. Cephalad from the sphincter the vagina has a short rounded region consisting of a thick walled tube through which the embryos must pass from the uterus into the orifice of the sphincter. This heavy walled portion of the vagina bears four large nuclei. Anteriorly, the outer wall of the vagina passes over into the single-walled uterus with its large sac-like cavity. On the dorsal side of the uterus at its junction with the vagina there is an enlargement of the wall in which are found two nuclei, the only ones associated with the uterus. Figures 11, 12 and 13, *u*, *v*, and *vs*, show the relations of these parts, together with a representation of the nuclei. The selective apparatus (fig. 11, *sa*), because of its extremely complicated structure, gives considerable trouble when an attempt is made to compare the nuclei of one individual with those of another. The eight nuclei shown in the drawing just cited have been found in every individual studied, but several other nuclei which occurred in this region in some individuals could not be found in others of the same species. The hard shelled embryos with which the body of a mature female is filled hide this structure almost completely in toto mounts, and in serial sections the embryos frequently cause it to be so torn that very little of the actual relations may be made out. Extending posteriad from the dorsal wall of this selective apparatus are two muscular strips whose function is evidently to support these organs since each finds a point of attachment on the dorsal wall of the body. One nucleus occurs in each of these (fig. 11, *ssa*). On either side of the genital orifice of the female there extends a broad fan shaped ligament which becomes attached to the dorsal wall of the body cavity (fig. 15, *fl*). In the broad portion

near the genital orifice each ligament contains a single large star-shaped nucleus.

Eorhynchus longirostris. In this form the vagina, from the genital orifice to the sphincter, is supplied with four nuclei, as shown in figure 27. The two nuclei associated with the sphincter, which can be made out clearly in serial sections, are not shown in this drawing. Anterior to the sphincter are four vaginal nuclei. The uterus, as in all other species studied, has but two nuclei and these occur at its posterior extremity. As a consequence of the difficulties involved in studying the selective apparatus, as pointed out above, the writer has been unable to establish any definite results regarding the occurrence of nuclei in that organ.

Eorhynchus emydis. In immature individuals of this species the female tract presents a very diagrammatic arrangement of the component structures. Figure 40 shows the nuclei with which the vagina below the sphincter is supplied. These four nuclei are arranged in pairs, one at either end of the region. The sphincter muscle has a single pair of nuclei. Anterior to the sphincter the thick walled portion of the vagina has four nuclei arranged in two pairs as shown for *Eo. gracilisentis*. The uterus has a single pair of nuclei at its posterior extremity. In the selective apparatus six pairs of nuclei could be seen distinctly. Of the two muscles (fig. 40, *ssa*) for the support of this organ each bears a single nucleus.

Eorhynchus tenellus. Reference to the figure will show that the female genital tract of this species (fig. 39) bears identically the same nuclei as have been described for *Eo. emydis*.

11. *The brain*

Eorhynchus gracilisentis. In the brain of *Eorhynchus* conditions are such as to render a careful analysis of the structure difficult. This organ (fig. 8, *br.*) is a conical mass of ganglion cells having an extreme length of 0.1 mm. The cells of which this mass is composed are small rounded bodies of uniform character so that there are no regions demarcated by variations in the size or structure of the component cells. The small, 0.005 mm., cells

are so closely packed together that even in a most careful reconstruction from thin serial sections one is never absolutely certain that in some cases two adjoining sections do not contain parts of the same nucleus. Reconstructions of two individuals from drawings of serial sections magnified nine hundred diameters gave in one instance one hundred and eight cells in the brain, in the other one hundred and nine. While these facts are not given with the idea of establishing an absolute constancy they may be taken to indicate that, even though the structure in this region is so extremely crowded, yet there is a close agreement in the results obtained from these two reconstructions.

Eorhynchus emydis. A reconstruction of the brain of a single individual of this species gave ninety-four nuclei as the total for that structure.

12. *The genital ganglion*

Eorhynchus gracilisentis. Eighteen cells are grouped together in the region of the cirrus in the male to form what is commonly spoken of as the genital ganglion (fig. 17 *gg*). As in the brain, these cells are all of the same type, giving no basis for a division of the ganglion into regions according to the structure of the component elements.

13. *Body musculature*

Eorhynchus gracilisentis. Two reconstructions of the body musculature each gave a total of thirty nuclei associated with that tissue. These were arranged in fifteen pairs, extending from the union of the body with the proboscis, caudad to the posterior extremity, one nucleus of each pair having a position slightly lateral to the dorsal canal. The distribution is such that a single cross section could not contain more than a single pair of nuclei. The difficulties involved in the determination of the number of nuclei present in this tissue consist chiefly in the discrimination between these nuclei and the embryos, which, in the female, are often so closely packed against the wall that only the most careful study will reveal whether a given structure is associated with the muscle layer or merely closely applied to it. The position of these nuclei in longitudinal section is shown in figure 5.

SUMMARY OF CELL CONSTANCY IN THE GENUS EORHYNCHUS,
WITH TABLE OF RESULTS

Consideration of the facts presented in the foregoing parts of this study has led the writer to a firm belief in a remarkable degree of constancy in all the somatic structures in the genus Eorhynchus. Failure to demonstrate this beyond a doubt for all the organs is due to physical limitations, primarily, rather than to conflicting data, for in every instance where a positive count has been made under conditions precluding the possibility of error in manipulation, the same number and arrangement of the nuclei has been found in every individual of the same species. Table 1 summarizes the results found for the five species studied.

TABLE 1

ORGAN OR TISSUE	EO. GRACILISSENTIS	EO. LONGIROSTRIS	EO. EMYDIS	EO. CYLINDRATUS	EO. TENELLUS
Subcuticula.....	6	6	6	6	6
Terminal proboscis ring of nuclei	12				
Terminal proboscis organ.....	5	5	4	4	4
Leminisci, 1 and 2.....	3	3	3	3	3
Retractors of hooks.....	4		4	4	4
Wall of proboscis-receptacle....	6	2+	6	6	6
Proboscis invertors.....	4	4	4	4	4
Proboscis sheath retractors:					
Dorsal.....	2	2	2	2	2
Ventral.....	2	2	2	2	2
Cement gland.....	8	16	8	8	8
Cirrus.....	4				
Vas deferens.....	2				
Muscle sac:					
Inside.....	2				
Wall.....	2				
Inverted part of male (posterior)	15				
Vagina.....	11	8	8	8	8
Uterus.....	2	2	2	2	2
Vaginal sphincter.....	2	2	2		2
Selective apparatus.....	8+	?	12		12
Stays of selective apparatus....	2	2	2		2
Ligaments at orifice of female...	2				
Brain.....	108 (?)		94 (?)		
Genital ganglion.....	18				
Body musculature.....	30				

GENERAL PROBLEMS RELATING TO CELL CONSTANCY

1. Cell size vs. body size

A problem such as the relationship existing between body size and cell size is often approached only from a teleological point of view. Given as the end result the adult body form, that becomes the goal toward which the development of the organism is directed. Thus the possibility that this final body form and size may be a mere incidental result attained through precisely determined series of processes of development is entirely ignored. In other words, a common conception of body form and size might be crudely expressed in the idea that nature establishes a mold for each particular type of body form and that, given a zygote of a definite size, the development of the individual must consist in attaining the boundaries of size and form set for that species, regardless of the number of cell divisions that may be required for that purpose. According to this interpretation, the size of the body is the constant factor while the number of cells which go to make up the organism is purely a matter of circumstance.

The problem of the origin of the adult body and the relationship between body size and size of the component cells is one of long standing. While the following discussion does not attempt to bring all the literature upon the subject into relationship, it will serve to give some idea of the nature of the contentions on the various sides of the problem. Conklin is one of the staunch supporters of the view that not only is cell size fixed but also that it bears no direct relation to body size. In *Crepidula fornicata*, in which large and small individuals occur, he has shown ('98) that the difference is due entirely to the larger number of cells in the larger individuals:

In *Crepidula* therefore the cell size is constant, and variations in the size of the body are due to variations in the number of cells present. The dwarfs are what they are by reason of external conditions and not because of inheritance; they are in short a physiological and not a morphological variety. In such a case the shape and size of the body as well as the number of cells in the entire organism, are greatly modified by the direct action of the environment.

A general presentation of the recent results upon the relations between body size and cell size is given by Minot ('98, p. 65):

Cells do not differ greatly from one another in size. The range of their dimensions is very limited. This is particularly true of the cells of any given animal. Recent careful investigations have been made upon the relation of the size of cells to the size of the animals, and it has been found that animals are not larger, one than another, because their cells are larger, but because they have more of them. . . . For example, a large frog differs from a small frog or a large dog from a small dog by the number of cells.

To the writer the foregoing statement has the appearance of too strong a generalization from a limited group of facts. In reality the cells of some animals do differ greatly from one another. Thus in *Eorhynchus emydis*, while the adult body form is perfectly presented in a small individual 1.7 mm. long, the maximum length of this species is over 30 mm., and both of these individuals have identically the same number of somatic cells. Even a most conservative calculation of the relative size of the subcuticular cells of the small and the large individuals shows that their ratio based on volume is 1:140. Since the subcuticula is a syncytium, this calculation is based on the ratio of the volumes of the subcuticula in the two individuals. The volume is determined by taking the product of the length, by the circumference, by the thickness of the subcuticula. Since the number of nuclei present in the small and the large individuals is the same, the ratio of the volumes of the entire subcuticula of the two individuals is the same as the ratio of the individual cells composing it. It would seem that the range of their dimensions in this case is *not* very limited.

Closely associated with this problem of cell size and body size, and growing directly out of it, is the question of the relation between the number of cells present in the organs of the different individuals. In the analysis of any given organ four possibilities of this relation exist: (1) Body size and cell size are both fixed. Of necessity in this instance the number of cells is just as sharply fixed. No case of this sort is known to the writer; (2) Size of the cells is fixed but the body size may vary. Conk-

lin's results on *Crepidula* exemplify this condition. The number of cells in any given organ may consequently vary within undetermined limits; (3) Body size is fixed while the size of the component cells may vary. There could be no precise limit to the number of cells in this instance; (4) Body size and cell size may both vary. This last possibility is still further divisible into two conditions: (a) if cell size and body size vary independently no necessary connection exists between the size of the body and the number of component cells, while (b) if body size varies directly as the size of the cell the number of cells in any given organ becomes a fixed quantity, as in the case outlined under (1). It is in accordance with the last possibility that the explanation of the condition found in the genus *Eorhynchus* is to be sought. In general it is in organisms having this fixed correlation between body size and cell size that cell constancy is to be found.

Regarding the cell lineage of *Nereis*, Wilson ('92, p. 377) writes: "The entire ontogeny gives the impression of a strictly ordered and predetermined series of events, in which every cell division plays a definite role and has a fixed relation to all that precedes and follows it." How readily this conception of ontogeny lends a support to the theory of cell constancy. In another connection, Wilson, ('00, p. 390) has stated that the number of cells produced for the foundation of certain structures is often fixed. For example, in annelids and gasteropods "the entire ectoblast arises from twelve micromeres segmented off in three successive quartets of micromeres from the blastomeres of the four-cell stage."

By way of explanation of this condition as indicated by Wilson and by Morgan, two possibilities present themselves, either (1) a constancy in numbers of cellular elements is the primitive condition of development which, as Conklin points out, is retained only in the early stages of ontogeny of the metazoa, or (2) cell constancy is a manifestation of a tendency toward fixity which is acquired only at a late stage in the development of a race.

That there is a tendency for the same number of embryonic cells to be used in the formation of the organs and structures of

the body is evidenced by the results obtained by Morgan in his experiments upon the embryos developed from isolated blastomeres of echinoderms and of chordates. In 1896 he found that the larvae of *Amphioxus* developed from isolated blastomeres of the two and four cell stages tend to utilize the same number of cells for the formation of the organs of the body as do the normal larvae. In the formation of the gastrula stage the normal larvae invaginate about one-tenth of the total number of cells present in the blastula. Thus in a one-half or a one-quarter embryo, while the number of cells in the blastula is not as great as the number in the normal blastula, the actual number of cells that is invaginated tends to equal the number invaginated in the whole embryo. In other words, it seems as though a definite number of cells were necessary for the formation of a given organ of the body. "I have been personally most loath," he adds, "to accept this conclusion, because it seemed a priori very improbable that a numerical question could enter into this problem, but I see no other alternative than to accept this view of the matter." It would seem that if this be the case in organ formation, the requirement is one rather of the number of cells entering into the composition of the organ, than a mere quantitative regulation or partition of the amount of protoplasm.

The controversy over the relation between the cell size and body size may be taken too seriously. The writer has pointed out the various possible relations between body size and cell size. The problem does not involve a fundamental conception under which all data must be subsumed and made to agree. Different groups of animals may vary widely in respect to the possibility of a direct correlation between the two, the only problem of real concern is to what extent any given condition is fixed within a given group of organisms. In fact Morgulis ('11) has shown experimentally that the relation of cell size to body size may be altered in the individual through a change of physiological conditions. In connection with his observations on complete inanition he has said ('11, p. 259): "In the case of *Diemyctylus* it was found that the volume of both the cells and nuclei of different tissues diminishes

as the animal becomes smaller, and that with a return to normal diet, when the animal grows very rapidly, its cells again increase in volume."

2. *The beginning of constancy*

Throughout the entire animal kingdom observations, chiefly incidental, have been recorded indicating that an organism or a single organ contains a fixed number of cells or of nuclei. Originally this condition was looked upon as of uncommon occurrence. Various workers among the protozoa have recorded the tendency for a single individual during sporulation or colony formation to give rise to a fixed number of cells. Thus in *Tillina* four spores are usually formed from a single individual, but this condition has not been absolutely fixed, for occasionally but two spores are produced. This would seem to indicate a transition from a reproduction by simple binary fission to a higher type where a larger number of individuals is produced from the parent cell. A step higher in the scale is *Colpidium* which, though normally producing four individuals in its temporary cyst, not infrequently forms eight. Among the colonial protozoa a single colony is frequently composed of a definite, constant number of cells: as an example of this *Gonium pectorale* might be cited. This is a sixteen cell colony in which each cell of the association in turn gives rise to a sixteen cell aggregate while still within the parent colony. In *Eudorina* is found an example where thirty-two undifferentiated cells arise from a parent cell producing a colony, as also in *Pandorina* which contains sixteen undifferentiated cells associated together. Thus in the protozoa there is a transition from the condition where reproduction is accomplished solely by simple binary fission, through a series of stages to that in which a definite number of cleavages result in the formation of a colony having a fixed number of individuals.

In the Metazoa, Conklin ('98) has distinguished a difference between the earlier and the later cleavages, attributing to the former the greater morphological importance:

The difference in the number of cells offers no difficulty in the doctrine of cell homology unless we assume that all divisions are differential, a thing which we know is not true. After blocking out the protoblasts of various regions and organs an indefinite number of non-differential divisions may occur either before or after the complete differentiation of the parts, and this probably explains the larger number of cells in the embryo of *Crepidula adunca* and the smaller number in the adult. In fact after the complete differentiation of all the tissues and organs, the number of cells may vary greatly in the different individuals of the same species or in the same individual at different times. In adult *Crepidula* the number of cells varies directly as the body size varies, the cell size remaining practically constant. These later divisions, in the main, are non-differential, and likewise it is probable that in the later stages of cleavage many non-differential and inconstant divisions occur. Not only is there greater variation in the number and size of cells in later as compared with earlier stages of cleavage, but there is also greater variation in the direction and time of division; all of which goes to prove that the earlier cleavages are more constant, more frequently differential, and therefore morphologically more important.

In citing these instances wherein among the Protozoa and in the embryology of the Metazoa constancy seems to have a beginning I do not wish to give the impression that from such isolated and fragmentary citations I would claim to have traced a probable actual rise of fixity in numbers of cells going to make up an adult individual. In fact I do not consider cell constancy as of simple origin, for, while it occurs in broadly separated groups of the animal kingdom, it has probably arisen independently in each from the primitive, more variable conditions through the processes which tend toward the elimination of variability during the phylogeny of the group.

3. Factors involved in the production of cell constancy

For the explanation of the causes and limitations of cleavage various factors have been suggested. Reference has already been made to the works of Morgan as indicating that the production of the anlagen of organs in individuals developed from isolated blastomeres tends to require the same number of cells as required in normal development, even though the abnormal individuals are smaller than the normal and have fewer cells. Conklin's work has also been cited, wherein he considers the earlier cleavages

morphologically of more importance, while the later divisions in *Crepidula* are believed to be non-differential and inconstant, but so far no one has offered a satisfactory explanation of why this is true. I do not here attempt to suggest any new factors as determining the number and arrangement of the cleavages involved in organ formation, but from the facts observed in the development of colonial protozoa of constant numbers, and in the ontogeny of the metazoa where constancy has been demonstrated, there must be some inherent factor determining that a certain number of cell divisions shall precede the formation of a given organ, and that when this number is realized the power of further nuclear division becomes lost. Moreover evidence gained from the study of the members of the genus *Eorhynchus* tends to discredit the possibility of cell size acting as such a factor, for in case size determines the time when a cleavage or division takes place, cell division would continue throughout the life of the individual, since these worms continue to increase in size from the time they reach the final host until they are expelled from it. There must be some factor within the original cell which determines every cell division, from the first cleavage to the establishment of the final adult condition. Whether this determining element is a 'force' which becomes dissipated in the processes of cleavage so that, when the definitive number of cells has been attained, no further progress is possible on the account of the lack of this 'force,' or whether the cause is to be sought in the presence of definite substances within the cell in the nature of either 'determiners' regulating cleavage or materials that are used up in the processes of mitosis can not be decided in the present stage of knowledge of the subject.

4. Cytomorphosis

In the genus *Eorhynchus* is found an evident exception to the generally accepted view of cellular changes accompanying advance of age in animal tissues. Probably the most important point of difference, or at least the one most easily observed, is the fact that as soon as the animal has reached the point

of development when it has taken on the body form of the adult, cell division either mitotic or amitotic, ceases to play any role whatever in the later history of the somatic cells. Among the numerous specimens of five species of this genus which I have examined there has not been, in any instance, the least indication of further increase in the number of cells constituting the body. This is evidenced by the entire lack of mitotic figures and also by actual count of the cells present. This gives good ground for doubting the generally accepted view that life must be accompanied by cell division as expressed in the contention that the cell has lost its lease on life as soon as it can no longer divide.

Nothing is known regarding the changes in *Eorhynchus* accompanying the differentiation of the embryonic cells into the tissues and organs of the adult, for no larval stages of any of the American species have been discovered, and none of the investigations upon the European species contain references to the changes in the structure of the cells at that time. In the later stages of development some slight changes have been observed in the structure of the nuclei but there are no strongly marked general cytological modifications such as usually accompany the advance of age. The nuclear change consists chiefly in the rearrangement of the chromatin. In the immature forms the nuclei, especially of the subcuticula and of the lemnisci, have their chromatic substance irregularly scattered throughout the nucleus, but with the advance to maturity this chromatic material assumes the form of a more compact solid mass lying in the center of the nucleus.

The conditions of existence for all of the cells of the body are so nearly uniform at all periods of life for the adult that it seems probable that death must follow as a consequence of the termination of the reproductive period of the individual or as the result of a combination of factors acting on the organism as a whole rather than as a gradual senescence of the individual cells.

5. *Brief review of known extent of cell constancy in the animal kingdom*

Lest the impression be conveyed that cell constancy is confined to occasional forms within a small group of animals the following condensed survey is given of the important work upon this topic. Martini has shown that constancy is present in most and probably in all of the organs of a new-born nematode. The muscle cells of *Oxyurias* are constant in number throughout life. Goldschmidt has demonstrated cell constancy in the nervous system of *Ascaris*. Brandes has found a definite number of brain cells in *Gigantorhynchus*, but has not pursued his studies to other organs of the body, nor has he deduced any general conclusions. Looss found constancy in the oesophagus of several nematodes. Apathy, working on the central nervous system of Hirudinea, emphasized the constancy found there. Hirschfelder found constancy in the midgut of Rotifera, while Martini following him has carefully worked out constancy in the entire body of *Hydatina senta*, so that he can recognize nine hundred and fifty nine individual cells, each of which he is able to locate in every of individual of that species. Woltereck has shown a constancy in the cells of the larva of *Polygordius*. Even in the Chordata constancy has been established by Martini in the various organs of *Oikopleura*, and *Fritillaria*. Aside from these definite records given as proofs of constancy by these writers, there exist masses of isolated facts such as have been cited in connection with the reviews of the work upon *Acanthocephala* in this article, but no attempt has been made to catalogue all of these scattered references. Miss Erdmann ('12) has given a very general survey of the literature upon cell constancy and the problems closely associated with it.

6. *The significance of cell constancy*

Cell constancy has a most interesting relationship to the problems of comparative anatomy and evolution. Evidently it can occur only in those forms which have a determinate cleavage. In what way has this condition been brought about? Were all forms of development originally of this type or has this condition been

developed only in certain groups of animals? If the latter be the case, then the question arises as to the factor or factors operating in widely separated groups, which could accomplish such an end. Up to the present time no direct attempt has been made to answer this question. There seems to have been a tendency, at least among some workers, to expect too great a future for the study of cell constancy. This has probably grown out of the failure to realize fully the limitations of the field. There is the possibility of a comparative anatomy, such as Martini has predicted, in which two forms may be compared one with the other in a much more intimate way than has been undertaken heretofore. In this case individual cells rather than gross anatomical structures would be used as units for comparison. An examination of the facts brought out in the earlier parts of this paper will show that they constitute just such a study in comparative anatomy. Necessarily such a study is limited to those groups which have acquired constancy.

The fact that cell constancy has never been demonstrated in any marked degree in organisms which hold an unquestioned position in the main line of descent of the animal series seems to indicate that it is not the primitive condition. The Nematodes, the Tunicates, the Rotifers, and the Acanthocephala, forms which most clearly display the phenomena of constancy, stand either as side branches from the main line of descent of animal life or as highly modified groups which have lost most of their indications of close relationship with other forms. For instance the rotifers have reached a point in development but little higher than the trochophore stage found in the development of higher forms and have become permanently fixed. The tunicates, coming off from the stem which has given rise to the Vertebrates, have, in a similar manner, either failed to proceed farther or, in case at one time they stood on a higher level, have through degeneration regressed to a condition where they have become fixed as an aberrant group. Parasitism has so profoundly affected the somatic portions of the Acanthocephala that little is left to serve as an indication of earlier phylogenetic relations. Especially, in this group, species and even genera differ from each other in slight

degree when compared with the range of variation in the more plastic groups of animals. This tendency toward uniformity of body characters has led to, or possibly has resulted from, a corresponding stability in the numbers of cells which go to make up the organs of the body in the genus *Eorhynchus*. It is true that within this genus the parasitic habit has contributed toward the elimination of physical factors which might induce radical changes. The result of this is that variability is practically confined to minor variations such as those of body size, and in general those which concern slight rearrangements of the component cells, rather than those involving the production of entirely new types of structures. The future of such a group of organisms is very clear. The stability which has been produced serves as an effective barrier to evolution. Under present conditions those organisms displaying marked cell constancy are incapable of producing new creations in the lines of organic progress, for the elimination of variability has precluded the possibility of progressive evolution.

I wish to express my indebtedness to Prof. H. B. Ward for the many helpful suggestions and kindly criticism of methods and of general outline of the work, rendered throughout the period of its preparation.

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ABBREVIATIONS

<i>br.</i> , brain	<i>lm.</i> , longitudinal body muscle
<i>bu.</i> , bursa	<i>ms.</i> , muscle sac
<i>bw.</i> , body-wall	<i>po.</i> , terminal proboscis organ
<i>c.</i> , cuticula	<i>prn.</i> , nuclear ring of proboscis
<i>cc.</i> , circular canals	<i>r.</i> , receptacle of proboscis
<i>cg.</i> , cement gland	<i>s.</i> , subcuticula
<i>ci.</i> , cirrus	<i>sa.</i> , selective apparatus
<i>cm.</i> , circular body muscles	<i>sn.</i> , subcuticular nuclei
<i>cr.</i> , cement reservoir	<i>sr.</i> , sperm reservoir
<i>fl.</i> , fan ligament	<i>ssa.</i> , stay of selective apparatus
<i>gg.</i> , genital ganglion	<i>ta.</i> , anterior testis
<i>go.</i> , genital opening	<i>tp.</i> , posterior testis
<i>ht.</i> , terminal hook	<i>u.</i> , uterus
<i>hb.</i> , basal hook	<i>v.</i> , vagina
<i>hm.</i> , middle hook	<i>vd.</i> , vas deferens
<i>ip.</i> , proboscis inverter	<i>ve.</i> , vas efferens
<i>l.</i> , <i>l.2</i> , lemnisci	<i>vs.</i> , vaginal sphincter
<i>loc.</i> , longitudinal canal	

EXPLANATION OF FIGURES

A camera lucida was used in making all the drawings included in the figures.

Figs. 1 to 16 Represent *Eorhynchus gracilientis* (Van C.).

Fig. 1 Proboscis of hematoxylin-stained specimen in balsam. $\times 97$.

Fig. 2 Hooks from the same specimen as shown in figure 1. $\times 310$.

Fig. 3 Embryos from body of mature female. $\times 310$.

Fig. 4 Portion of body wall showing a subcuticular nucleus. Sagittal section. $\times 310$.

Fig. 5 Sagittal section to one side of that shown in figure 4, showing muscle nuclei. $\times 310$.

Fig. 6 Sagittal section through proboscis showing terminal proboscis organ and hook retractors; hematoxylin. $\times 310$.

Fig. 7 Sagittal section through proboscis receptacle of inverted specimen showing terminal organ of the proboscis. $\times 310$.

Fig. 8 Sagittal section through base of proboscis receptacle showing location of brain. $\times 310$.

Fig. 9 Cement gland of male; toto mount. $\times 97$.

Fig. 10 Transverse section through tip of proboscis showing terminal ring of twelve nuclei. Iron hematoxylin. $\times 310$.

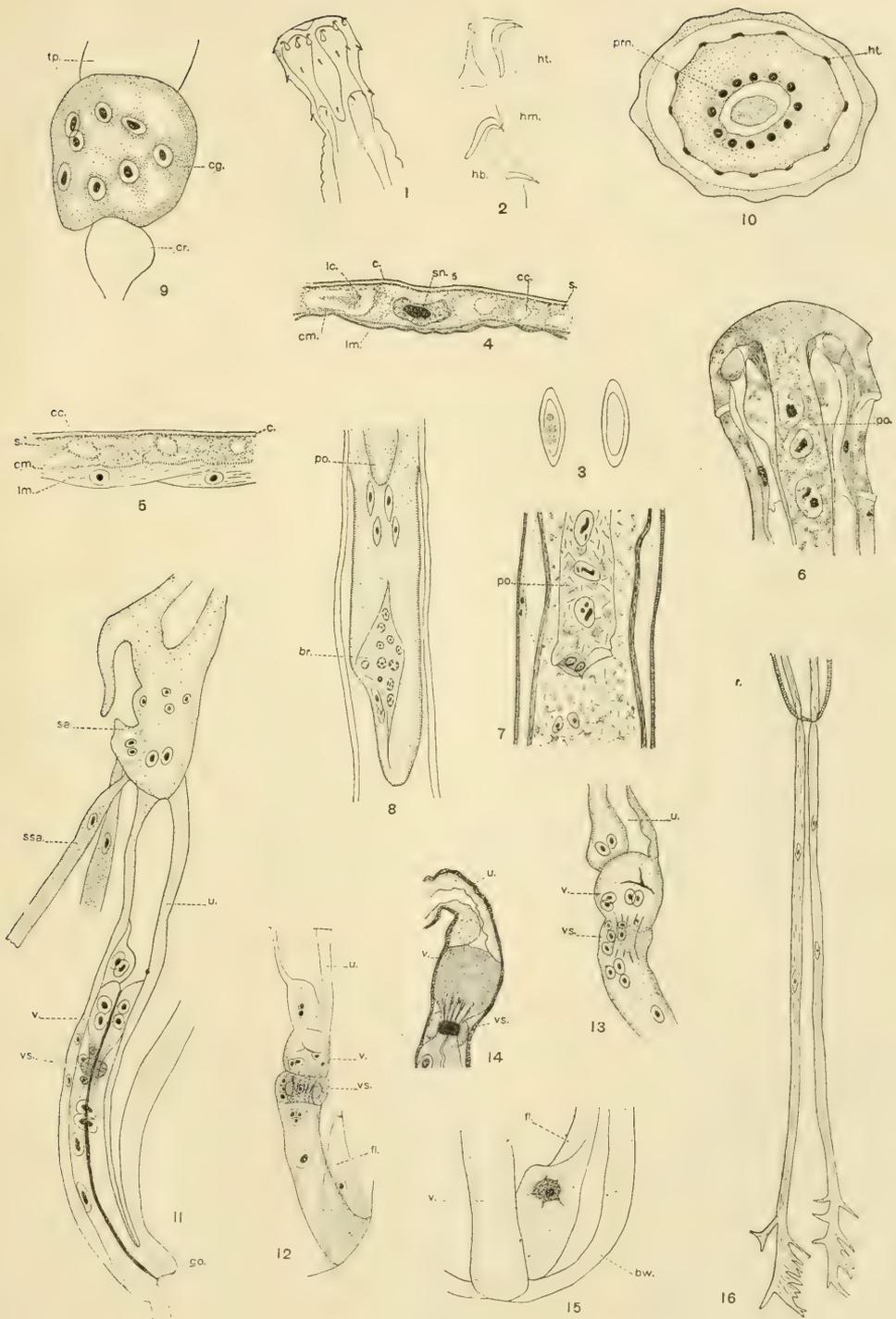
Figs. 11 and 12 Reconstructions of female genital tract. $\times 310$.

Fig. 13 Female genital tract in toto mount; hematoxylin. $\times 310$.

Fig. 14 Sagittal section of same, in region of vaginal sphincter. $\times 310$.

Fig. 15 Fan-shaped ligament at side of female genital orifice. $\times 310$.

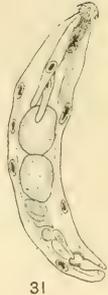
Fig. 16 Retractors of the proboscis receptacle, from dissection. $\times 310$.



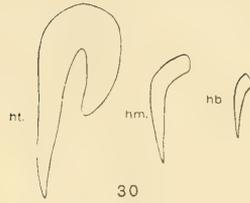
- Figs. 17 to 19 *Eorhynchus gracilisentis*. (Van C.).
- Fig. 17 Genital organs in posterior region of male; reconstruction from sagittal sections.
- Fig. 18 Reconstruction of male copulatory apparatus. $\times 310$.
- Fig. 19 Male copulatory apparatus everted; toto mount. $\times 97$.
- Figs. 20 to 28 *Eorhynchus longirostris* (Van C.).
- Fig. 20 Cement gland. $\times 97$.
- Fig. 21 Proboscis of fully mature female; in balsam. $\times 97$.
- Fig. 22 Anterior end of body; proboscis retracted; toto mount. $\times 30$.
- Fig. 23 Portion of body wall in sagittal section. $\times 310$.
- Fig. 24 Embryos. $\times 310$.
- Fig. 25 Nuclei from muscle sac, posterior to cement reservoir. $\times 310$.
- Fig. 26 Sagittal section through anterior end of body, showing location of brain and terminal proboscis organ. $\times 97$.
- Fig. 27 Reconstruction of female genital tract, showing divisions and the nuclei. $\times 97$.
- Fig. 28 Dorsal retractor of proboscis receptacle; toto mount. $\times 97$.
- Fig. 29 *Eorhynchus tenellus* (Van C.), proboscis of mature male. $\times 97$.
- Fig. 30 Hooks of *Eo. tenellus*. $\times 310$.
- Fig. 31 *Eorhynchus rutili*; copied from Lühe, 1911. About $\times 20$.



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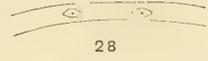
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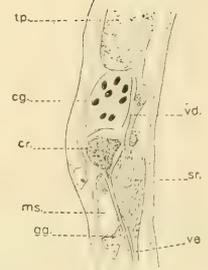
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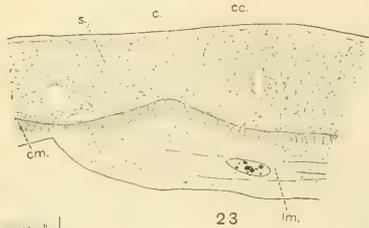
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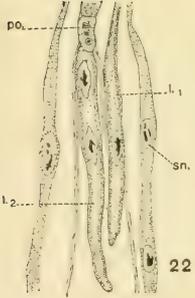
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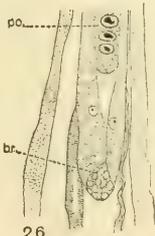
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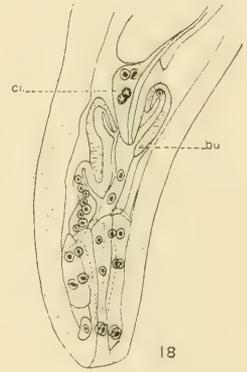
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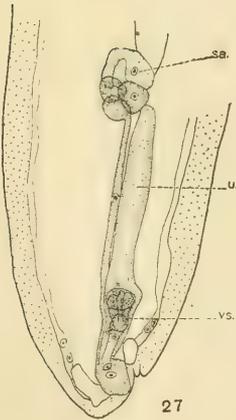
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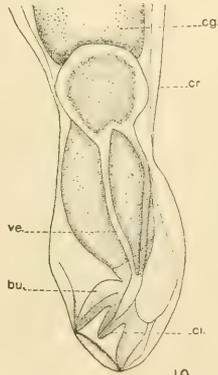
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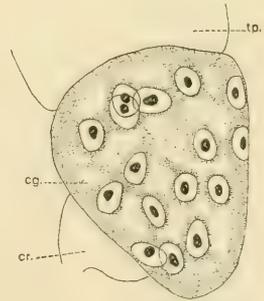
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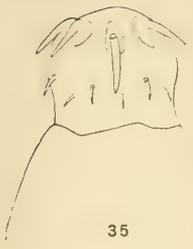


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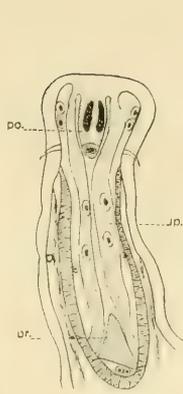


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- Fig. 32 *Eorhynchus emydis* (Leidy); proboscis of fully mature female. $\times 97$.
Fig. 33 Hooks of *Eo. emydis*. $\times 310$.
Fig. 34 Embryos of *Eo. emydis*. $\times 310$.
Fig. 35 Proboscis of *Eo. cylindratus* (Van C.) $\times 97$.
Fig. 36 Hooks of *Eo. cylindratus*. $\times 310$.
Fig. 37 Embryos of *Eo. cylindratus*. $\times 310$.
Fig. 38 *Eo. tenellus*; reconstruction from sagittal sections of anterior end of body. $\times 97$.
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Fig. 41 *Eo. emydis*; Cement gland of mature male. $\times 97$.
Fig. 42 *Eo. emydis*; Cement gland of immature male. $\times 97$.
Fig. 43 *Eo. emydis* (juv.); lemnisci in toto mount. $\times 97$.
Fig. 44 *Eo. emydis* (juv.); proboscis receptacle and adjacent organs; toto mount stained in hematoxylin. $\times 97$.
Fig. 45 *Eo. agilis*; longitudinal section, showing especially the terminal proboscis organ. Copied from Hamann, 1895, plate 3, figure 28.
Fig. 46 *Eo. rutili*; anterior end of body. Copied from Saeftigen, 1884, plate 5, figure 6.



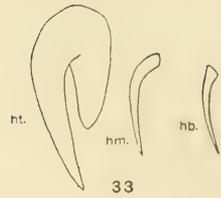
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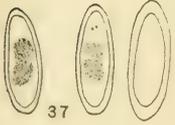
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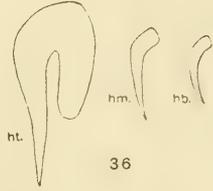
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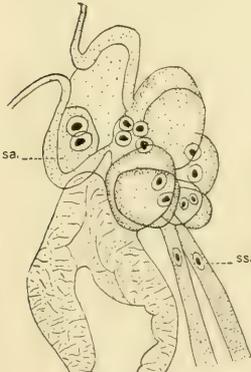
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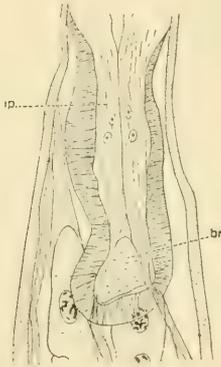
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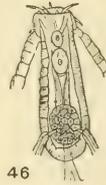
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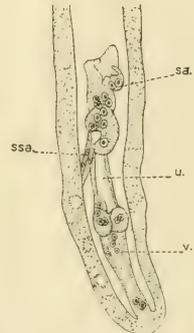
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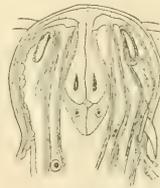
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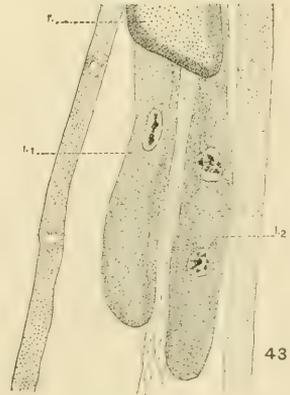
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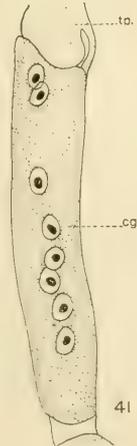
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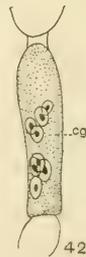
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THE INNERVATION OF THE INTEGUMENT OF CHIROPTERA¹

JAMES EDWARD ACKERT

From the Zoological Laboratory of the University of Illinois

TWENTY-ONE FIGURES (FOUR PLATES)

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INTRODUCTION

As is well known, the skin of bats is very sensitive to tactile stimulation. These animals in captivity give vigorous responses when various parts of their bodies and membranes are touched. Even eighteenth century investigators thought the integument of bats was especially adapted for the perception of delicate

¹ Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 29.

tactile stimuli, for Spallanzani and Cuvier observed that bats deprived of sight avoided small objects with accuracy. Cuvier found the wings to be supplied with an enormous number of nerves, and thought that during flight the blinded bat, on approaching the object, sets up air currents, which, reacting on the sensitive patagium and external ears, enable the animal to avoid the obstacle. That the sense of touch is more highly developed in bats than in other mammals was asserted by Schöbl ('71), who described 'Terminalkörperchen' at the bases of the hairs. Moreover, Redtel, two years later, maintained that it is possible for these animals to perceive the slightest difference of external air pressure upon their wings.

The extreme sensitiveness of the integument of these animals and the possibilities of modern technique seemed to justify a further search for sensory structures in their skin. Moreover, at the time this work was begun, no investigator had made an extensive study of the innervation of the skin of bats since Schöbl published in 1871 his account of the terminal corpuscles in the flying membrane.

The work has been carried on in the Zoological Laboratory of the University of Illinois under the direction of Prof. Frederic W. Carpenter, to whom I am indebted not only for his personal interest in the progress of the work, but also for his constant advice and helpful criticisms.

MATERIAL AND METHODS

The material for this investigation consisted of forty-one bats of which thirty-one were cave bats (*Myotis lucifugus*) from Indiana. The remainder, the common red bats (*Myotis subulatus*), were taken in the vicinity of Urbana, Illinois.

Most of the material was prepared by an *intra vitam* methylene blue method. The blood was washed out of the freshly etherized animal, and a 1 per cent solution of methylene blue in distilled water injected into the arterial system through the heart. After leaving the animal, with its vessels full of staining fluid, freely exposed to the air for oxidation, the fluid was washed out with

Ringer's solution, and small pieces of tissue fixed in a cold 8 per cent solution of ammonium molybdate in distilled water. The tissues were then dehydrated, cleared in xylol, and imbedded in paraffine. Sections of 20μ were thick enough to enable one to follow the nerve fibers some distance, and sufficiently thin to admit ample light. For checking results other methods of preparing material were employed. The killing and fixing fluids used for this purpose were corrosive sublimate and acetic acid, Zenker's fluid, ammoniacal alcohol, and 10 per cent formol. The stains used included silver nitrate (Cajal method for nerve fibrils), carmalum as a counter stain for methylene blue, Mallory's connective tissue stain, Heidenhain's iron hematoxylin, Hanson's hematoxylin and orange G, and Delafield's hematoxylin and eosin, the last of which proved the most satisfactory for general use.

OBSERVATIONS AND DISCUSSION

GENERAL STRUCTURE OF THE INTEGUMENT

1. *Integument of the body*

The skin of the body of bats is covered with hair which, as Allen ('93) has found, varies in different regions in texture and amount. In general, the crown of the head, the neck, the sides of the under surface of the body, the rump and the pubis have a thick pelage, while the distal portions of the ears, the soles of the feet, the mammae and the external genitalia are almost naked. The snout is scantily clothed, but shows a limited number of vibrissae which arise from wart-like structures.

In different regions of the body the skin varies greatly in thickness. The integument of the face is the deepest; that of other parts of the body diminishes in depth gradually in the following order: palmar region, plantar region, rump, ventral thoracic region, crown, and dorsal thoracic region.

As a rule, some difficulty is experienced in distinguishing all the layers commonly found in the human integument. In the epidermis the Malpighian or deeper stratum can be readily made out. Its deepest layer is made up of subcolumnar cells.

The intermediate layer of polygonal cells is for the most part absent, though in places (e.g., the face) it appears as a single sheet of isolated, more or less flattened cells, whose nuclei are somewhat reduced in size (fig. 1). Numerous pigment granules are present in this layer (fig. 1, *pg*).

The stratum corneum is thickest in the palmar and plantar regions. It is made up of several layers of cornified epithelium, the outer ones of which are usually in the form of loose scales. The deeper layers are more compact, and appear to consist of flat, enucleate cells. In certain regions of the body (lining of the mouth, lumbar region) these layers resemble to some extent the stratum lucidum of the human skin, but the presence of this stratum can be made out definitely only in the palmar and plantar regions (fig. 2, *sl*).

The surface of the epidermis is frequently interrupted by hairs, and also by the openings of ordinary sudoriparous and of modified sweat glands as Diem ('07), Porta ('10) and others have shown. The ordinary sweat glands and the modified sweat glands may open into the hair follicle, or independently on the surface. The distribution of skin glands over the body is very variable. Though not numerous in the region of the rump, sweat glands are, however, present. This is in accord with Diem's results, but opposed to those of Hoffman ('98). The writer was unable to find sweat glands in the sole of the foot, and agrees with Toldt ('07) that these glands do not occur in the ball of the thumb. Toldt found numerous glands in the 'Säugescheibe,' and also large groups of glands in the region of the neck and of the external genitalia. The upper lip is more abundantly supplied with skin glands than any other part of the body.

As is frequently the case, the superficial layer of the corium, the stratum papillare, is raised into ridges and papillae which project into the epidermis. These are most marked in the upper lip, where simple and compound papillae are present. The interlacing strands of connective tissue and the reticulum of elastic fibers which together form the ground work of the corium are comparatively fine and closely packed, thus causing this layer to be somewhat dense. Mallory's connective tissue stain

shows that the general direction of these strands and fibers is parallel with that of the stratum Malpighii. While it is not possible to determine a boundary between the stratum papillare and the stratum reticulare, yet the deeper connective tissue bundles of the latter are obviously more loosely interwoven than those of the superficial layer of the corium. As in other mammals, the corium contains blood vessels, hair follicles, sebaceous glands, sudoriparous glands, striated and smooth muscle fibers, nerve trunks, medullated and non-medullated nerve fibers, tactile corpuscles, and nerve endings. The last three structures mentioned will be described in detail later.

As has been noted, the upper lip of the bat is richly supplied with skin glands. One type of these, the modified sweat gland, differs somewhat from the typical sweat gland, so a description of its structure may not be out of place here. Compared with a hair follicle, this gland is enormous in size. It consists of a long, uncoiled secreting portion with an extended funnel-shaped duct. The secretory portion is lined by a single layer of columnar cells with finely granular protoplasm and round or oval nuclei (fig. 3, *cc*). Leydig, Schöbl and Sabussow ('10) have called attention to the fact that these large modified sweat glands (in the flying and interfemoral membranes) have a coating of smooth muscle fibers, which, by their longitudinal course, cause a slight spiral striping of the gland. This coating of muscle fibers (fig. 3, *mf*) lies between the layer of columnar cells and an external covering or basement membrane (fig. 3, *bm*). The latter is homogeneous and without nuclei. The duct of the gland is lined throughout by short, somewhat irregularly cubical cells, arranged in a single layer, and surrounded by a delicate basement membrane. Not infrequently secretion products are found in the lumina of the glands. The products are more or less similar in appearance to what Wimpfheimer ('07) terms degeneration products ('detritus') found in uncoiled sweat glands in young moles.

It is worthy of remark that pigment cells occur in the corium both of the body integument and of the flying and interfemoral membranes (fig. 2, *pc*). In the corium of the integument they

are numerous, and appear to be scattered about promiscuously. Their form is very variable. They may be spherical, oval, elongate and slightly spiral, heart-shaped, pear-shaped, raggedly lobulate, and with or without processes (fig. 5). In size they vary from 34μ in length and 25.5μ in width to 374μ in length and 60μ in width. The cell body is filled with fine brown granules. In hematoxylin-eosin preparations a few of the granules usually take the dull blue stain of the hematoxylin, while in the methylene blue material some or all of the granules may stain a bright blue. Figure 5, *b* represents a pigment cell containing stained and unstained granules.

2. *Flying and interfemoral membranes*

The flying membrane of bats is a skin duplicature formed by the lateral extension of the dorsal and ventral integument of the body. The proximal parts of the membranes are covered with fine hairs similar of those of the pelage, while over the distal areas extremely fine, more or less modified hairs occur sparsely. In the natural condition there is a manifold wrinkling and plaiting due to numerous elastic bands within the membrane (Schöbl).

Externally the flying membrane is made up of small, hexagonal, plate-like cells which form a continuous membrane. Each cell contains pigment granules which are collected into an intra-marginal zone much as Schöbl has described (p. 4). This investigator reports that the center and border of the cell (in *Vesperugo serotinus*) are free from pigment granules. In *Myotis lucifugus* and *M. sublatatus* the writer found pigment in both of these regions, but in smaller quantities than in the intra-marginal zone. The cells of the outer (dorsal) surface of the flying membrane contain more and darker pigment granules than do those of the inner (ventral) surface (Schöbl). In fact this surface in places contains almost no pigment.

As in the integument of the body the epidermis of the flying membrane stands out in the sections in contrast to the cutis. The Malpighian layer also can be readily distinguished from the stratum corneum. According to Schöbl's studies the Mal-

Malpighian stratum is composed of two layers of scattered cells. The writer, however, finds that one layer of cells occurs quite as frequently as two. The nuclei of the deeper layers of both dorsal and ventral sides are slightly more oval than those of the more superficial layers, the latter being somewhat flattened. From the shape of the nuclei one would infer that when a single layer occurs it is the outer one. In the Malpighian stratum of the dorsal side of the patagium numerous pigment granules are present, while in this stratum on the ventral side very little pigment occurs. Aside from being somewhat thinner, the stratum corneum does not differ from the corresponding structure in the skin of the body.

The tissue enclosed between the dorsal and ventral Malpighian strata of the patagium constitutes the corium, which varies in thickness in different regions. In both the flying and interfemoral membranes it is thickest near the body, while in the more distal areas it gradually becomes thinner.

The corium is made up of three poorly defined strata of connective tissue, a central, somewhat loose one, corresponding to the stratum reticulare of the body integument, and two others—one on either side—of denser tissue, more or less similar to the stratum papillare. The chief arteries, which are accompanied by the larger veins and nerve trunks, cause this stratum to be much thicker in those regions where they occur than elsewhere. Although the outer surfaces of this stratum are thrown into folds to some extent, the writer has been unable to find papillae.

In the stratum reticulare are contained the larger blood vessels and nerves, and the striated muscle bundles and elastic bands (Balken) first described by Leydig, whose results were later confirmed by Schöbl. Here also are found the secreting portions of sweat glands, and the proximal third of hair follicles. The outer stratum of the corium contains the central portions of the hair follicles, their sebaceous glands, and the sweat glands. Each follicle, with the sebaceous, sudoriparous and modified sweat glands associated with it, is surrounded by a capillary network.

NERVE LAYERS OF THE INTEGUMENT

1. Nerve layers of the body integument

In the subcutaneous tissue and in the reticular stratum of the body integument, are large medullated nerve trunks and branches which, for convenience, are called the first nerve layer. By dichotomous branching these nerves break up into a loosely intertwined meshwork consisting of an enormous number of medullated nerves. These interwoven nerves, which are not actually united in a plexus, constitute the second nerve layer (fig. 4, *snl*). Arising from the latter are medullated nerves which pass toward the periphery. Near the outer surface of the corium they begin to divide. The resulting non-medullated branches pass directly to the Malpighian stratum, forming the third nerve layer (fig. 4, *tnl*). Ordinarily so much pigment is present here that it is impossible to follow the fibrils to their endings. However, in places where the epidermis has accidentally been torn, one can readily trace the fibrils well into the Malpighian stratum, noting branching fibrils which pass outward and terminate in or between the cells of the stratum granulosum. As these can be traced more readily in the membranes where little or no pigment is present they will be considered more fully later (p. 313). Varicosities are numerous both in the second nerve layer and along the fibers which pass to the third layer (figs. 14, 15, 16, 17). The greater number of these enlargements, however, occurs on the smaller fibers. Varicosities in the third nerve layer, that is, on the surface of the corium or in the Malpighian stratum, have not as yet been observed by the writer. As the literature on nerve layers in the skin of bats deals almost entirely with these layers in the patagium, the brief historical survey will be given in the consideration of the flying membrane.

2. Nerve layers of the flying and interfemoral membranes

As early as 1796 Cuvier called attention to the abundance of nerves in the flying membranes of Chiroptera. Leydig, a half-century later, while differing somewhat with Cuvier as to the number of nerves present, admitted that these membranes are richly innervated.

The first investigator, however, to make an intensive study of the arrangement of the nerves in the flying membranes was Schöbl ('71), who states that the nerves of the patagium are naturally divided into five layers. The first layer, situated in the innermost stratum of the flying membrane, contains the larger nerve trunks, the main blood vessels, the chief muscles and the elastic bands. The second nerve layer is double, one part lying above, and its duplicature below the first layer. The nerve trunks of this layer branch dichotomously again and again, forming an irregular network. The third layer of nerves, which is also double, lies external to the previous one, on a level with the finest blood vessels. As to the size of the nerve trunks of this layer, Schöbl states that they consist usually of two, very rarely of four, non-medullated fibers. The fourth nerve layer, likewise double, lies outside of the third. It consists throughout of an irregular net of single non-medullated fibers. The meshwork in this layer, however, arises not by an interlacing of fibers, as is the case in the other two layers, but by direct anastomosis of single non-medullated fibers. On certain fibers of this layer Schöbl noted a number of enlargements or swellings ('Krootenpunkten'), which were triangular, square, or polyhedral in form, having a fine granular appearance, but exhibiting no nuclei. He also occasionally saw more or less similar spindle-shaped enlargements in the course of a single fiber, especially the larger ones. The fifth and last nerve layer, also double, lies immediately over the previous one on the surface of the cutis, ordinarily remaining attached to the deepest cells of the Malpighian stratum. The fibers of this layer are likewise non-medullated, and have a diameter ranging from 0.9μ to immeasurable fineness. This layer arises from the previous one by the division of the finest fibers of the latter. At the places of division of the fibers, the swellings which were found so frequently in the last nerve layer seldom occur in this one, and the spindle-shaped variety is lacking entirely. This layer of extremely fine non-medullated nerve fibrils lying immediately at the surface of the corium, partly between the lowest cells of the Malpighian stratum, Schöbl holds as a terminal. He further

states that in preparations in which the lowest cells of the Malpighian stratum remain undisturbed on the corium, *no free endings* of the finest non-medullated nerve fibrils are found, and that fibrils passing further toward the surface between the cells of the stratum granulosum, are never to be found either on the surface of the preparation or in cross section. He pointed out, however, that occasionally round or elliptical, swollen structures resembling fine nerve endings are to be seen, but these almost always prove to be nodal points of division of nerve fibrils. These minute swellings occurred so seldom that Schöbl attributed their presence to faulty technique.

Sabussow ('10), working on the innervation of the flying membrane, did not wholly accept Schöbl's idea of the distribution of the nerves of this part of the body. This investigator found large nerve trunks in the innermost stratum of the patagium, but held that Schöbl's second nerve layer lay in the same plane as the first, and consequently could not be said to exist as a separate nerve layer. Concerning Schöbl's third nerve layer Sabussow simply stated that it is not double. But this investigator confirms the existence of the fourth nerve layer of Schöbl, adding that, no matter how the membrane be torn, this layer can be seen to be double. He also confirms Schöbl's fifth layer, which is non-medullated and double; but instead of the few 'swellings' which Schöbl observed, Sabussow found numerous varicosities. The latter sums up the layers he found as follows: (1) a simple layer including the first two layers of Schöbl; (2) a broadly meshed double network with triangular enlargements in it; (3) a network of varicose fibers also double. Consequently according to Sabussow, there are five nerve layers in the patagium.

In transverse sections of my own preparations of the flying and interfemoral membranes there can readily be seen, here and there, regions which are approximately twice as thick as that of the remaining area of these membranes. It is in these thickened regions that the chief arteries, veins, nerve trunks and frequently the principal muscle bundles are found. These particular regions contain, as will be shown, one more layer of nerves than do the others.

The main blood vessels, accompanied by the chief nerve trunks, pass out from the body through the flying and interfemoral membranes in the stratum reticulare, giving off, here and there, important branches, which, as stated, are frequently found with the muscle bundles. These blood vessels, partly because of their own size, and partly on account of the increased amount of connective tissue around them, cause the elongated thickenings or ridges already referred to in these membranes. The medullated nerve trunks and their chief branches, both found in the innermost stratum (reticulare) and existing only in the aforesaid ridges, constitute the first nerve layer. The second, a double layer of nerves, arises from the first by repeated dichotomous branching, traverses the deeper part of the corium, and spreads throughout the entire area of the flying and interfemoral membranes. In methylene blue preparations this layer is seen to consist of a loose network of medullated nerve fibers, many of which contain comparatively large varicosities (fig. 13, *va*). The third and last nerve layer is likewise double. Numerous medullated fibers arising from the second nerve layer pass toward the two external (dorsal and ventral) surfaces of the membranes. Many of these fibers, on approaching the Malpighian stratum, divide dichotomously; others do so at the surface of the corium. Both lose their medullation. The forked branching continues to some extent in the Malpighian stratum, the larger fibrils giving off smaller ones, until finally delicate nerve threads end in minute enlargements, which will be described in detail later. These branchings of non-medullated nerve fibrils at the surface of the corium and in the stratum Malpighii constitute the third nerve layer. While varicosities of different sizes (figs. 13, 14, 15) appear in the nerve fibers leading up to this layer, the writer has not observed them in the latter.

According to the present observations, then, certain regions of the flying and interfemoral membranes are supplied with three layers of nerves, others with but two. Briefly stated their number and distribution are as follows:

1. A layer of medullated nerve trunks and numerous medullated branches, occurring in the stratum reticulare, but only in

the elongated ridges containing the largest blood vessels and much connective tissue.

2. A double medullated nerve layer in the deeper part of the corium, extending throughout the membranes.

3. A layer, likewise double, present in the entire Malpighian stratum, and consisting of numerous branches of non-medullated nerve fibrils.

A comparison of the foregoing results shows that the first nerve layers of Schöbl and of the writer coincide; that Sabussow's first layer included Schöbl's first and second layers and the writer's first, together with the innermost branches of his second nerve layer. A study of sections from different parts of the membranes has convinced the writer that Schöbl's second, third and fourth nerve layers may well be considered as one layer, the writer's second. Close to the body, where these membranes are thick, and where Schöbl probably made his observations, since he especially recommended this region for study, it is true that the writer's second layer is thicker dorso-ventrally than it is near the elbow, or in the region midway between the body and the tail. But at the periphery, between the elongated phalanges, and near the posterior border of the interfemoral membrane, where the skin duplicature is thin, this nerve layer is exceedingly compressed. The contention of Sabussow that Schöbl's second nerve layer lay in the same plane as the first, and consequently could not be considered as a separate layer, is not supported by the present observations. Schöbl's first layer is to be found in the stratum reticulare, while the second, arising by repeated dichotomous branching of the first, takes a position in the deeper part of the superficial layer of the corium. The writer's second nerve layer corresponds to Schöbl's second, third and fourth layers, while Sabussow's second layer includes the third and fourth layers of Schöbl, and the greater part of the writer's second. The third nerve layer of Sabussow and of the writer, respectively, corresponds to Schöbl's fifth.

NERVE ENDINGS IN THE INTEGUMENT

The nerve terminations in the integument of Chiroptera may be grouped into five classes as follows:

1. Free nerve terminations in the epidermis
2. Nerve endings on hairs
3. Special sensory end-organs
 - a. End-bulbs
 - b. Terminal corpuscles
4. Motor nerve endings on striated muscles
5. Nerve endings on modified sweat glands

1. *Free nerve terminations in the epidermis*

As was stated in an earlier part of this paper (p. 308), free nerve endings in the form of minute swellings were observed in the stratum Malpighii. These free nerve terminations or end-knobs can most readily be seen in sections of the ventral portions of the membranes, where little or no pigment is present. Especially desirable for this purpose are oblique sections, or those which contain small areas of the surface of the membrane (fig. 6). In such sections it is possible to focus down through the transparent stratum corneum, thereby obtaining distinct views of the deeply stained (blue) nerves of the third layer (fig. 6, *n*). The latter stand out in bold contrast to the weakly stained cytoplasm of the Malpighian stratum. In sections 20μ thick one can, by focusing, trace non-medullated nerve fibers, from the point of branching near the surface of the corium, on out among the deeper Malpighian cells. The larger fibrils and the smaller ones given off from them are plainly visible. Finally, among the cells of the stratum granulosum (fig. 6, *sgr*), the ultimate branches terminate in minute round or oval end-knobs (figs. 6, 7, *e*). Similar structures were mentioned but misinterpreted by Schöbl, who, observing a very limited number of minute round swellings resembling fine nerve endings in the stratum Malpighii concluded that they were foreign particles due to faulty technique.

The end-knobs take a deep blue stain similar to that of ordinary axis cylinders, and appear to be homogeneous in structure. They are oval or spherical, their size varies from 0.5μ in length

and 0.4μ in width to 0.9μ in length and 0.8μ in width. In the sections studied, these end-knobs appear to be numerous. Ordinarily, one to a cell is observed, though occasionally even two are seen close to a single cell boundary. Sometimes a tiny fibril appears to end without any enlargement (fig. 7, *x*). This, however, may be due to the failure of the methylene blue to differentiate the end-knob, as those who have worked with this stain will readily understand.

That these diminutive enlargements or end-knobs are real nerve terminations and not the nodal swellings sometimes seen where fibers divide has been satisfactorily proven. For example, in focusing on the surface of the transparent stratum corneum no knobs nor fibrils can be seen. A deeper focus brings into view end-knobs with a fine nerve fibril running into each. A still deeper focus shows tissue below the end-knobs and enables one to follow the nerve fibrils from the now indistinct terminal swellings back to the branch from which the nerve fibrils are given off. Where little or no pigment is present, these nerve terminations can be seen without difficulty.

For a time the writer was unable to determine whether the nerve end-knobs are situated in the stratum Malpighii or in the deepest layers of the stratum corneum. At length, however, a section was found in which a part of the ventral surface of the interfemoral membrane curled up, permitting an oblique view. The methylene blue stain was deep enough to show the margins of a number of consecutive superficial cells of the stratum granulosum (fig. 6), and little pigment was present. By focusing upon this obliquely turned portion of the surface of the membrane, it was comparatively easy to distinguish the flat, elongate, scale-like cells constituting the stratum corneum from the more oval, clearly defined, superficial cells of the Malpighian stratum. By focusing upon the curved surface it was possible to see a number of nerve end-knobs on or near the surface of the stratum granulosum, but as yet no end-knobs have been seen by the writer in the stratum corneum.

The question of the exact position of the end-knobs in respect to the epithelial cells naturally arises. It is certain that a large

number of the structures in question are situated on the surfaces of the cells (fig. 7, *es*). Others appear to be within the cytoplasm. However, it is frequently possible by focusing to see that these end-knobs are after all on the borders of the cells. If all were completely stained, it is not improbable that the remaining end-knobs could be shown to be intercellular.

So far as the writer has been able to ascertain the only reference to free nerve terminations in the epidermis of bats is that of Botezat ('08). The study of this investigator was made principally on the nerves in the epidermis of the dog's nose, but he mentioned the finding of intracellular end-knobs ('Endknöpfen') in the skin of the nose of the bat. He held that the free nerve terminations in the epidermis, not only of bats, but of all classes of vertebrates, are intracellular, though none of his figures indicates it. Retzius ('92) showed free nerve terminations (intercellular) in the epidermis of the lip of the human foetus. Of free nerve endings in the epidermis of the mouse and of the rabbit he made the following statement: "Die feinen, varicösen Nervenfasern verzweigen sich und endigen im Rete Malpighii interzellulär ohne jeden directen Zusammenhang mit Zellen." Van Gehuchten, in 1893, described free nerve terminations in the epidermis of the face, lip, ear, paw and tail of the white mouse and white rat. He likewise found the free nerve endings to be intercellular. He stated, "Partout nous avons trouvé l'existence de fibres nerveuses intra-épidermiques se ramifiant et se terminant librement entre les cellules épithéliales." While Dogiel ('03) did not hold intracellular endings as out of the question, yet he was strongly of the opinion that the free nerve terminations are intercellular.

2. *Nerve endings on hairs*

While the innervation of hairs has for some time been a field of fruitful investigation, there still remain some unsolved problems in connection with the hair of Chiroptera. In recent years especially, more attention has been directed toward the innervation of tactile or sinus hairs than toward that of the hairs of the pelage. The writer's descriptions will be confined wholly to the latter.

Observations made by different investigators on the innervation of the hairs of bats have been so conflicting that it seems advisable to give a brief review of the literature. Schöbl ('71) studied the innervation of the hair of the flying membrane, and set forth the following principal points: In the hairs of the bat, the nerves terminate in special corpuscles ('Terminalkörperchen') situated at the bases of the hair follicles. The hair receives a bundle of nerves which consists of from two to five medullated fibers. These twist many times in a spiral about the hair shaft forming a nerve wreath or ring. From this spiral ring two to four nerve fibers are given off, and these extend downward, ending in the terminal corpuscle beneath the hair follicle. A superficial nerve ring which consists of from one to two coils is formed by fibers from the fourth nerve layer. Boll ('71), working on similar material, confirmed Schöbl's observations.

The following year Stieda took exception to Schöbl's account, especially in regard to his 'Terminalkörperchen.' This observer concluded that the structure in question was not a nervous apparatus but rather a differentiated part of the hair follicle ('Haarkeime'). The nerve ring was not mentioned by Stieda.

Beil ('71) also denied the existence of Schöbl's end-corpuscles, although he was able to see the nerve ring. Concerning the structure of the latter, its course, or the endings of its fibers, he could determine nothing definitely. Above the sebaceous glands, however, Beil noted the entrance of two or three bundles of non-medullated nerves into the hair follicle.

Using the method described by Schöbl himself, Velecky ('72), investigating the flying membrane, likewise did not find the so-called end-apparatus; nor did the use of gold disclose these 'Terminalkörperchen.' By the latter method, however, he demonstrated non-medullated nerves which approach, from below, the cells of the epithelium of the outer root sheath of the hair, and spread into the intercellular spaces, forming a net.

In the same year appeared a more important piece of work by Jobert, in which he described in considerable detail the innervation of the hair of the bat's wing. The principal points brought out were as follows: (1) All the hairs of the skin are supplied

with nerves and are perceptive. (2) The affirmation of Schöbl that into each hair follicle there enters one nerve is not true. Each hair is supplied with many nerve fibers, five to six or more, which approach the follicle, together or separately, and from different sides. They may unite into two or three small trunks. On reaching the neck of the hair the nerves divide, lose their medullation, and are distributed on the hyaline membrane more or less like radiations, ending freely at about the same level. (3) The nerve ring of Schöbl does not exist, neither do the 'Terminalkörperchen.' (4) At the level of the superficial subepithelial network of nerves, minute threads are seen which surround the follicle and disappear in the epithelial sheath.

Arnstein ('76) recognized two different kinds of nerve terminations on hairs: (1) The free endings on the hyaline membrane in the form of a 'palisade;' (2) The nerve network which occurs in the outer root sheath.

Bonnet ('78), who investigated the innervation of the hair follicles of a number of mammals including the bat, confirmed Arnstein's observations on the endings of nerves on the hyaline membrane. Bonnet's idea was that a nerve ring exists in connection with each hair. The small fibers which constitute this structure lie outside of the straight fibers, which terminate in a 'palisade,' and surround them much as hoops surround a barrel, in the form of a ring consisting of six or more pale fibers. Of the root sheaths in the region of the sebaceous glands Bonnet says, "This is a rendezvous of the various small medullated nerve fibers which come to the hair partly above and partly below the sebaceous glands. These fibers going to the follicle spread out forming a woven net of minute medullated fibers."

In describing the innervation of the hair of certain mammals, including bats, Szymonowicz ('01) pointed out that the medullated fibers approach the follicle below the sebaceous glands, divide, losing their medullation, and penetrate to the hyaline membrane, where some of the fibrils encircle the hair, while others end on the hyaline membrane. The latter fibrils branch regularly, and run parallel with the long axis of the hair. This investigator observed perceptive menisci in a strongly developed

outer root sheath of a common hair on the face of *Vesperugo serotinus*.

According to the observations of Sabussow ('10) the hair of the flying membrane of bats is supplied with several medullated nerve fibers whose number is never less than two. These fibers approach the hair follicle, divide, spread around the hair spirally, more or less in the form of a ring, give off small fibrils from the latter, branch, and finally end in the form of a 'palisade' on the hyaline membrane. The fibrils of the 'palisade' may contain varicosities along their courses, or their distal ends may be lance-shaped. The spiral ring around the 'palisade' consists of small varicose threads. This Sabussow holds as a second kind of nerve ending on the hair. He asserts that he never saw these two kinds of endings, namely, the 'palisade' and the varicose threads of the spiral ring, at the same time in the same hair. From this he concludes that there exist two kinds of hairs, each of which is supplied with one of these nerve terminations.

Parallel with the spiral ring just described and more superficial, Sabussow observed a broadly meshed network of fibers resembling a nerve ring, and apparently surrounding the hair above the sebaceous glands. This network or ring, which belongs to the second nerve layer, could by focusing be seen to give off more or less flattened fibers resembling the 'palisade.' Being unable to find any definite connection between the 'palisade' apparatus first described and this one which comes from the subepithelial network, Sabussow inferred that the two were independent.

In the writer's deeply stained methylene blue preparations of the bat's skin, both of the body and of the membranes, the hair follicles with their numerous nerves stand out in bold contrast to the surrounding, weakly stained connective tissue (figs. 8, 9). The nerves which supply the hairs arise from the second nerve layer, pass outward to approximately the level of the inner third of the hair follicle, where, at first, they appear to pass along from one hair to another. But upon close examination it is seen that nerves may be distributed in one of two ways: (a) The whole fiber may end directly in a single follicle (figs. 8, 9, *ff*); (b) Upon

approaching hairs the nerve may divide, one or two branches going to a follicle, the others passing out to the epidermis (figs. 8, 9, *fe*). By far the greatest number of the nerves in question are distributed in the first way. The numerous fibers form a veritable network, which might justly be termed a nerve layer, but which for simplicity is not so considered by the present writer.

As to nerve endings on the hair, it may be said that they occur at three different levels and in three separate layers of the follicle: (1) A superficial nerve ring situated above the orifices of the sebaceous glands and giving off nerve threads in the connective tissue sheath (fig. 9, *sn*); (2) Fine varicose or flattened nerve fibrils which lie immediately below the sebaceous glands, and end on the hyaline membrane parallel to the long axis of the hair (fig. 9, *eh*); (3) Nerve fibrils at the level of the lower third of the follicle, which take a horizontal position in the outer root sheath (fig. 9, *eo*). A further consideration of these types of nerve endings follows.

1. *Superficial nerve ring.* Medullated nerve fibers approach the hair above the opening of the sebaceous glands. At the outer border of the connective sheath, they divide, spreading around the follicle and forming a loose ring of from two to six or more fibers. From the ring are given off non-medullated fibrils, some of which are interwoven into a delicate network, while others appear to end freely in the connective tissue sheath of the follicle. This ring doubtless corresponds to the "broadly meshed network resembling a ring" described by Sabussow ('10) above the sebaceous glands. As is seen in figure 9, *f*, the non-medullated fibrils show no tendency to pass downward to a nerve ring below.

2. *Varicose or flattened nerve fibrils.* Immediately below the sebaceous glands medullated nerve fibers, chiefly of type (a), enter the region of the hair follicle, penetrate the connective tissue layers, divide, losing their myelin, and encircle the hair in a nerve ring. The number of fibers constituting the nerve ring varies from two to eight or even more. From the inside of the ring fibrils are given off which divide dichotomously. The branched fibrils assume a position parallel to the long axis of the

hair, and usually end in slight enlargements (fig. 9, *eh*), some of which are merely small varicosities, while others resemble the minute end-knobs seen in the free nerve terminations in the epidermis. In certain cases there are no enlargements, but in these instances the terminal fibers are flattened. This type of nerve ending undoubtedly corresponds to the well known nerve ring and 'palisade' described first by Arnstein ('76), and recognized since by Bonnet ('78), Szymonowicz ('01), and Sabussow ('10). Merkel ('80) described a similar end-apparatus on a common hair in the lip of a cat. The "termaisons en fourchette" of the Hoggans ('83) and the nerve rings of Retzius ('94), Van Gehuchten ('96) and Ostroumow ('00) are probably corresponding nervous structures.

3. *Nerve fibrils in the outer root sheath.* At the level of approximately the lower third of the root of the hair, medullated nerves penetrate the connective tissue layers of the follicle. When the hyaline membrane is reached they divide and run for a short distance on or near its surface. These nerve fibers give off a few strong non-medullated fibrils which pierce the glassy membrane, and end in the outer root sheath, usually taking a horizontal position in the latter (fig. 9, *eo*). The nerve endings of this type are found in a slight swelling of the root sheath, which may correspond to the superior swelling described in typical sinus hairs. So far as the writer has been able to ascertain, nerve endings of this type have not previously been described in the pelage hair of the bat. While such examples are not numerous, yet they seem to him to be genuine. Nerve endings in the form of tactile corpuscles were described by Szymonowicz ('01) in the outer root sheath of a hair in the face of *Vesperugo serotinus*. The same observer, in 1909, mentioned the finding of Merkel's corpuscles in this layer of the follicle in man. Retzius ('94) described a nerve fiber in the outer root sheath of a hair of a mouse, and Vincent ('13) found nerve fibrils in this layer of the sinus hair of the rat.

3. *Special sensory end-organs*

The literature dealing with special sensory end-organs in general has recently been reviewed by a number of investigators: Szymonowicz ('95), Trejakoff ('02), Dogiel ('03), Schäfer ('10). Therefore only a brief survey of the observations upon such end-organs in the skin of bats will be given here.

Arnstein ('76) found in the flying membrane of a bat an end-bulb which he thought resembled the well known cylindrical end-bulb of Krause. It was possible for him to trace an axis cylinder into the organ, but he was unable to make out the ending of the fiber. In one instance, however, he saw it break up into fine fibrils. Arnstein was of the opinion that these end-bulbs occurred in the flying membrane where no hairs were present.

Schumacher, in 1907, mentioned the presence of a large number of layer-like corpuscles ('Lamellenkörperchen') among the phalanges.

Sabussow ('10) investigated the flying membrane of two species of bats (*Vesperugo noctula* and *Vespertilio daubentonii*). He stated that in weakly stained preparations he could see terminal bulbs which were divisible into two classes according to size. Some were so small that upon slight magnification it was difficult to see them; others were comparatively large, had a zigzag course, and could be recognized with ease. The latter could also be seen in material prepared by Apathy's after-gilding gold chloride method. The general characteristics peculiar to all end-bulbs which Sabussow observed were: (1) a longitudinal course of the fibers of the enveloping connective tissue membranes, apparent in gold preparations, and (2) a delicate wavy appearance of these membranes seen in methylene blue material. The connective tissue nuclei did not stain in methylene blue preparations, but on account of the difference of refraction, Sabussow thought that by focusing he could see them. He noted that the core of the bulb was narrow, but was unable to make out its finer structure.

The consideration that led Sabussow to classify these structures under the cylindrical type of end-bulbs was chiefly the

way in which the nerve fiber ended in the interior of the bulb. He observed that medullated nerve fibers divided at Ranvier's nodes, giving off several medullated branches. Occasionally, one of these branches entered an end-bulb, and passed through the whole interior of the organ to its opposite extremity. This naked axis cylinder in the bulb became slightly expanded, and ended either with a sharp point or in a thickening resembling a button.

Sabussow, in his figure 10, pictured an end-bulb stained with methylene blue, which he called a variation of the cylindrical end-bulb type. He described it as follows:

Within the bulb the axis cylinder expands, and, in the middle, broadens into a wide, paw-like plate with deep notches in its edges. From this plate there is given off a fiber which bends backward and upward, and in turn widens into a similar paw-like plate. The substance of the plate has a granular appearance, with here and there small masses of stain.

In my own methylene blue preparations of the integument two kinds of special sensory end-organs have been observed. (1) A small elongate end-bulb into which a single medullated nerve fiber enters, passes approximately to the opposite end, and terminates in a slight enlargement (fig. 18); (2) A large, round, cellular corpuscle innervated by a single fiber which disappears among the cells of the organ (figs. 19, 20). A more detailed description of each type follows.

a. End-bulbs. These structures occur in the corium near hair follicles, but clearly outside of the root sheaths. Ordinarily they are found below the level of the sebaceous glands, parallel with the long axis of the hair. Their size is approximately 1.5μ in length by 0.5μ in width. In general appearance they are regularly club-shaped in outline, the interior being filled with a semi-fluid substance. The medullated nerve, on entering the bulb, loses its myelin, the sheath of which becomes continuous with the sheath of the end-bulb. After passing through half the length of the organ, the axis cylinder expands slightly into a flat plate (fig. 18, *p*) which gives off two or three short heavy branches, and terminates near the end of the bulb in a small enlargement

(fig. 18, *en*). The deeply stained blue plate stands out in bold contrast to the weakly colored bulb about it. The distal branch arising from the thickened axis cylinder usually bends to one side, breaks up into an irregular, elongate, granular mass, and as such extends back through the expanded part of the bulb (fig. 18, *br*). In the portions of the organ surrounding the plate and the recurrent granular mass no layers nor nuclei are visible. With the exception of the lack of nuclei the appearance of this end-bulb is practically identical with that of the structure which Sabussow ('10) showed in his figure 10, and which he termed a modified end-bulb of Krause. The absence in my preparations of the nucleated capsule characteristic of the cylindrical end-bulb of Krause can be explained by the fact that such structures do not ordinarily stain in methylene blue. Although these organs are somewhat smaller than the cylindrical end-bulbs in question, their location and structure are such that the writer is inclined to think that they are modified cylindrical end-bulbs of Krause.

As has been shown, the writer confirms one type of Sabussow's end-bulbs, the first, which closely resembles Krause's cylindrical end-bulb (fig. 18, Sabussow's fig. 10). But the Russian author's second type, namely, the large one containing a nucleated sheath, and having a zigzag course, has not yet been seen in the preparations used for this study. However, there are present in this material structures (portions of medullated nerve trunks) which correspond so closely to the descriptions and pictures of his second type of end-bulb (Sabussow's plate 1, figs. 5, 6, 7, 8, 9) that it appears very probable that the two are identical. In his figure 8, he shows 'end-bulbs,' which, to the writer, seem clear examples of cut medullated fibers separating out from a common trunk. Sabussow himself points out that medullated fibers in this region branch repeatedly at the nodes of Ranvier. His figure 7, a picture of two so-called end-bulbs, represents apparently a portion of a medullated nerve fiber, which at one of these nodes, divides into branches. In his descriptions he states that the axis cylinder passes through the whole bulb to its very end. This is precisely what is found in a portion of a medullated nerve (fig. 21) when cut obliquely. The 'ending'

of the axis cylinder appears pointed or hooked, according to the position of the nerve when cut. The connective tissue nuclei, which did not stain in methylene blue, but which Sabussow thought he could make out by focusing, are in the opinion of the writer nuclei of the sheaths of Schwann or of the perineurium.

In the present investigation no gold chloride method was used, but material prepared by the Cajal silver nitrate method, Bielschowsky's method, or with methylene blue counter-stained with carmalum, has failed so far to reveal the presence of this second type of 'end-bulb' as described by Sabussow.

b. Terminal corpuscles. These comparatively large spheroidal corpuscles are found in the stratum reticulare of the corium, usually at some distance beneath the lower level of the hair follicles. In methylene blue preparations they stain deeply, exhibiting a cellular appearance, and frequently showing one or more nuclei (figs. 19, 20). Their size, which is fairly constant, is approximately 20μ in length by 10μ in width. Each corpuscle is innervated by a medullated fiber which arises from the second nerve layer. The fiber passes into the deeper part of the corium, and after giving off a few branches, enters the corpuscle, where it disappears among the cells. Occasionally, the fiber, on approaching the organ in question, forms a slight spiral coil (fig. 19). Thus far it has been impossible to trace the course of the nerve fiber within the corpuscle. To establish the identity of these organs with any of the known types of corpuscles is difficult. The layer-like capsules characteristic of Pacinian corpuscles are not apparent, but their absence may be due to incomplete staining. They more nearly approach the size of the type in question than that of any other type of corpuscles commonly found in the mammalian skin. Their location is identical with that of Pacinian corpuscles. From the fact set forth it seems possible that these spheroidal, cellular bodies may be Pacinian corpuscles.

To these two types of sensory end-organs may be added terminal varicosities, which are abundant in the region of hair follicles, outside of the root sheaths. Comparatively strong nerve fibers can be seen to enter these structures, where they break up into fine fibrils, and are surrounded by neuroplasm. It

is possible that these organs are varicosities in which the fibers beyond the enlargements fail to stain. But they are found constantly in deeply colored preparations, and moreover, are somewhat greater in size than those ordinarily occurring in the course of a fiber. Arnstein ('76) described varicosities in the outer root sheath of sinus hairs in bats, and Sabussow mentioned the presence of these structures on the courses of nerves outside of the root sheaths, but it appears that terminal varicosities external to the hair follicles have not previously been observed in Chiroptera.

4. *Motor nerve endings on striated muscles*

Voluntary muscles in the integument of the face, especially in the upper lips of bats, are well developed. In sections stained *intra vitam* with methylene blue these muscles are ordinarily deeply colored, the cross striations being of a slightly darker hue. Under such conditions it is usually possible to make out only the muscle fibers and their nuclei; but in regions somewhat removed from the larger blood vessels, where the blue stain is weak, one can see medullated nerve trunks among the muscle bundles. Along the muscle fibers which are stained only sufficiently to see their outline, it is possible to trace medullated nerve fibers (fig. 11, *nv*) which give off a small number of branches. The latter in terminating, form motor end-plates (figs. 11, 12, *mep*) on the muscle fibers.

It is not the purpose of the writer to enter into a discussion of the literature on this important subject, but merely to describe his observations and to mention wherein they agree or disagree with those of a few recent workers. For a review of the literature on motor nerve endings see Boeke ('09), Dogiel ('06), Huber and DeWitt ('97).

As a rule, the medullated nerve fibers can be traced to the border of the muscle fiber. At or near the edge of the latter, the medullation stops and the nerve fibers soon begin to separate into their component fibrillae, and finally end in a more or less regular end-net or arborization (figs. 11, 12, *ea*). This is in accord

with the observations of a number of investigators, including Boeke, who described motor end-plates on muscles in the tongue of the bat.

At the point where the nerve fibers enter the enlarged motor end-plate there is a slight elevation of the surface of the muscle (fig. 12, *el*). The position of end-plates on muscle fibers has been in doubt for some time. In the preparations used in this study they appear to be beneath the sarcolemma (fig. 11, *sa*). This shows especially well in cross sections of muscle fibers in the tongue (fig. 12, *sa*). Most investigators are now in accord in regarding this structure as under the sarcolemma.

In weakly stained preparations the branched endings can be seen to lie in more or less irregularly shaped matrices. The latter are of two kinds: (1) A weakly stained area containing numerous deeply colored granules of various sizes; (2) A somewhat smaller area without granules.

In shape, the former are irregularly circular or even triangular. The granules, which vary greatly in size, stain almost if not quite as deeply as the nerve fibrils themselves (fig. 11, *mag*). To structures corresponding to these Kühne ('87) gave the name soles ('Sohlen'). The smaller areas or soles, which appear to be free from granules in this stain, are oval or pear-shaped, the axis cylinders always entering the narrowed end. Huber and De-Witt ('97), Dogiel ('90) and Retzius ('92) stated that the sole does not stain in methylene blue preparations, whether examined at once or fixed in ammonium molybdate and studied in sections. The material, from which the present observations were made, was prepared according to the latter method. The irregularly shaped matrix in which the axis cylinder terminates is typically granular, but the nuclei seen by Huber and others in such preparations counter stained with carmalum or picro-carmin do not stain in methylene blue. In each of the two matrices or soles described above, the end-arborizations are nearly similar. The fact that Huber and the other observers mentioned failed to see soles whose granules stained in methylene blue preparations, may have been due to the inconsistency of the stain. This possibility, together with the fact that nerve terminations on motor plates

are very similar in each kind of sole described, and that the size of the so-called second kind comes within the possible range of that of the first, leads the writer to think that perhaps the apparently different kinds of soles found by him in the striated muscles in the epidermis of the bat are in reality one and the same type. In the one, the granules have stained, in the other they have not.

5. *Nerve endings on modified sweat glands*

So far as the writer has been able to ascertain, the literature contains no reference to the innervation of sweat glands in bats. As was noted elsewhere (p. 305), the modified sweat glands (fig. 10) have a coating of smooth muscle fibers which are arranged longitudinally (fig. 10, *mu*). In a weakly stained methylene blue preparation from the interfemoral membrane of *Myotis lucifugus*, such sweat glands have been observed with numerous stripes running at right angles to the smooth muscle fibers (fig. 10, *fi*). These stripes, which occur at comparatively regular intervals, extend hoop-like around the secretory portion of the gland external to the muscle fibers. The structures in question are much smaller than the muscle fibers, have a wavy course, and take the deep blue stain characteristic of nerve fibrils. A number (two to five) of delicate non-medullated nerve fibers (fig. 10, *no*) can be traced to the sides of these glands, but whether they connect with these circular stripes, the writer is at present unable to ascertain.

That sweat glands are under the control of the sympathetic nervous system is generally recognized. As is well known, preganglionic neurites leave the spinal cord through the ventral roots of the spinal nerves, and, after a shorter or longer course, terminate in some sympathetic ganglion in a very characteristic manner. Here the preganglionic neurites branch repeatedly, dividing into numerous small varicose nerve fibers, which interlace to form intracapsular plexuses around the cell bodies of the sympathetic neurones. It is likewise well known that in the sympathetic ganglia of Mammalia such intracapsular pericellular plexuses may be very simple, consisting of only a few varicose fibrils, as well as

very complex. The general structure of these pericellular plexuses, the absence of definite observations upon endings of sympathetic neurites (post-ganglionic) on sweat glands, and the striking hoop-like arrangement of these fibrillar stripes around the glands, lead the writer to question whether, perhaps, the post-ganglionic neurites may not form simple plexuses about the glands more or less similar to the pericellular plexuses about the cell bodies of the sympathetic neurones. Such an arrangement of the terminal fibers of a post-ganglionic neurite would be most effective. The nerve threads lying immediately upon the smooth muscle fibers and the bases of the gland cells could form functional connections with them. This view of the endings of post-ganglionic neurites on modified sweat glands seems somewhat more plausible from the fact that these circular fibrillar structures appear about the glands only in their secretory portions, and likewise only in the regions covered by the longitudinal smooth muscle fibers.

WHAT SENSORY ORGANS ARE CONCERNED WHEN BLINDED BATS
AVOID OBSTACLES WHILE ON THE WING?

Although the present problem is primarily a morphological one, yet a discussion of the function of the integumentary sensory structures described may not be out of place here. Of especial interest is the question of the means by which blinded bats avoid obstacles while on the wing. A number of investigators, experimenting with living bats, have maintained that the organ concerned in these delicate reactions is the skin. A brief summary of their opinions follows.

As already noted, Cuvier thought the flying bat, on approaching an object, sets up air currents, which react on the patagium, enabling the animal to avoid the obstacle.

Jobert ('72) observed that on pinching the skin, the animals responded faintly as compared with their vigorous reactions when hairs were pulled out. This led him to think that the sensitiveness of the flying membrane is due to the hairs. He inferred that currents of air affect the hairs and that each movement of the lat-

ter is transferred to the nerve ring in such a way that objects are perceived and avoided.

In 1873, Redtel liberated a blinded bat in a room in which had been placed numerous threads. The animal avoided the threads successfully. From the abundance of nerves found by Schöbl in the flying membrane, and from his own experiments, Redtel inferred that it was possible for bats to perceive the slightest change of air pressure upon the wings.

Sabussow ('10), like Jobert, was of the opinion that air currents were set up between the object and the approaching blinded animal, and that by means of these currents the nerves of the hairs were affected.

A few investigators have held that the organs for the perception of the delicate stimuli, which bring about these avoiding reactions, are located in the internal ear.

Jurin ('98), experimenting upon living bats, observed that when their organs of hearing were destroyed, they were unable to avoid obstacles placed in their way. A mutilation so severe as this, however, would certainly produce shock effects which might affect very considerably the results of the experiments.

Hahn ('08) caused a large number of mutilated bats (blinded, ears cropped, etc.) to make a given number of flights in an enclosure through which numerous wires had been stretched. He agreed with Jurin that objects are perceived by the flying animals chiefly through sense organs located in the internal ear. The evidence for this was obtained by closing the external auditory meatus with plaster of Paris, whereupon he found that the percentage of 'hits' (collisions with wires) was much higher for this experiment than for that of any of the others. The fact should not be overlooked that the placing of a hard substance like the one used in the meatus, and possibly against the sensitive tympanic membrane, is likely to interfere seriously with the normal functioning of the nervous system.

It is thus seen that the weight of evidence favors the view that condensations (pressures) of the atmosphere set up between the obstacle and the blinded bat stimulate sensory structures in the integument.

The question of what integumentary organs are concerned naturally arises. Organs capable of being stimulated by such condensations would have to meet certain requirements: (1) They must be distributed over the head and flying membranes at least, as these parts are foremost in flight; (2) They must be superficially located, for stimulations from air condensations are doubtless very slight.

While no special nerve structures which appear to form the sole basis for the perception of air pressures have been observed by me, yet the presence of large numbers of free nerve terminations (end-knobs) near the surface of the epidermis seems significant. These structures comply with both of the requirements set forth. They are widely distributed over the body and membranes, and their superficial position among the outermost cells of the stratum Malpighii makes them especially well placed for the reception of light touch stimuli. Their number in the epidermis is enormous.

The superficial nerve rings (and their terminal fibers), though not located as near the surface of the integument as the nerve end-knobs, are so situated about the necks of the follicles as to be affected by even the slightest movements of the hairs. These nervous structures are also widely distributed over the skin and their position is somewhat superficial. Von Frey ('96), in his researches on the sense of pressure in man, has shown that pressure nerve fibrils terminate in a ring surrounding the hair follicle, this form of termination serving as an end-organ. This writer states that on account of the position of the ring, the fibrils are stimulated by any pressure exerted upon the hair. The other nerve endings on hairs of bats are farther from the surface, so that movements of the hair sufficient to stimulate them would probably have to be more pronounced than those produced by condensations of the atmosphere.

An examination of the anatomical evidence thus indicates that two types of sensory end-organs in the skin of Chiroptera meet the requirements mentioned for the perception of air pressures. These are the free nerve terminations, and the superficial nerve rings of hairs.

End-bulbs and terminal corpuscles no doubt are tactile in function, but their depth below the surface of the epidermis precludes any probability that they aid in the perception of condensation pressures of the air.

In connection with the question as to which of the two sensory endings mentioned above functions to the greater extent in the perception of atmospheric pressures, it should be pointed out that the area of the integument supplied by superficial nerve rings is insignificant in comparison with the area supplied with nerve end-knobs. Likewise, the number of terminal fibers of the rings is not to be compared with the enormous number of end-knobs in the epidermis.

As is well known, the human cornea is very sensitive to delicate tactile stimuli. Cohnheim ('67) has shown that the only type of perceptor to be found in the cornea is that of free nerve terminations.

Goldscheider, in 1886, determined by experimentation the location of tactile spots on his arm, and then removed for study pieces of skin containing them. Here, also, the only sensory structures revealed by a histological examination were free nerve terminations.

The evidence thus leads to the conclusion that free nerve terminations are more important in the tactile reaction than are the superficial nerve rings of hairs.

It is not, of course, to be inferred that all the free end-knobs function alone as pressure perceptors, for, as is well known, the sensory nerves of the human skin mediate at least four different qualities of sensations, namely, pressure, warmth, cold and pain. But the number of nerve end-knobs in the skin is so great, and the latter in the bat is so sensitive to delicate tactile stimuli, that the number of free nerve terminations in the epidermis functioning as pressure perceptors must necessarily be very large.

To sum up, then: The writer is inclined to think that the most reasonable explanation of the avoidance of obstacles by blinded bats involves the assumption that condensations of the atmosphere are set up between the object and the approaching bat, and

that these condensations are perceived by the blinded animals chiefly by means of the free nerve end-knobs in the epidermis, but also in part by the superficial nerve rings of the hair follicles. As the flight of these animals, when close to objects, is tolerably slow, it seems probable that such sensory impulses could be transmitted to the central nervous system, and motor ones be carried back to the muscles of the wings in time for the bats to avoid obstructions in their way.

SUMMARY

GENERAL STRUCTURE OF THE INTEGUMENT

1. The integument of Chiroptera has a general covering of hair, although the soles of the feet, the mammae, the external genitalia, and the distal parts of the ears and of the flying and interfemoral membranes are almost naked.

2. The skin consists of epidermis and corium. The epidermis is made up of a well developed stratum corneum (whose deepest layers, the stratum lucidum, can be seen distinctly only in the palmer and plantar regions) and of a Malpighian stratum. In the integument of the body the Malpighian stratum contains the three layers commonly found in the mammalian skin, while this stratum in the membranes consists at most of but two layers, and frequently of but one. The corium is composed of an external stratum papillare, containing both simple and compound papillae, and of an internal stratum reticulare.

3. Pigment granules are abundant in the Malpighian stratum, while in the stratum corneum they are much less numerous. In the flying and interfemoral membranes more pigment is present in the dorsal than in the ventral duplicature of the epidermis. Isolated pigment cells are of frequent occurrence throughout the corium.

NERVE LAYERS OF THE INTEGUMENT

1. In the integument and subcutis of the body three layers of nerves are found. The first (most internal) layer consists of medullated trunks in the subcutaneous tissue. By dichotomous branching these nerves break up into a loosely intertwined meshwork, consisting of an enormous number of medullated nerves,

and forming the second nerve layer. Arising from the latter are medullated fibers which pass to the stratum Malpighii. Here they divide, forming a simple, non-medullated network, which constitutes the third nerve layer.

2. Certain regions of the flying and interfemoral membranes have three layers of nerves, others but two. These are (1) A layer of medullated nerve trunks with numerous medullated branches, occurring in the stratum reticulare, but only in the elongated ridges containing the largest blood vessels and much connective tissue; (2) A double, medullated nerve layer in the deeper part of the corium extending throughout the membranes; (3) a layer, likewise double, present in the entire Malpighian stratum, and consisting of a large number of branching non-medullated nerve fibrils.

3. Numerous varicosities are found in the corium on branches from the second nerve layer.

NERVE ENDINGS IN THE INTEGUMENT

1. Free nerve terminations occur in the Malpighian layer. Small medullated fibers from the third nerve layer can be traced out among the deeper Malpighian cells to the stratum granulosum, where they terminate in minute end-knobs, probably intercellularly.

2. Nerve fibers supplying the hair follicles may be distributed in two ways: (a) The whole fiber may end directly in a single follicle; (b) On approaching hairs a fiber may divide, one or two branches going to a follicle and the others passing out to the epidermis.

3. Nerves end on pelage hairs at three levels and in three different sheaths of the follicles. These endings are: (1) A superficial nerve ring situated above the orifices of the sebaceous glands, and giving off nerve threads in the connective tissue sheath; (2) fine, varicose or flattened nerve fibrils which lie immediately below the sebaceous glands, and terminate on the hyaline membrane parallel to the long axis of the hair; (3) Nerve fibrils at the level of the lower third of the follicle, which usually take a horizontal position in the outer root sheath.

4. Two types of special sensory end-organs are found in the skin: (1) A small elongate *end-bulb* into which a single medullated nerve fiber enters, passes approximately to the opposite end, and terminates in a slight enlargement; (2) A large, round, cellular *terminal corpuscle* innervated by a single fiber whose branches disappear among the cells of the organ.

5. Terminal varicosities are abundant in the region of the hairs outside of the sheaths of the follicles.

6. In the skin of the face, especially, striated muscles are well developed. Motor end-plates occur on these muscles. In the integument the end-plates appear to be beneath the sarcolemma, and in the muscles of the tongue these plates are clearly below the sarcolemma.

7. Small fibers resembling sympathetic post-ganglionic neurites extend hoop-like around the large modified sweat glands external to the longitudinally arranged smooth muscle fibers.

8. Blinded bats, when on the wing, probably perceive obstacles through the sense of touch by the effect of condensations of the atmosphere (produced on approaching the object) upon the free nerve terminations in the epidermis and the superficial nerve rings of the hair follicles.

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EXPLANATION OF FIGURES

All figures are from bat integument except figure 12, which is from bat's tongue. All, with the exception of figures 2 and 3, were drawn with the aid of the camera lucida. Figures 2 and 3 were drawn with the aid of the Spencer projection apparatus. The magnifications follow the descriptions of the figures.

PLATE 1

EXPLANATION OF FIGURES

1 Part of a transverse section of the skin of the face; *pg*, pigment granules; *mal*, stratum Malpighii; *sc*, stratum corneum. Fixed in corrosive-acetic, and stained in hematoxylin and eosin. $\times 375$.

2 Portion of transverse section of integument at base of thumb. *co*, corium; *mal*, stratum Malpighii; *pc*, pigment cell; *sc*, stratum corneum; *sl*, stratum lucidum. Fixed in corrosive-acetic, and stained in hematoxylin and eosin. $\times 250$.

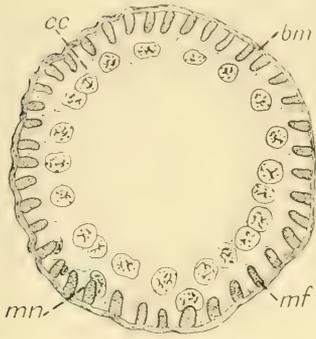
3 Transverse section of modified sweat gland from wing membrane; *bm*, basement membrane; *cc*, columnar cells; *mf*, smooth muscle fibers; *mn*, nucleus of smooth muscle fiber. Stained intra vitam with methylene blue, fixed in ammonium molybdate, and counter-stained with Mayer's carmalum. $\times 750$.

4 Part of a transverse section of skin of face; *ep*, epidermis; *hf*, hair follicle; *sgl*, sebaceous gland; *sul*, second nerve layer; *tnl*, third nerve layer. Stained intra vitam with methylene blue, and fixed in ammonium molybdate. $\times 375$.

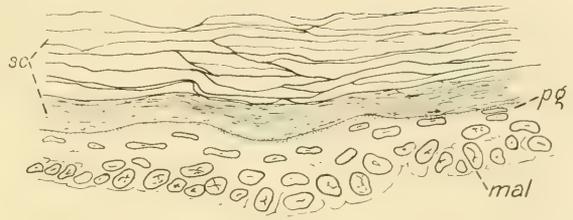
INNERVATION OF INTEGUMENT OF CHIROPTERA

JAMES EDWARD ACKERT

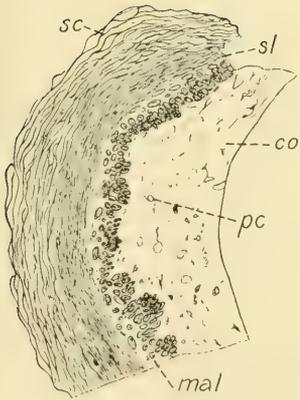
PLATE I



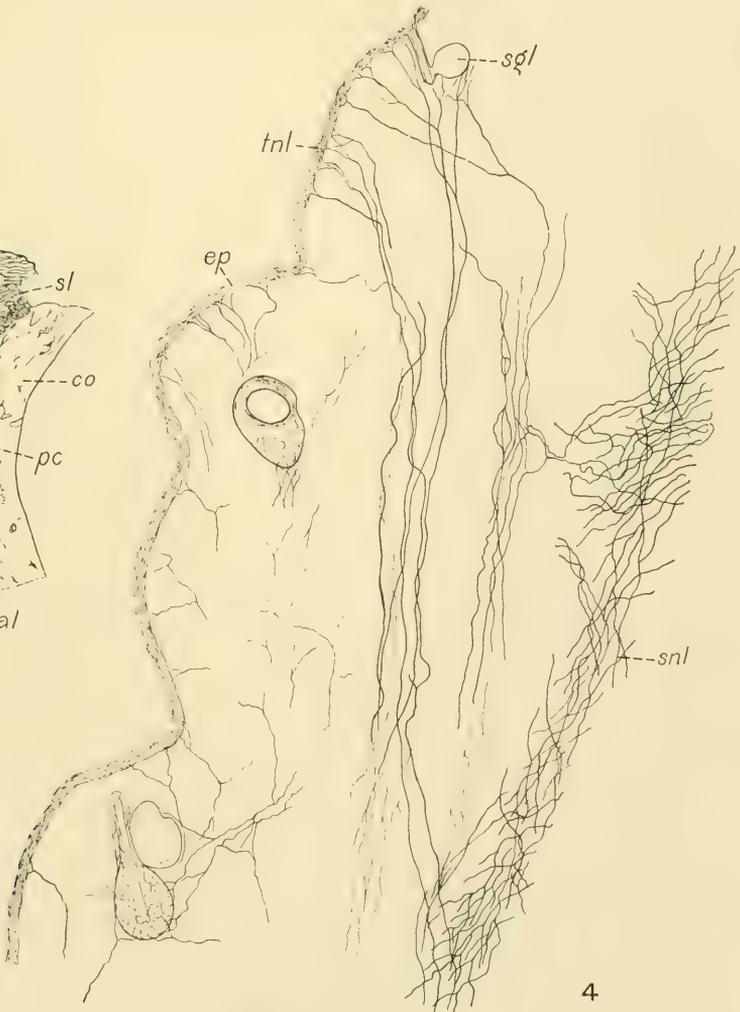
3



1



2



4

PLATE 2

EXPLANATION OF FIGURES

6, 7 Portions of epidermis of interfemoral membrane; *e*, end-knob (free nerve termination); *es*, end-knob on surface of cell; *n*, nerve of third layer; *sgr*, cell of stratum granulosum; *x*, terminal fiber without end-knob. Stained intra vitam with methylene blue, and fixed in ammonium molybdate. $\times 575$.

10 Portion of modified sweat gland from interfemoral membrane; *fi*, hoop-like fibril resembling sympathetic nerve fibril; *mu*, smooth muscle fiber; *no*, non-medullated nerve. Stained intra vitam with methylene blue, and fixed in ammonium molybdate. $\times 575$.

11 Striated muscle from upper lip; *ea*, end arborization; *mag*, matrix of motor end-plate; *mu*, striated muscle fiber; *nu*, nucleus; *nv*, motor nerve fiber; *sa*, sarcolemma. Stained intra vitam with methylene blue, and fixed in ammonium molybdate. $\times 1500$.

12 Transverse sections of striated muscles of tongue; *ea*, end arborization; *el*, elevation where axis cylinder pierces the sarcolemma; *ma*, matrix of motor end-plate without granules; *mep*, motor end-plate; *sa*, sarcolemma. Stained intra vitam with methylene blue, and fixed in ammonium molybdate. $\times 1500$.

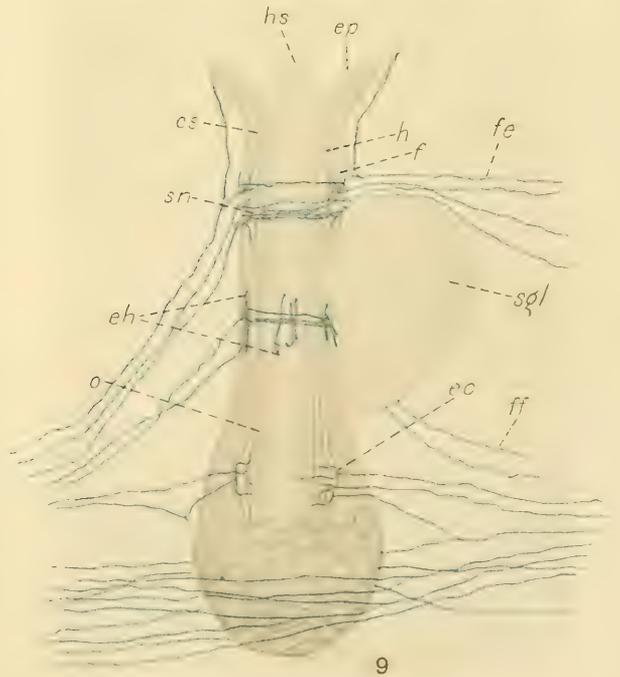
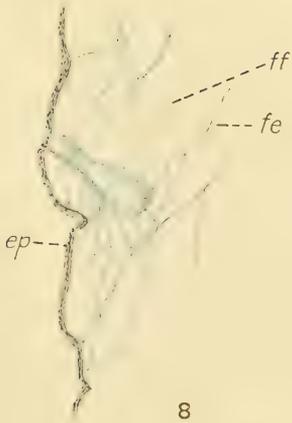
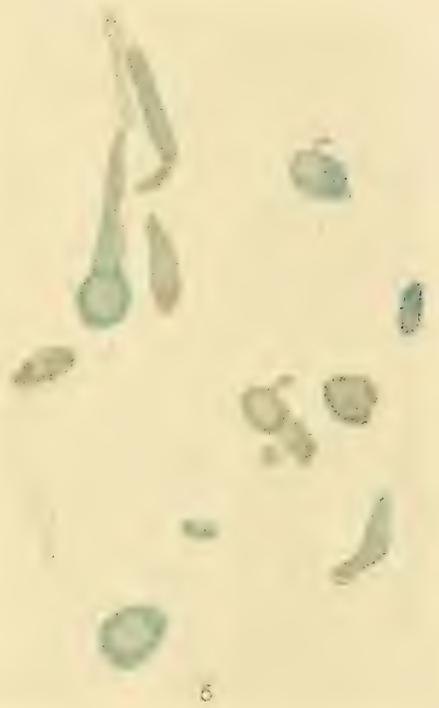


PLATE 3

EXPLANATION OF FIGURES

5 Pigment cells in the corium of skin of face; *a*, cell with granules unstained; *b*, cell containing both stained and unstained granules; *c*, cell with granules stained. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 750$.

8 Portion of transverse section of skin of back; *ep*, epidermis; *fe*, nerve fiber giving one branch to hair follicle and one to epidermis; *ff*, whole fiber ending in hair follicle. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 166$.

9 Longitudinal section of hair follicle; drawn from a methylene blue preparation, but some features of the hair sheaths added from other preparations; *cs*, connective tissue sheath; *eh*, nerve endings on hyaline membrane; *eo*, nerve endings in outer root sheath; *f*, fibrils from superficial nerve ring; *fe*, nerve fiber giving one branch to hair follicle and one to epidermis; *ff*, whole fiber ending in single follicle; *h*, hyaline membrane; *hs*, hair shaft; *o*, outer root sheath; *sgl*, sebaceous gland; *sn*, superficial nerve ring. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. Other features from preparations fixed in Zenker's fluid, and stained in hematoxylin and eosin. $\times 750$.

13 Portion of transverse section of wing membrane showing varicosity on fiber terminating in epidermis; *mal*, stratum Malpighii; *sc*, stratum corneum; *va*, varicosity. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 1500$.

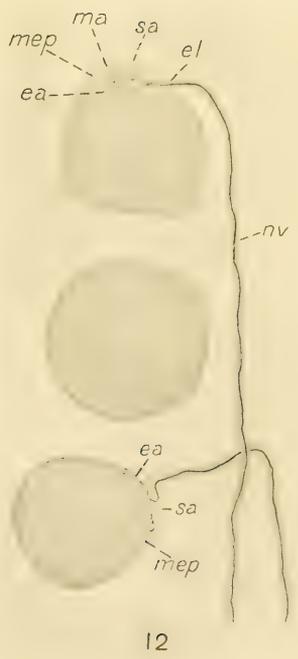
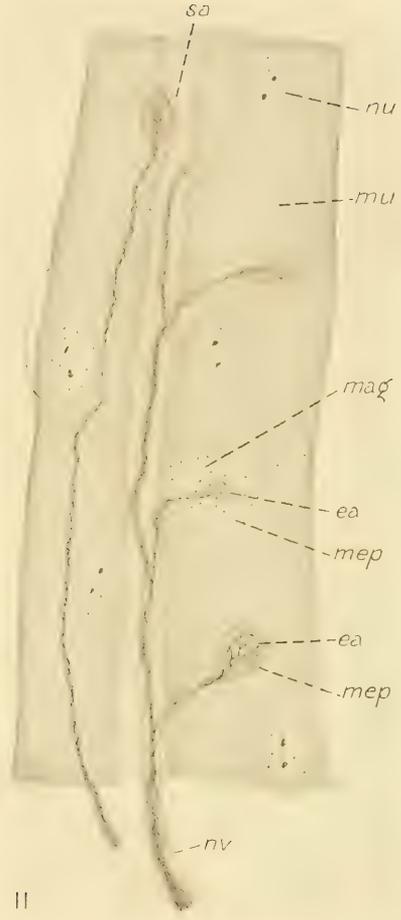
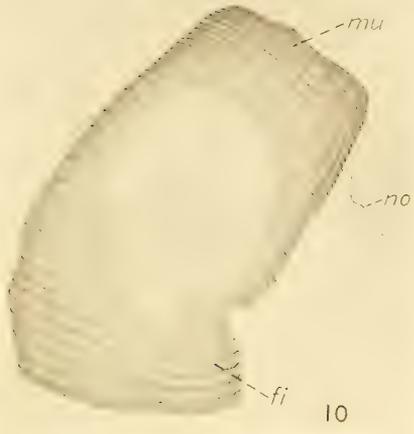


PLATE 4

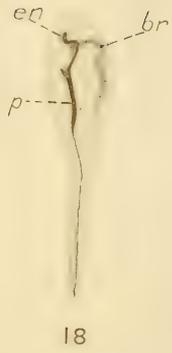
EXPLANATION OF FIGURES

14, 15, 16, 17 Varicosities on nerve fibers in skin of face. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 1500$.

18 End-bulb from upper lip; *br*, distal branch of axis cylinder; *en*, enlarged ending of axis cylinder; *p*, plate (expanded axis cylinder). Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 1500$.

19, 20 Terminal corpuscles in skin of back; *nu*, nucleus. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 750$.

21 Section of nerve trunk from interfermoral membrane; *ax*, axis cylinder. Stained *intra vitam* with methylene blue, and fixed in ammonium molybdate. $\times 166$.



THE PRONEPHRIC DUCT IN ELASMOBRANCHS

GEORGE A. BATES

From the Tufts College Medical School

SIXTY-ONE FIGURES (FIVE PLATES)

HISTORICAL RÉSUMÉ

The development of the pronephric duct in elasmobranchs has been studied by a number of observers, with very different conclusions as to its origin. These differences have arisen, in part, from the difficulty of demonstrating cell boundaries and limiting membranes in embryos preserved by the usual methods and, in part, from the difficulty of interpreting oblique sections. The theory that the vertebrates stand in phylogenetic relations with the annelids and the consequent attempt to homologize the nephridial system of the two is, in a measure, responsible for the discussion and the resulting conflict of opinions. The question in controversy is: Whether the duct takes its origin in whole or in part from the mesoderm, or whether it arises from or is contributed to by the ectoderm. At first there seems to have been no hesitancy in pronouncing the origin to be entirely mesodermic, and all the early papers (Semper, et al) so described it.

In 1888 Johannes Rückert ('88) stated that "the duct is developed (in the group) from the ectoderm." Van Wijhe ('89) claimed to have found evidence for the same conclusion. Several papers followed in rapid succession describing similar conditions in other groups; one author comparing the growth of the duct to that of the lateral line nerve which, he claimed, is developed from the ectoderm in the same way as the pronephric duct. The question raised by such comparison has been answered by Harrison ('03, '10). Within more recent years the statements have been very conflicting, some stating that it is, at least in part, ectodermal

in origin, while others claim that the cells concerned in its development arise wholly from the mesoderm.

It has been generally assumed that in the phylogenetic history of vertebrates the pronephric duct once opened directly to the exterior through the ectoderm, which assumption had its origin in the theory of the phylogenetic relationship of annelid and vertebrate. This is presented very clearly by Haddon ('87) and also in his "Introduction to the study of embryology" ('87, p. 239). In these publications he recapitulates the discussion from the time ('75), when Hensen determined for the rabbit that the pronephric duct is of ectodermal origin, to Perényi ('87) who claimed the same thing for the frog.

He quotes Spee ('84) for the guinea-pig and Flemming ('86) as confirming Hensen for the rabbit; also Van Wijhe ('86) as saying that the duct arises from the ectoderm in the thornback ray (*Raja clavata*). He says: "The origin of the segmental duct from the epiblast being now known to occur in Elasmobranchs, Anura, Lacertilia and Rodents we are justified in assuming that this is the general fact." Upon this assumption he proceeds to present an hypothesis by which he homologizes the pronephric duct of vertebrates with the nephridia of the chaetopod worms. He says: "There can be little doubt that the segmental duct arises from the epiblast. This discovery will necessarily lead to a modification in our views concerning the morphology of the vertebrate excretory organs." He quotes Hatschek as having described a single nephridium in *Amphioxus* which is in all respects comparable with a vermian nephridium and then makes the following statement:

We have, then, only to assume that a pair of similar vermian nephridia occurred in each body segment of the ancestral vertebrate, and that the nephridia of each side of the body opened externally into a lateral groove [which he has previously described as developed in the epiblast much as the neural tube is developed]. It would further only be necessary for the groove to deepen and next to form a canal (just as does the neural groove) to bring about the vertebrate arrangement. Thus, in vertebrates, as in invertebrates, the nephridia open by epiblastic pores, but in the former the area into which they open is precociously converted into a canal which subsequently acquires a secondary opening to the ex-

terior through the cloaca. On this hypothesis the nephridia of vertebrates always open by their original epiblastic pores, primitively directly to the exterior, secondarily into a canal separated from the epiblast.

Hertwig ('92, pp. 358-9) states the case very clearly and brings the discussion down to the date of his own publications. He speaks of the view entertained by almost all investigators that "the duct arises from the mesoderm and grows by proliferation of its own cells as far as the hind gut (proctodaeum) and thus was constricted off from neither the outer nor the middle germ-layers nor yet derived from them cell-material for its increase." He claims, however, that this interpretation has become untenable, quoting various authors to substantiate the fact that, in several classes of vertebrates, the duct in process of growth is in close union with the outer germ-layer and that it is prolonged backward by means of cell-proliferation in that layer, while in front it is being constricted off from that layer or, as he terms it, the parent-tissue. He further states that "the pronephric duct grows at the expense of the outer germ-layer and moves, as it were, along the latter, with its terminal opening behind, as far as the hind gut." This interpretation led, later, to the assumption by various authors of the view that not only the pronephric duct but the entire urinary system was derived from the ectoderm (Hertwig '92, p. 358).

Hertwig, later, shows that such views cannot be made to harmonize entirely with conditions found in the lower vertebrates and, making allowance for all observations, he summarizes the subject thus: "The pronephros is developed from the middle germ plate, and that then its posterior end comes into union with the outer germ-layer and in conjunction with the latter grows farther backward as the pronephric duct." He quotes Van Wijhe and Rückert in support of this explanation and then interprets the pronephric duct "at its first appearance as a short canal-like perforation of the wall of the body, which begins in the body-cavity with one or several ostia and opens out upon the skin by a single orifice. Originally the outer and inner openings lay near together, later they moved so far apart that the outer opening of the canal united with the hind gut."

It will be seen that these statements present the two phases of the problem, i.e., the origin of the duct from the mesoderm and the contribution of the ectoderm to its development. The phylogenetic theoretical consideration is also suggested.

Among the many observers who have discussed the development of the pronephric duct may be mentioned Semper ('74, '75), Balfour ('78), Spee ('84), Beard ('87), Haddon ('87), Rückert ('88), Van Wijhe ('86, '88, '89), Mitsukuri ('88), Rabl ('96), Gregory ('98, '00). Of these Rückert, Van Wijhe and Rabl have entered actively into the controversy, the two former being advocates of the theory of ectodermic origin or contribution to the duct, and the latter directly and radically opposed and an ardent supporter of the theory of its exclusive mesodermic origin.

Rückert ('88) seems to have taken the initiative in his very radical position, stating "that the duct is developed wholly from the ectoderm." In his figure 36, plate 16, he shows three mitotic figures at the point of union of the ectoderm and anlage of the duct. He states that two layers, ectoderm and anlagen, are fused together at the point of contact, making the thickness of the wall much greater, and this, in conjunction with the presence of the numerous mitotic figures in this region, gives much reason for his contention. He says: "Die auffallend zahlreichen Mitosen, welche sich stets in dieser Region des Ectoblast finden, geben einen weiteren Beleg für diese Auffassung. Ein in dieser Hinsicht ziemlich prägnanter Schnitt (Horizontal-schnitt) durch das hintere Ende des Vornierenganges ist in Fig. 36 dargestellt."

Van Wijhe claims that the ectoderm contributes to the structure of the duct, and compares its growth to that of the nerve of the lateral line organ which, according to his interpretation, is developed in part at least from cells contributed by the ectoderm. He admits the possibility of the duct growing by the division of its own cells, but considers it improbable, his general conclusion being that the ectoderm is in considerable measure responsible for its growth. He says:

Was nun seine Abstammung betrifft, so betheiligt sich das Ektoderm sicher an seiner Bildung, indem er in ähnlicher Weise wie die Nerven der Seitenorgane weiterwächst. Ebensowenig wie bei diesen Nerven möchte ich eine ausschliessliche Abstammung von der Haut behaupten,

da die Möglichkeit nicht ausgeschlossen ist, dass Zellen des Pronephros unter fortgesetzten Theilungen den Gang in seiner ganzen Länge mitbilden helfen. Doch kommt mir Letzteres nicht wahrscheinlich vor.

He quotes Balfour ('87, p. 127). Here Balfour describes the pronephros as

. . . . arising as a solid knob from the somatic layer of the mesoblast and growing outward toward the epiblast. The knob consists of from 20 to 30 cells agreeing in character with the neighboring cells of the intermediate cell-mass and are, at this period, rounded. It is mainly, if not entirely, derived from the somatic layer of the mesoblast. From this knob there grows backward a solid rod of cells which keeps in very close contact with the epiblast, and rapidly diminishes in size toward its posterior extremity. Its hindermost part consists in section of, at most, one or two cells. It keeps so close to the epiblast that it might be supposed to be derived from that layer were it not for the sections showing its origin from the knob above mentioned. We have in this rod the commencement of what I have called the segmental duct.

Van Wijhe, commenting on Balfour's statement, thinks that Balfour was working with the technical methods of his time, which were defective. The solid knob was the pronephros and did not arise from the middle, but from the lateral plate, and he conjectures that, because Balfour interpreted the knob as consisting of from 20 to 30 cells, he must have drawn his conclusions from a single section instead of from a series, as he himself had done. He states that his series cut the knob into 23 sections. He further states that the close contact of the duct with the epiblast, spoken of by Balfour, is not a mere touching or contact, but an actual fusion and quotes Beard, Rabl and Rückert as substantiating his statement. He quotes Rückert as having seen an invagination of the ectoderm on the duct and claims to have observed, in exceptional cases, the same thing. He states that the contribution of cells by the ectoderm to the structure of the duct is made certain through the occurrence of a mitotic figure in one of his sections, in which one daughter cell was situated in the skin and the other in the duct. He says: "Die Betheiligung des Ectoderms an der Bildung des Ganges wird sicher gestellt durch Kerntheilungsfiguren, bei welchen der eine Tochterkern in der Haut, der andere in der Anlage des Ganges liegt. Dies zeigt Fig. 5b, welche einem Schnitt durch einem Embryo von *Scyllium catulus* mit 37 Somiten entnommen ist."

OBSERVATIONS

The object of the present paper is to present what the writer believes to be conclusive evidence of the development of the pronephric duct, from its origin in the pronephros to its union with the cloaca, from cells derived originally from the mesoderm. No attempt has been made to describe the development of the pronephros, except in the merest outline which may be of use in introducing the account of the development of the pronephric duct itself.

The investigation was undertaken at the Harpswell Laboratory at South Harpswell, Maine, at the suggestion of Dr. H. V. Neal, who also kindly furnished the material, ready prepared, and to whom I gratefully render my thanks, not only for the material but for the kind advice and helpful criticisms. The material used was *Acanthias* embryos prepared by the vom Rath picro-osmoplatic method. This renders cell outlines and limiting membranes very distinct, and makes it possible to differentiate between different cells and cell layers. In the writer's opinion, it is the sharp differentiation of cell outlines that has made it possible to distinguish with certainty, in all cases, the layer to which any individual cell belongs.

The pronephros arises from the middle plate, or mesomere, of a number of body somites—in *Acanthias*, six—by a proliferation of cells from the somatic wall. These proliferations soon fuse and the pronephric ridge (*Vornierenwulst*) thus formed extends laterally until it comes into contact with the ectoderm. At the line of contact the cells of the outer surface of the ridge form a cord, which later acquires a lumen and becomes converted into the pronephric duct. The cord of cells which is the anlage of the duct, rapidly grows caudally from the pronephric body, between the somites and the ectoderm, until it reaches the cloaca.

During the process of backward growth the tip or growing end of the anlagal duct is at first entirely free from the ectoderm. As, however, the line of growth is obliquely backward and outward, it almost immediately comes into contact with the adjacent ectoderm. The relation of the two structures, duct and ectoderm, is

very close at the point of contact, but as the former elongates, its more proximal portion gradually separates from the latter and lies free in the space between the ectoderm and the somites. It is only at the point where the growing tip of the duct impinges upon the ectoderm, if anywhere, that any contribution by the ectoderm to its growth can take place; and it is here that the various observers claim to have found evidence of such contribution.

In an embryo *Acanthias* measuring 4.5 mm., corresponding nearly to No. 18 of Scammon's *Normentafeln*, the development of the pronephros is well advanced. The growth of the pronephric anlage toward the ectoderm is very irregular. The ridge does not extend outward with an even margin but seems to be thrust out in processes, sometimes massive and again in long, slender, pointed projections, as seen in figures 6, 7a and b, and 8. When the ridge comes into contact with the ectoderm it does not fuse with it but is merely pressed against it. This fact is demonstrated in figure 6, where the mass has been pulled away from the outer layer in the process of preparation, leaving the limiting membrane intact, but leaving its impress upon the ectoderm. The ectoderm at this stage is a one-celled layer and, while the pronephric anlage has made its impression upon it and it has become thinned out, yet it remains intact, as will be seen by its unbroken, limiting membrane. Dr. Gregory ('97) figures several instances where the separation of the two layers has occurred. She thinks that cells from the ectoderm have been torn away with the pronephric anlage. I have examined her figures and am convinced, by my own observations in similar cases, that the ectoderm is intact and that the mistake lay in her inability to follow cell outlines and limiting membranes by the method of preparation employed. When the pronephric anlage has finally reached the ectoderm the outer cells arrange themselves so that the long axis of the nuclei coincide with the long axis of the anlage. This arrangement is preparatory to the formation of the anterior portion of the anlage of the pronephric duct which extends the entire length of the pronephric body on its outer margin. At its caudal extremity the pronephric body is prolonged into the space between

the ectoderm and somites. At first free, as seen in figure 9, it later impinges upon the ectoderm, as shown in figures 1, 2 and 3, drawn from sections of an embryo of 5 mm., corresponding closely to No. 19 of Scammon's *Normentafeln*. The growing tip of the anlage of the duct extends along the ectoderm in close contact with that layer, and in this stage has reached a length corresponding to that of three somites. Within the pronephric body are numerous cells in mitosis and others are found also in the growing duct. In figure 1 the cavity of the somite (nephrocoele) has begun to bend toward the anlage of the duct, marking the beginning of the first pronephric tubule. The relation of the ectoderm to the anlage of the duct is very clearly shown in figure 3, which is drawn under a one-twelfth oil immersion objective in order to bring out definitely the existence of a limiting membrane between the two layers. Figures 1, 2 and 3 represent consecutive sections, figure 3 showing the growing tip and its relations to the somites as well as to the ectodermic layer. They show the extent of the growth of the anlage at this stage and demonstrate its mesodermic origin and also the fact that the contribution of cells from the ectoderm at this early period, when the anlage has just come into contact with it, is improbable, because a limiting membrane is present between them. This will be made still more convincingly manifest by sections to be presented in subsequent figures.

A cross-section of an embryo of 4 mm. (*Normentafeln* No. 16) illustrates Balfour's description of the beginning of the development of the pronephros from the middle plate. Van Wijhe, in his discussion of Balfour's account, states that the solid knob described by Balfour formed a part of the pronephros as demonstrated by his series of sections. It will also be recalled that the segmental duct of Balfour came into close contact with the ectoderm, and, but for its origin in the mesoderm, might be interpreted as being derived from it (the ectoderm). Van Wijhe claimed that this close contact was an actual fusion. It seems to the writer that the sections represented in figures 1, 3, 4 and 5 settle the question in favor of Balfour's interpretation. The demonstration of the limiting membrane between the growing tip of the anlage of

the duct and the ectoderm, at the point of contact, shows beyond question the true relation between the two layers.

Figure 9 gives a comprehensive view of the entire embryo at the time when the anlage of the duct has a length of about three somites on one side and a little more than two on the other, showing the asymmetrical growth of the two sides and also the entire independence of the anlage at its first appearance. It will immediately come into contact with the ectoderm as shown in figure 3. It also shows the relation of the pronephros to the ectoderm. The appearance of the growing tip and its relation to the ectoderm present some peculiar and characteristic features at the point of contact. The ectoderm becomes thinner, apparently by the drawing out or extension of its cells. This modification of the ectoderm is very pronounced in many places, but particularly at the point where the growing tip of the duct impinges upon it. In many instances, where the two layers are closely attached, the ectoderm becomes very thin, while the extreme point of the anlage is reduced to a few cells, usually to only a single cell in diameter, but there is always to be distinguished a limiting membrane between the two. If this were not so the structure would present the appearance of thickening, as described by Rückert, and also, but for the limiting membrane, one would conclude that the thinning of the ectoderm was caused by the splitting off of cells to be contributed to the growing tip of the duct.

The peculiar modification of the ectoderm seems to be the result of some influence, probably pressure, exerted by the growing tip of the duct. It often happens that in the process of preparation the two structures are separated, and then an indentation is left on the ectoderm where the anlage has pressed against it. Many instances of this kind, as well as cases where the same condition exists with the structures still intact, are clearly displayed in the series of cross sections. It will be observed, however, that no matter how close the contact may be, there is always to be demonstrated a limiting membrane separating the duct from the ectoderm. It will readily be appreciated that any method of preparation which obliterated cytoplasmic outlines and made limiting membranes invisible would show the two layers as a single mass, or

even a syncytium, and the location of the line of separation would be impossible.

It is obvious that if there be any contribution to the duct from the ectoderm it must take place at the point where the two layers come into contact, which is at the growing tip of the former. In order to cover this phase of the problem several series of cross sections have been drawn. The first series is from an embryo of 5 mm. and shows the terminal cell at figure 10. The section has cut through the cell at its extreme edge and left only a fragment of the cytoplasm adhering to the ectoderm. The fragment is composed of clear protoplasm and is quite thick, as may be seen from the fact that the next section has not cut entirely through it. This clear cytoplasm seems to be a constant feature in the structure of these cells and, in many instances, appears to be extra material forming the medium of attachment between the tip of the duct and the ectoderm. The next section has cut across the cell, including the nucleus.

Two features are especially striking in this series; first, the peculiar mode of attachment of the anlagen cells to the ectoderm by means of the clear cytoplasm above-mentioned, as observed in sections 10, 12, and 13, and, second, the thinning out of the ectoderm. In figures 10, 11 and 12 the contact of the anlage seems to have exerted no possible influence, as there is considerable space between the two layers.

As the sections begin to involve the larger portion of the anlage where several cells are included in each cross section of the duct, this space appears and disappears showing how loose the attachment between the layers is, except at the outer edge of the anlage where the clear cytoplasm seems to act as the direct medium of union. Particular attention is called to the distinctness with which the line of separation between the ectoderm and anlage is shown in the form of the limiting membrane. If the material had been prepared by any method which made this line invisible, the distinction between the two layers would have been well nigh impossible. It would have presented the appearance of a syncytium with the consequent difficulty in distinguishing to which layer the cells belonged. In order to be satisfied of the

truth of this the writer examined many series of sections fixed and stained by the most common methods (e.g., corrosive-acetic, Zenker's fluid, picro-acetic, Bouin's fluid, for fixing; and borax-carmin, Delafield's hematoxylin, alum-hematoxylin for staining), and found, in every instance that the difficulty of distinguishing cell outlines was so great that interpretation was practically impossible. Figures 16 to 24 are drawn from sections of an embryo of 6 mm., showing the terminal cell at figure 16 and the anlage of the duct entirely detached from the ectoderm at figure 24. The sections are consecutive and are intended to demonstrate the same conditions as the previous series, with the additional feature that the duct may be followed from its contact with the ectoderm behind to the point farther forward where it becomes entirely separated from that layer and lies free in the space between ectoderm and the somites. In this series the modification of the ectoderm is very conspicuous, as is also the peculiar mode of attachment by means of the cytoplasmic outgrowths from the cells of the tip of the duct. Particular attention is called to figure 22, where the ectoderm seems to be hollowed out for the reception of the anlage, and yet there is not only a limiting membrane, but also a considerable space between the two layers.

Figures 25 to 35 are from a series of cross sections of an embryo measuring 7 mm., beginning with the terminal cell at the growing tip of the duct and continuing forward until the anlage is practically free of the ectoderm. Prominent among the many interesting features in this series is the constant independence of the duct in its growth along the ectoderm. It will be noted that in the entire series there is constantly present a considerable space between ectoderm and duct. The modification of the ectoderm is a marked feature to be particularly noted in figures 28, 31 and 35, where the ectoderm is very much affected. Another feature, very clearly exhibited, is the way the anlage is attached to the ectoderm. The medium of attachment (already several times alluded to) is always the product of the mesodermic cells of the anlage and appears to be thrust out not unlike the pseudopodia of an amoeba. It seems to be the same in substance as that composing the processes thrown out by the pronephros before

that structure reaches the ectoderm which were referred to when the growth of the pronephros was described. This substance seems to fasten itself to the ectoderm and thus unites that layer with the duct. It will be remembered that Van Wijhe claimed that the relation was one of fusion, not a mere contact. The presence of this substance acting as a medium of union between the two layers would, in a way, seem to bear out his statement, although not in the sense in which he intended it; for it is entirely independent of the ectoderm, its relation to that layer being wholly secondary. It will be readily seen, however, that in places where the entire surface of contact is covered by this substance it might very easily be interpreted as a fusion, were the method of preparation such as to render the limiting membrane invisible. In some places it seems to exert tension upon the ectoderm. This is especially apparent in figure 35, where it forms a broad band and has apparently pulled the ectoderm toward itself, as if it had contracted and drawn the ectoderm out of line. The peculiar bend in the ectoderm brings the ectodermal cell, dorsal to the duct, into such relation with the latter that, but for the fact that the cell outlines and limiting membrane are so clearly manifest, the structures might be interpreted as being continuous, and the presence of a mitotic figure in the ectoderm at the point where this layer bends towards the duct would seem to present corroborative evidence of ectodermic contribution to the latter. But the independent character of the two layers is so clearly demonstrated in the section under discussion that no such inference is possible.

Figures 33, 34 and 35 present much the same conditions; except that in figure 35 the mitosis is in metaphase and the cell is cut transversely so that the axis of the spindle is in the wrong direction for the daughter cell to be contributed to the duct. This was the case in the example drawn by Rückert in his figure 36, as cited above and pointed out by Rabl ('96). In figures 33 and 34 the mitotic figures are so placed that in division they would be parallel to the surface of the ectoderm and thus, but for the clearly demonstrated limiting membrane, the possibility of ectodermic contribution might be suggested. Another feature to be noted in this, as well as in the preceding series, is the irregular manner of

the growth of the duct. This irregularity is much more marked in earlier than in later stages, and, in cases where the dividing line between it and the ectoderm is indistinguishable, would present conditions where the relative position of parts of the two layers would seem to warrant the conclusions reached by some observers. This is particularly obvious in the case of Rückert's figure 35, where the three sections present these conditions very markedly.

Figures 36 to 39 present a series of sections from an embryo of 10 mm. In this series the anlage is not so irregular in outline and the terminal cells are larger and not so closely attached to the ectoderm. It will also be observed that the anlage becomes freed from ectoderm much more abruptly, its attachment covering only four sections of 10 microns each. A change is also observable in the ectoderm; the cells being much thicker and more shortened, while the nuclei, in most cases, lie with their long diameter at right angles with that of the layer itself. It still, however, shows modification at the point of contact with the duct; at figure 36 profoundly so. Figure 36 is an example of the close contact between the ectoderm and anlage of the duct, and demonstrates how readily the conditions might be misinterpreted if the limiting membrane were invisible and the structures thus blended into a syncytium. The striking modification of the ectoderm and the relative position of the ectodermic cell dorsal to the duct might easily lead to the suggestion of ectodermic contribution. With the limiting membrane so clearly demonstrated such inference would seem to be unwarranted.

Figure 39a is a very interesting example of the close contact between the two layers. It shows a terminal cell of the duct and is very closely attached to the ectoderm. At either end of the cell the clear cytoplasm of attachment is very conspicuous. The whole cell seems, indeed, to be composed of it, the center around the nucleus being translucent. If the limiting membrane between this terminal cell and the ectoderm were invisible the distinction between the two layers, duct and ectoderm, would be impossible.

Figures 40 and 41 are drawn from sections of a 9 mm. embryo. The ectoderm has become very much thickened and the cells

shortened. The nuclei are so placed that their long diameter is at right angles to the surface. The duct seems to lie in a groove in the ectoderm which has been hollowed out for its reception. The two sections are not consecutive, but are given to illustrate the change in the character of the ectoderm and the close relation existing between it and the duct. Figure 40 is a section at the extreme end of the anlage and has cut through the growing tip at a point beyond the terminal cell into the clear cytoplasm which the cell throws out in its advance along the ectoderm. This peculiar feature of the growth of the anlage will be more clearly demonstrated in the frontal sections to be described later. It will be observed that the ectoderm seems to be grooved for the reception of the duct, and also that there is a well defined limiting membrane between the two layers. These conditions are very clearly shown in figure 41 which cuts through the anlage more anteriorly and demonstrates more definitely the groove in the ectoderm, as also the arrangement of the ectodermic cells for its formation.

In figures 42 to 47, drawn from sections of an embryo of 11 mm., the duct is much larger than in the preceding series and comes into contact with the ectoderm only at the extreme tip. The ectoderm shows less modification and its cells have become much shorter and more thickened. In some places the connection between the anlage and the ectoderm is very close, as at figures 42 and 43. This is at the extremity of the growing tip, but elsewhere the two layers are widely separated and the connection between them has been completely severed. The ectoderm shows the usual evidences of modification, although the irregularity of its contour is much less great and seems to be in less degree dependent upon the presence of and contact with the anlage.

Thus far the study has been confined largely to cross sections and the evidence presented of the origin of the duct from the pronephros and hence from the mesodermic somites, seems convincing. This receives confirmation by the study of frontal sections where comprehensive views may be had of the two structures, ectoderm and duct, and their relation more extensively

observed. Figure 48 is from a section of a 20 mm. embryo and shows the duct in contact with the cloaca. It has not yet broken through the cloacal wall.

In figure 49, from an embryo of 4.5 mm., the anlage has just begun its growth from the pronephros and is pushing its way along the ectoderm. Figure 50 is the next section where the extremity of the growing tip of the anlage is shown. These two sections illustrate very clearly the origin of the structure from the pronephric body. The terminal cell is elongated into a finger-like process composed of the clear cytoplasm, before alluded to, and seems to be feeling its way, as if it were creeping along the ectoderm. The phenomena suggest the pseudopodia of an amoeba. This feature is very strikingly brought out in the subsequent figures of frontal sections, figures 51 to 54. These also show that the irregular character of the ectodermic layer is not always due to the presence of the growing tip of the duct, as the peculiar thinning of the wall seems to occur all along its course, independent of any influence that could be exerted by the pressure of the growing terminal cell. In figures 55 and 61, the duct is very large, compared with the thickness of the ectoderm, and this disproportion seems to preclude the origin of the thicker duct from the thinner ectodermic layer.

We come now to the consideration of the significance of the presence of mitotic figures in duct and ectoderm which were thought to be of so much importance by Rückert and Van Wijhe. In his figure 36 Rückert shows a section with three mitotic figures in the region of the point of union between the ectoderm and duct. As will be recalled, he attributes much importance to the fact that the fusion of the duct with the ectoderm makes the tissue at the point of union much thicker and also lays stress upon the presence within the mass, of numerous mitotic figures. To this argument Carl Rabl long ago made what to the writer's mind is an entirely adequate answer. He claims that only one of the cells in mitosis is to be considered—the one in the ectoderm immediately contiguous to the duct. This cell is in metaphase (Mütterstern) and is so placed that one sees it in the direction

of the axis, which is parallel to the surface of the ectoderm, not perpendicular to it, as it must be, if, in its division, it is to contribute a daughter-cell to the duct. He says:

Am hinter Ende des Ganges sind hier drei Theilungsfiguren zu sehen. Diejenige, welche sich unmittelbar an den Vornierengang anschließt und auf die es daher zunächst ankommt, ist ein Mütterstern, der so gestellt ist, dass man auf ihn in der Richtung der Achse sieht; die Achse steht also parallel zum Ektoderm, nicht senkrecht, wie sie stehen müsste, wenn die Figur beweisend sein sollte. Die beiden anderen Theilungsfiguren sind, meiner Ansicht nach, irrelevant.

Rückert, in his figures 35 to 41, shows several sections which display much the same conditions as are presented in the cross sections figured in the present paper, and in the light of the writer's own observations and the repeated instances where the same relative position of duct and ectoderm have existed, it is difficult to escape the conclusion that the same interpretation is inevitable in both cases. So far as the mitotic figures are concerned it seems certain that two of them are in the duct and one in the ectoderm. All three of the cells are in metaphase, and the two in the duct are so placed that the direction of division would be such as to preclude the possibility of exchange of cells between ectoderm and duct, even though the mitosis were in the ectoderm. This is what Rabl meant, it would seem, although he considered only one of the mitotic figures.

Numerous mitotic figures are illustrated in my drawings, several of which are of particular interest. Figure 35 shows a cell in mitosis in the ectoderm, which is thinned out at one place and the duct is attached to it just above this. The attachment is by means of the peculiar clear protoplasm, before alluded to. In this case it seems to have contracted and drawn the ectoderm out of line so that the mitotic cell is deflected toward the anlage. It will be readily observed that, if the method of preparation did not make it possible to demonstrate clearly the outline of the cell and the limiting membrane between the two layers, the structures would appear to be continuous. It is true that the mitosis is in metaphase, with the axis in the wrong direction for possible contribution of a cell to the duct, but suppose the same conditions to

have existed in figures 33 and 34, might not the conclusion of such contribution have been reached? Indeed this is what did happen in the case figured by Van Wijhe. In this case, too, it seems to the writer, Rabl has given the right answer, where he claims that both parts of the dividing cell are in the anlage of the duct.

Figure 57 shows a mitosis which seems closely comparable to that figured by Van Wijhe. The section was stained with Delafield's hematoxylin and, as it originally appeared, figure 57 was very strikingly like Van Wijhe's figure. By accident it was broken apart (in the act of focusing a one-twelfth oil immersion objective upon it), and when the duct parted from the ectoderm, it left the latter intact. The drawing, figure 58, was made with the duct moved back to the ectoderm, where it so lies that the latter overlaps it, but it very clearly illustrates the fact that the two layers are entirely separate and that they were never fused, in the sense in which the term is repeatedly used; and also that contribution from the dividing cell in the ectoderm to the duct was impossible.

Figure 60 presents a similar instance. Here the terminal cell of the duct is very closely united to the ectoderm and particularly so with an ectodermic cell in mitosis. It is obvious that if the limiting membrane were not present and the structure were a syncytium, the interpretation would be very difficult and contribution of a daughter cell to the duct from the mitosis would seem more than probable. These are examples out of many instances and might be indefinitely multiplied. At figure 56 is shown a mitotic figure in the terminal cell of the duct and, at 55, in the anlage just above the terminal cell.

Other mitotic figures appear very frequently in the duct and in many instances in such positions and relation to the ectoderm as to lead to misapprehension as to their significance. One such is shown in figure 59. Here a mitotic figure occurs in the duct in such relation to the ectoderm that, but for the clearly distinguishable cell outline and the presence of a limiting membrane, it would be difficult to determine to which layer the cell belongs. Under conditions as above presented the question could not be raised.

Figure 49 shows the caudal extremity of the pronephros in an embryo of 4.5 mm., with the duct just beginning, and here, just at the junction of the pronephros and duct, is a mitotic figure. Its presence at this point and the early stage of development seemingly can have but one meaning, namely, that the anlage is growing by the division of its own cells. This is not an isolated example as may be seen by reference to the figures.

All these examples, coupled with other evidence, seems to point inevitably to the conclusion that, not only is the anlage of the duct a direct outgrowth from the pronephros, and therefore mesodermic in origin, but its subsequent growth is accomplished by the division of its own cells and it in nowise receives contribution of cells from the ectoderm.

Respecting the theoretical phase of the problem, it seems clear that the relation of the anlage of the duct is secondary and that the duct is not developed from an ectodermic groove. If the nephridia of vertebrates ever opened to the surface through ectodermic pores, and later into a canal arising from the ectoderm, ontogeny has failed to repeat phylogeny in Acanthias, for there is no evidence of such arrangement in the embryological development of this form.

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ABBREVIATIONS

<i>Ect.</i> , ectoderm	<i>Pro.du.</i> , pronephric duct, or anlagal duct
<i>G.tp.</i> , growing tip of the anlagal duct	<i>Pron.</i> , pronephros
<i>Lim.m.</i> , limiting membrane	<i>Sp.c.</i> , spinal cord, or neural tube
<i>Nt.c.</i> , notochord	

PLATE 1

EXPLANATION OF FIGURES

1 Frontal section of a 5 mm. embryo, showing the pronephros arising from six somites, the sixth participating but little in this section. The fifth curves outward toward the anlage of the pronephric duct, *a*, with which it is to unite, thus forming a complete tubule. The duct will arise on the lateral edge of the pronephric body, where the nuclei are beginning to arrange themselves in line, *b*. At the caudal end the anlage of the duct projects into the space between ectoderm and somites. The extent to which it has grown may be seen by comparing with figures 2 and 3.

2 The same parts as in figure 1, illustrating method of growth. Note the numerous mitotic figures in the pronephric anlage and one in the anlage of the duct, indicating rapid growth; ectoderm a single cell thick.

3 Growing tip of figure 1 under high power, to show its relation to the ectoderm.

4 and 5 From cross sections of a very early embryo. They correspond to some figures in Balfour's Monograph and show a very early pronephros and its origin from the middle plate.

5 Same, more enlarged, showing contact with ectoderm at *a*.

6 and 7 From embryos of 4.5 and 5 mm., illustrating the manner of growth of pronephros toward ectoderm, the pronephros having been pulled away in figure 6, leaving its impress. This separation, caused by shrinking in preparation, demonstrates the independence of the two structures. Figure 7 illustrates the manner of growth of the pronephros; the clear translucent cytoplasmic processes at *a* and *b* are very characteristic.

8 Shows some isolated cells bearing such processes

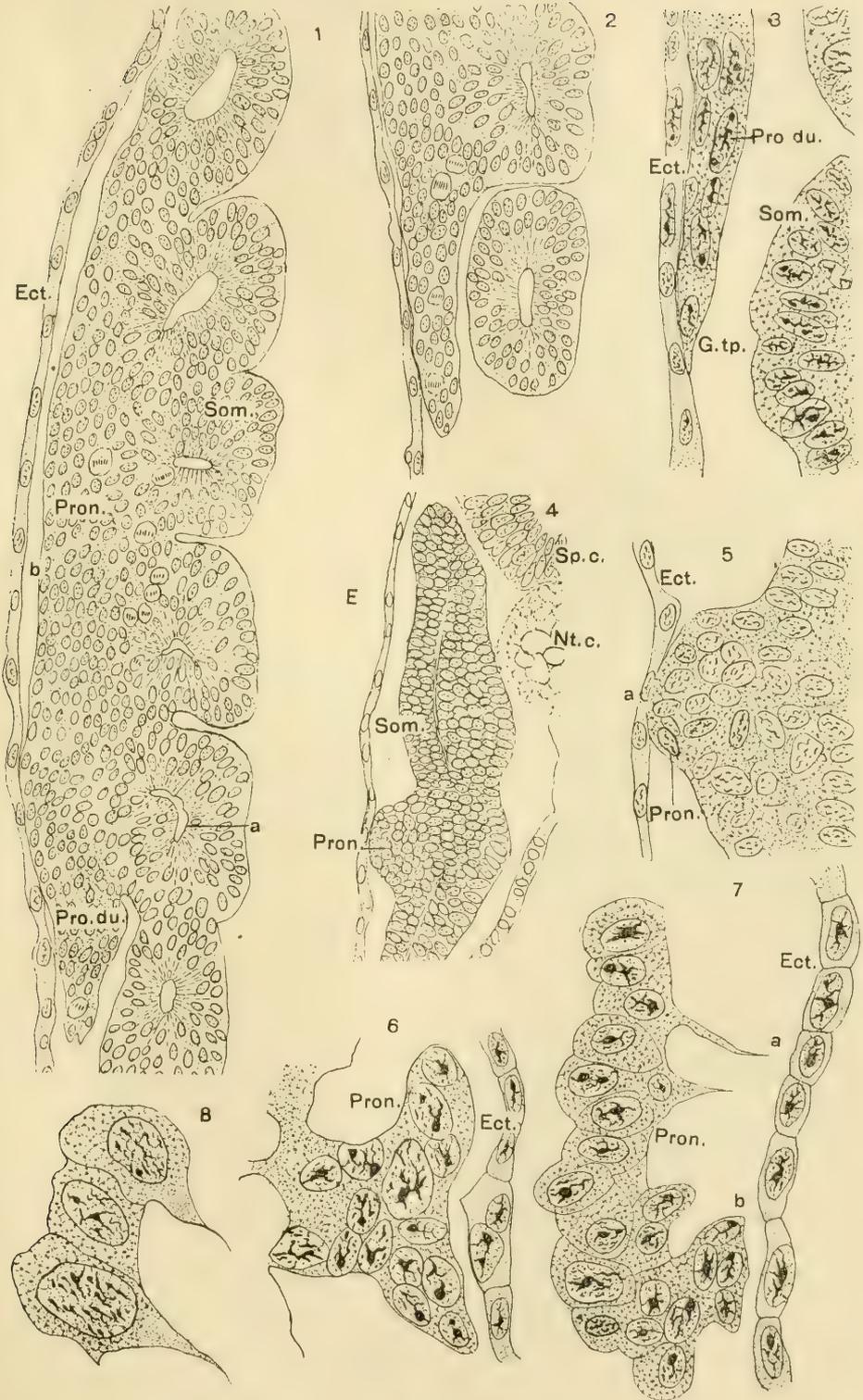


PLATE 2

EXPLANATION OF FIGURES

9 From an embryo of 5 mm., showing the anlage of the pronephric duct growing from the pronephric anlage at *a*. The ducts of the two sides are asymmetrical, one side extending over two and the other three somites. The independence of duct and ectoderm is seen at *c*. The two were in close contact in the living embryo but have separated in preparation. The drawing also shows the distance to be traversed by the duct to reach the cloaca.

10 to 15 Consecutive sections $6\ \mu$ thick, of a 5 mm. embryo. In figure 10 the duct is attached to the ectoderm by clear protoplasm. Figure 12 shows the cell body and nucleus of the same cell. These sections show that the medium of connection of ectoderm and duct is derived entirely from the duct and also the limiting membrane between the two layers.

16 to 24 Consecutive sections, $4\ \mu$ thick, of a 6 mm. embryo, showing the anlage of the duct from the terminal cell, *a*, to where it is free from the ectoderm. Note modification of ectoderm at point of contact with duct, the means of attachment of the two and the limiting membrane. The outline of the anlage of the duct is more regular than in last series.

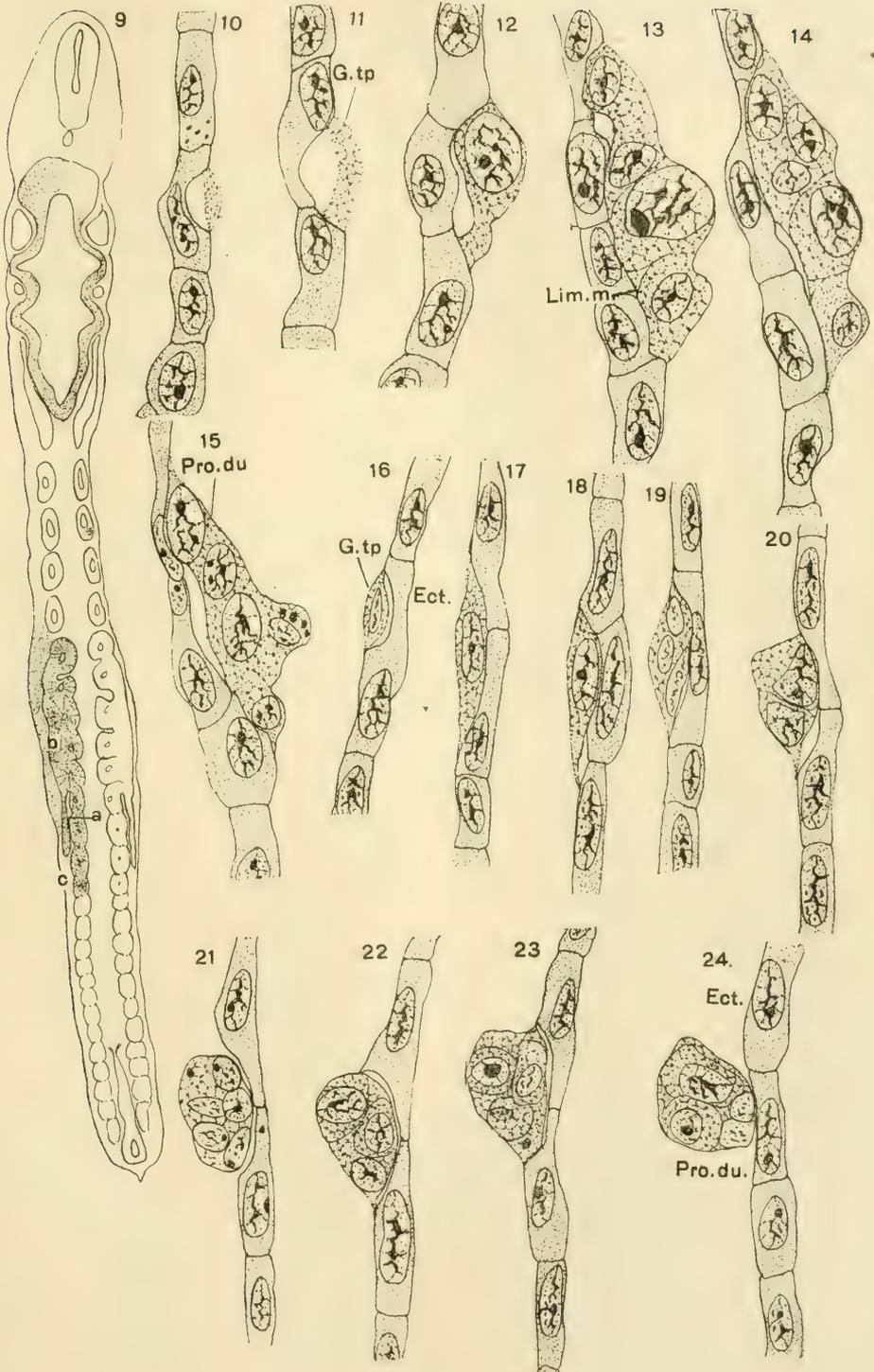


PLATE 3

EXPLANATION OF FIGURES

25 to 35 Cross sections, $6\ \mu$ thick, of a 7 mm. embryo. Note the medium of attachment of duct and ectoderm and its obvious origin from the former. In figure 30 the cytoplasm of the cell is a thick layer, attached to the ectoderm at only two points. This and figure 31 show the irregular form of the growing tip, which becomes more regular as it frees itself from the ectoderm. Figures 33 to 35 show mitotic figures in the ectoderm, those in figures 33 and 34 being in metaphase with the axis of the cell corresponding with that of the ectoderm; that in figure 35 being at right angles to the same axis. The anlage of the duct is attached to the ectoderm by clear cytoplasm, which, in figure 35, seems to have contracted and pulled the ectoderm out of line.

36 to 39 Consecutive cross sections of a 10 mm. embryo, presenting much the same features as the last series, except that the duct is larger and the connection between it and the ectoderm is not so close and it becomes free more abruptly.

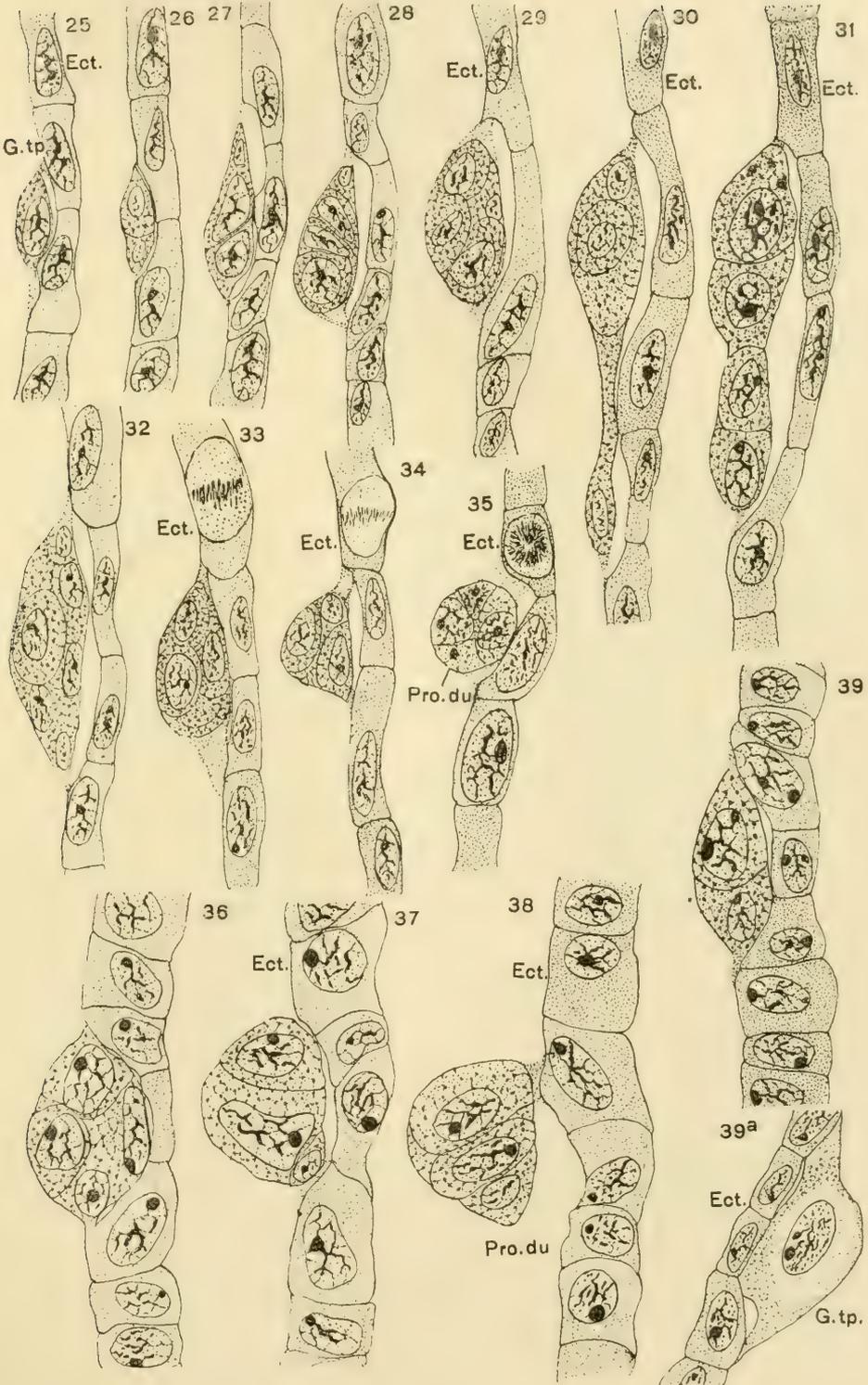


PLATE 4

EXPLANATION OF FIGURES

40 and 41 Selected sections of a 9.5 mm. embryo. In figure 40 the clear protoplasm at the extreme tip might be interpreted, but for the limiting membrane, as a part of the ectoderm, it conforms so perfectly to the contour of that layer; while the ectoderm seems hollowed out for its reception, the clearly defined line makes it easy to distinguish the layers. Figure 41 shows the ectoderm as if grooved for the reception of the duct. These sections show the ectoderm much thickened and the cells placed so that the nuclei have their axes at right angles with that of the layer.

42 to 47 Cross sections of a 11 mm. embryo, showing much the same features as the last, except that the separation of the duct from the ectoderm is more abrupt and the duct is rounded and more regular. The ectoderm is less modified and much thickened, with the cells shorter.

48 The anlage of the duct where it reaches the cloaca in a 20 mm. embryo. At this point the duct bends sharply inward to reach the cloaca, hence the section is oblique. The wall of the duct is of columnar epithelium. It has not yet broken through to the cloaca, but the walls are fused, and the drawing shows the duct as a triangular loop attached to the cloaca, *c*, by its base.

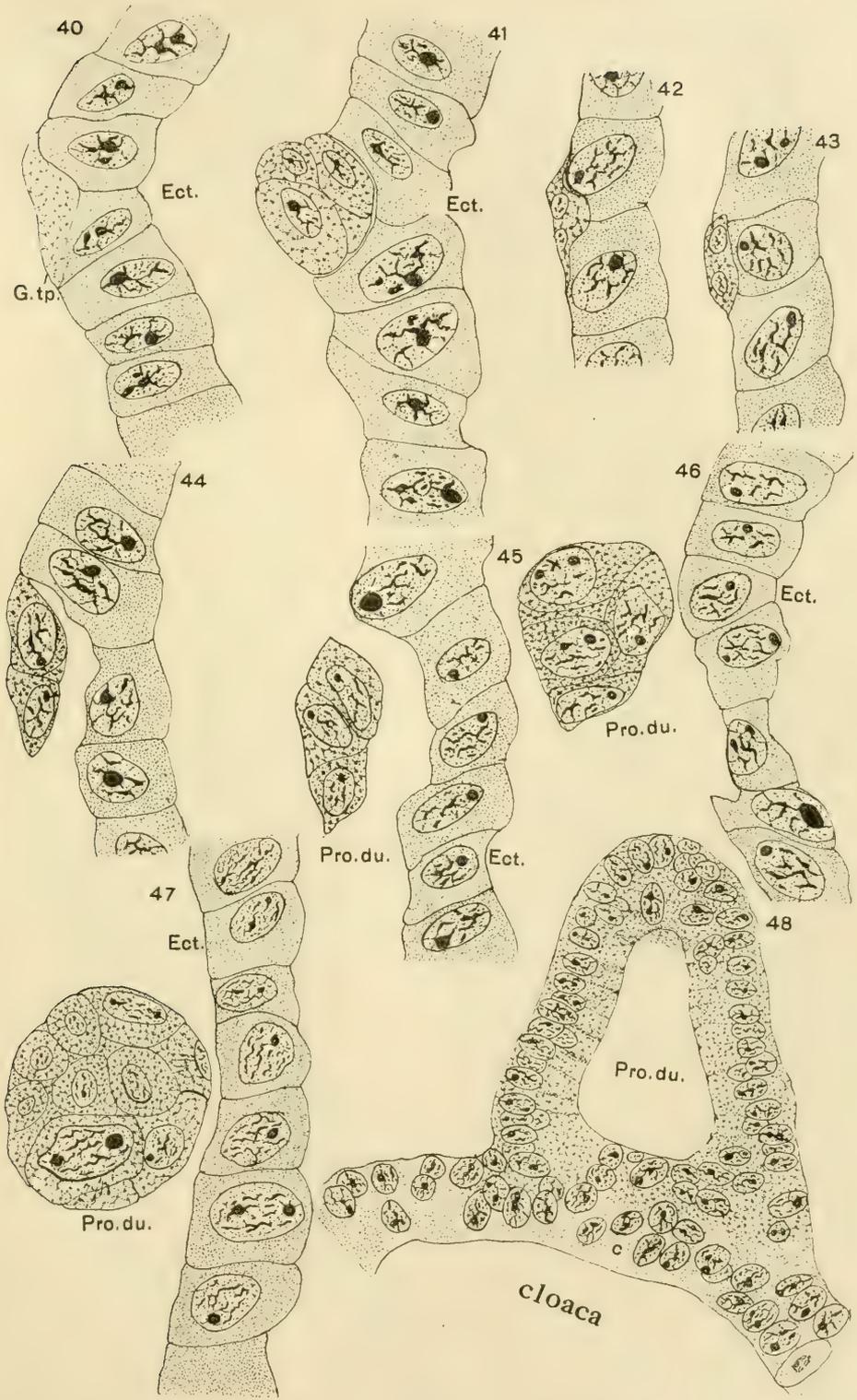


PLATE 5

EXPLANATION OF FIGURES

49 From a frontal section of a 4.5 mm. embryo (about 25 somites). The anlage of the duct is beginning to grow back from the pronephros and has just become attached to the ectoderm, which is much thinned and bent outward just above the point of attachment.

50 From a frontal section of a 5 mm. embryo, shows the close contact of ectoderm and the anlage of the duct, and demonstrates the impossibility of distinguishing between the layers, were the limiting membrane invisible.

51 to 54 Frontal sections of 5.5 and 6 mm. embryos, demonstrating the character of the growing tip of the anlage of the duct.

55 to 60 Showing mitotic figures in duct and ectoderm. Figure 56 is in the terminal cells of the anlage of the duct. Figures 57, 58 and 60 are comparable to the figure of Van Wijhe. The mitotic cells in the ectoderm are about to divide and, but for the limiting membrane, they might be interpreted as contributing cells to the anlage of the duct. This is especially evident in figure 57. Figure 58 shows the same section after the anlage has separated from the ectoderm and has been moved back into place, showing the entire independence of duct and ectoderm; figure 60 is much the same.

61 From a frontal section of a 7.5 mm. embryo, shows the disproportion in size between the anlage of the duct and the ectoderm. It also shows that the ectoderm may be modified at other points than at the growing tip. The close relation between duct and ectoderm and the limiting membrane are also shown.

STUDIES ON GERM CELLS

I. THE HISTORY OF THE GERM CELLS IN INSECTS WITH SPECIAL REFERENCE TO THE KEIMBAHN-DETERMINANTS.

II. THE ORIGIN AND SIGNIFICANCE OF THE KEIMBAHN-DETERMINANTS IN ANIMALS.

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¹ Contributions from the Zoological Laboratory of the University of Michigan, and the Marine Biological Laboratory, Woods Hole, Mass.

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GENERAL INTRODUCTION

Studies of the history of the germ cells in animals have proven that in many cases these cells originate in a perfectly definite way and at such an early embryonic period as to represent the first cellular differentiation that takes place in ontogeny. In certain animals such a determinate segregation of the germ cells cannot be established with the data available without certain assumptions, to which objections may be made. Limiting ourselves, therefore, to the instances where the germ cell cycle is completely known, it is possible to divide the history of the germ cells from one generation to the next into the following periods:

1. Primary cellular differentiation, i.e., the formation of one or more primordial germ cells during the segmentation of the egg;
2. A short period during which in some cases the primordial germ cells increase slightly in numbers by mitosis;
3. A long period of *rest* characterized by cessation of cell division, either active or passive change of position, separation of the germ cells into two groups which become the definitive germ glands, accompanied by the general growth of the embryo until the larval stage is almost attained;
4. Multiplication by mitosis of the primitive oogonia or spermatogonia to form a definite number (Miastor and perhaps

others) or indefinite number (so far as we know) of oogonia or spermatogonia;

5. In some cases the differentiation of oogonia into nurse cells and ultimate oogonia, and the spermatogonia into Sertoli cells and ultimate spermatogonia;

6. The growth of the ultimate oogonia and spermatogonia to form primary oocytes and primary spermatocytes;

7. Maturation;

8. Fertilization (if not parthenogenetic).

This list of periods differs from the series usually recognized in that it starts with the *beginning* of the germ cell cycle instead of at a comparatively late stage, i.e., with oogonia and spermatogonia. Certain of these periods, especially those of maturation and fertilization, have been emphasized by investigators much more than others. Many of the fundamental problems of heredity and development are, however, concerned with the events which take place during the less known stages.

For a number of years the writer has been particularly interested in the segregation of germ cells during embryonic development, and has studied especially certain visible substances which are present in the egg before cleavage begins, and later become part of the material contained in the primordial germ cells. In the eggs of certain Chrysomelid beetles this substance was termed the 'pole-disc' (fig. 7, A, *g.c.d.*, p. 403), and the granules of which the pole-disc is composed were called 'germ-cell determinants' because they enable us to determine which cells will become germ cells. Since this term is likely to be misinterpreted, the granules of the pole-disc and other similar substances that have been found in the eggs of animals are, in this paper, called 'Keimbahn-determinants,' since they furnish the means of recognizing the germ-cell material in the undivided egg or in cleavage stages, and thus make it possible for us to determine the 'Keimbahn' from one generation to another.

The following events may be listed in the history of the Keimbahn-determinants:

1. Localization of the Keimbahn-determinants in the oocyte or mature egg;

2. Association of one or more cleavage nuclei with part or all of the Keimbahn-determinants to form one or more primordial germ cells;

3. The apparently equal distribution of the Keimbahn-determinants between the daughter germ cells at each mitotic division (Sagitta possibly excepted);

4. The disappearance of the Keimbahn-determinants in the oogonia and spermatogonia;

5. The reappearance of the Keimbahn-determinants in the oocyte or mature egg.

In the general history of the germ cells there may be two periods of differentiation:

1. The segregation of the primordial germ cells during cleavage stages.

2. The differentiation of nurse cells and ultimate oogonia, or Sertoli cells and ultimate spermatogonia in the germ glands. This second differentiation does not occur in *Miastor* and certain other animals, and even when it does occur it is doubtful whether the nurse cells and Sertoli cells should be considered as true somatic cells or simply as abortive oogonia or spermatogonia which have been unsuccessful in the struggle for development. A casual examination is liable to delude one into thinking that the differentiations mentioned above are widely separated in the germ cell cycle, but a little closer study shows that they really occur during a relatively short period in the entire history. For example, in certain insects, where nurse cells arise from oogonia, this process takes place just before the growth period during which the Keimbahn-determinants became localized in preparation for the primary cellular differentiation.

It is evident from the general outline as stated above that the most important period in the germ cell cycle is that extending from the formation of the ultimate oogonia and spermatogonia to the complete segregation of the primordial germ cells. Our knowledge of events during the latter part of this period is comparatively great, whereas we know practically nothing about the early stages involving the genesis of the Keimbahn-determinants and their localization in the oocyte or mature egg.

Embryological investigation has gradually progressed from the study of germ layers back to the study of the segmentation of the egg, and from this to the organization of the ovum, and from here to the genesis and localization of organ forming substances in the oocyte.

In the following pages the results of some investigations made by the writer are described, and a discussion of the results obtained by other investigators is given, in an attempt to determine the origin, nature, and significance of the Keimbahn-determinants.

I. THE HISTORY OF THE GERM CELLS IN INSECTS WITH SPECIAL REFERENCE TO THE KEIMBAHN-DETERMINANTS

I. INTRODUCTION

The Keimbahn in animals was first described by Metschnikoff ('65, '66) in the paedogenetic larvae of the fly, *Miastor*. Since that time various investigators have been able to trace the germ cells in many other species of insects, belonging to several different orders, from early cleavage stages to the definitive germ glands, and have discovered that a complete Keimbahn can also be demonstrated in species belonging to other classes and phyla, notably the Crustacea, the Nematoda, and the Chaetognatha. The writer ('09) has published an account of our knowledge of the origin and early development of the germ cells in insects up to the year 1908, but no complete account of the Keimbahn in other groups of animals has ever appeared.

The data regarding this phase of the germ cell cycle are widely scattered in the literature; frequently buried in treatises on general embryology, and less often contained in contributions devoted to this subject alone. The accounts found in current reference books and text books are for the most part obsolete or inaccurate. In the following account statements, with figures, of the more important discoveries of other investigators have been included in order to allow a general consideration of our entire knowledge regarding the Keimbahn-determinants.

2. DIPTERA

A. *Historical account*

The segregation of the germ cells in the early embryonic stages of animal development was first discovered in certain Dipterous insects. In 1862 Robin described, in the nearly transparent eggs of *Tipulides culiciformes* the appearance of four to eight buds at one pole just previous to the formation of the blastoderm. He called these buds 'globules polaires' and thought that they were protruded at the anterior end. Weismann ('63) likewise discovered bud-like protrusions at a corresponding stage in the development of the egg of *Chironomus nigroviridis* and *Musca vomitoria*. He corrected Robin regarding their orientation by proving that they arise at the posterior end and not at the anterior end of the egg. Because of their position he applied to them the term 'Polzellen,' a term that has persisted until the present time. Weismann was unable to follow the history of the pole cells and so did not succeed in determining their true significance.

Metschnikoff ('65, '66) and Leuckart ('65) were the first to announce that the pole cells are really primordial germ cells, and the first to trace them from their initial appearance until they entered into the constitution of the definitive germ glands. Their results, obtained from the study of the eggs of *Miastor* and *Simula*, were confirmed by Grimm ('70) and Balbiani ('82, '85) in *Chironomus*.

Pole cells have also been described among the Diptera, in *Musca* by Kowalevsky ('86), Voeltzkow ('89), and Escherich ('00); in *Calliphora* by Graber ('89), and Noack ('01); in *Chironomus* by Ritter ('90), and Hasper ('11); in *Lucilia* by Escherich ('00); and in *Miastor* by Kahle ('08) and Hegner ('12).

Among insects belonging to other orders typical pole cells have been found only in parasitic Hymenoptera (Silvestri, '08), and in Chrysomelid beetles (Lecaillon, '98; Hegner, '08, '09; Wieman, '10a) although germ cells have been described at an early stage in the development of the butterflies, *Euvanessa antiopa* (Woodworth, '89) and *Endromis versicolora* (Schwan-

gart, '05); in the aphids (Metschnikoff, '66; Balbiani, '66-'72; Witlaczil, '84; Will, '88); in the honey-bee (Petrunkevitch, '01-'03); and in *Forficula auricularia* (Heymons, '95).

Since the original work contained in this paper was undertaken in order to determine the origin and significance of certain peculiar inclusions in the primordial germ cells of various animals the writer has been particularly interested in any extra-nuclear substances visibly different from the general cytoplasm. One of the principal characteristics used for the purpose of identifying germ cells in the embryos of animals is the presence within their cytoplasm of yolk substance. Many of the authors cited above noticed yolk globules in the pole-cells. For example, in *Chironomus*, Weismann ('63) states that each pole-cell possesses "ein oder zwei Dotterkörnchen;" and Metschnikoff ('66) described dark yolk masses in the pole-cells of *Simula* and *Miastor*. These examples indicate the general presence of yolk-like substances in the primordial germ cells of the Diptera, but it remained for later more detailed investigations with finer methods to determine the origin and fate of these cytoplasmic inclusions. Five papers have appeared which contain information bearing on these problems; (1) Ritter ('90) on *Chironomus*, (2) Noack ('01) on *Calliphora*, (3) Kahle ('08) on *Miastor*, (4) Hasper ('11) on *Chironomus*, and (5) Hegner ('12) on *Miastor*.

Chironomus. As stated above, the pole-cells of *Chironomus*, were first described by Weismann ('63) who, however, did not recognize them as germ-cells. Grimm ('70) succeeded in tracing the pole-cells in *Chironomus* until they became surrounded by other cells, forming two germ-glands, thus confirming Metschnikoff's ('66) account in *Miastor*. *Chironomus* was later studied again by Weismann ('82), by Balbiani ('82, '85), by Jaworowski ('82), by Ritter ('90) and by Hasper ('11). Only the work of the last two needs to be considered here since that of the other writers mentioned was not carried on with modern methods nor in such great detail.

Ritter ('90) used the section method and was thus able to study the structure of the germ cells more carefully and to trace them more accurately during embryonic development. He found

that the first pole-cell appeared at the posterior end of the egg when there were a large number of nuclei scattered about in the yolk. A second pole-cell was protruded close behind the first. Each carried out of the egg part of a flat mass of granules which, in section, formed a wreath around the nucleus. The two original pole-cells increased by division to four and then to eight. Two divisions of each pole-cell nucleus now occurred, resulting in eight quadrinucleated cells; these seemed to move of their own accord through the blastoderm which closed after them. They now lay at the posterior end of the germ-band from whence they were possibly moved anteriorly by the growing forward of the entomesoderm. The mass of pole-cells finally divided into two groups which occupied a position on either side of and dorsal to the hind-intestine; there they remained until after the larva hatched, when they became the definitive sex-organs.

Ritter was the first to determine the fact that the 'yolk masses' contained in the pole-cells of *Chironomus* are derived from a definite structure and are not chance acquisitions from the yolk granules in the egg. After giving a brief sketch of the polar bodies and male and female pronuclei, he says:

In dem nächsten Stadium sind in dem Dotter keine Zellen mehr zu sehen; dagegen tritt an demjenigen Pol, an welchem später die Polzellen erscheinen, also an dem hinteren, ein eigenthümlicher wulstartiger Körper auf, welcher durch das Hämatoxylin sehr dunkel gefärbt wird. Er erscheint auf mehreren Schnitten und stellt eine etwas nach oben vorgewölbte Platte dar, welche vielfach runde Fortsätze zeigt und aus feinkörnigem Protoplasma besteht. Er bleibt bis zum Austritt der Polzellen an derselben Stelle.

Ritter then gives a fragmentary account of the early divisions of the cleavage nucleus, at the end of which, the two first pole-cells appear each containing a "grossen Kern und um denselben herum kranzförmig einen Theil des obengenannten dunklen wulstförmigen Körpers." This darkly staining body he called 'Keimwulst.'

That the 'Keimwulst' played an important rôle in the segregation of the germ cells was quite obvious to Ritter, but he was in error when he stated that this body contained the first cleavage

nucleus and "dass nach der Theilung des Furchungskernes die Theilprodukte theils in dem dunklen wulstförmigen Körper verbleiben, theils aus demselben herausrücken."

In 1911 Chironomus was again studied by Hasper, who published a complete description of the Keimbahn in Chironomus confinis and C. riparius. At the posterior pole of the eggs at the time of deposition is a disc-shaped mass of granules (fig. 1 A, kbpl) called by Hasper the 'Keimbahnplasma,' which is identical with the Keimwulst' of Ritter. Hasper characterizes this 'Keimbahnplasma'

. . . als dichte, scharf konturierte, wurst- oder flaschenförmige, gerundete oder auch in 2 Klumpen getrennte, mit wenigen Vacuolen versehene Masse präsentiert, die am hintern, im Ovarium nach hinten gekehrten Ende des Eies etwas unter der Oberfläche liegt, in schaumigem Protoplasma eingebettet, zuweilen aber auch noch ganz von Dotter umgeben. Es ist diese wichtige Differenzierung des Ooplasmas nichts anderes als jene spezifische Substanz, die bei der Determinierung des ersten von der Entwicklung dargebotenen embryonalen Materials eine entscheidende Rolle spielt und die daher im Folgenden als Keimbahnplasma noch mehrfach Erwähnung finden wird (pp. 549-550).

Ritter ('90) advanced the idea that the cleavage nucleus of Chironomus divides within the 'Keimwulst' and that here the first cleavage division occurs, one daughter nucleus remaining in the 'Keimwulst' and becoming the center of the primordial germ-cell, the other giving rise to somatic nuclei. This is probably the basis for Weismann's ('04) statement regarding his conception of the germ-plasm that

If we could assume that the ovum, just beginning to develop, divides at its first cleavage into two cells, one of which gives rise to the whole body (soma) and the other only to the germ-cells lying in this body, the matter would be theoretically simple. . . . As yet, however, only one group of animals is known to behave demonstrably in this manner, the Diptera among insects

There is, however, nothing in the literature to warrant the above statement, since Ritter's hypothesis has been disproved by Hasper.

The primordial germ-cell is really recognizable as such in Chironomus at the four-cell stage (fig. 1, B, *p.g.c.*). One of the

first four cleavage nuclei migrates to the posterior end, and, separating from the rest of the egg together with the 'Keimbahnplasma' and the cytoplasm in which this substance lies, forms the 'Urgeschlechtszelle.' The primordial germ cell is undergoing division by mitosis at the time when it is protruded from the egg and during this process the 'Keimbahnplasma' is apparently equally divided between the daughter cells.

Während die ersten Teilungen rasch aufeinander folgen, kommt die letzte gar nicht mehr zur Vollendung, d.h. sie erstreckt sich nur auf die Kerne, so dass schliesslich 8 zweikernige Genitalzellen im hintern Polraum liegen. Und damit ist die Entwicklung der Keimbahn für lange Zeit überhaupt abgeschlossen; denn während der nun folgenden Embryonalperiode ist sie durch ein durchaus passives Verhalten ausgezeichnet (p. 553).

One of these binucleated germ-cells is shown in figure 1, C.

It is unnecessary to trace the history of these primordial germ-cells (pole-cells) since it has been shown repeatedly that they give rise to the oogonia or spermatogonia in the definitive germ-glands. Portions of the 'Keimbahnplasm' persist at least until the larva hatches (fig. 1, D). The origin and nature of the 'Keimbahnplasm' was not discovered by Hasper but the name applied to it and the fact that the author adopts my term 'germ-cell determinants' (Keimzell-determinanten) in discussing it, indicate that he considers it of fundamental importance in the segregation of the germ-cells.

The possibility of determining the origin of the 'Keimbahnplasma' of *Chironomus* led the writer to study the oogonia in the terminal chamber and the various stages in their growth up to the time of deposition. Larvae were collected and allowed to develop in the laboratory and the ovaries were dissected out of the adults which were obtained from these larvae. However, the material procured has been found lacking in both the earlier stages of the development of the oocytes and the late stages in the formation of the ovum. It has been considered best, therefore, to reserve a study of this material until a complete series can be secured.

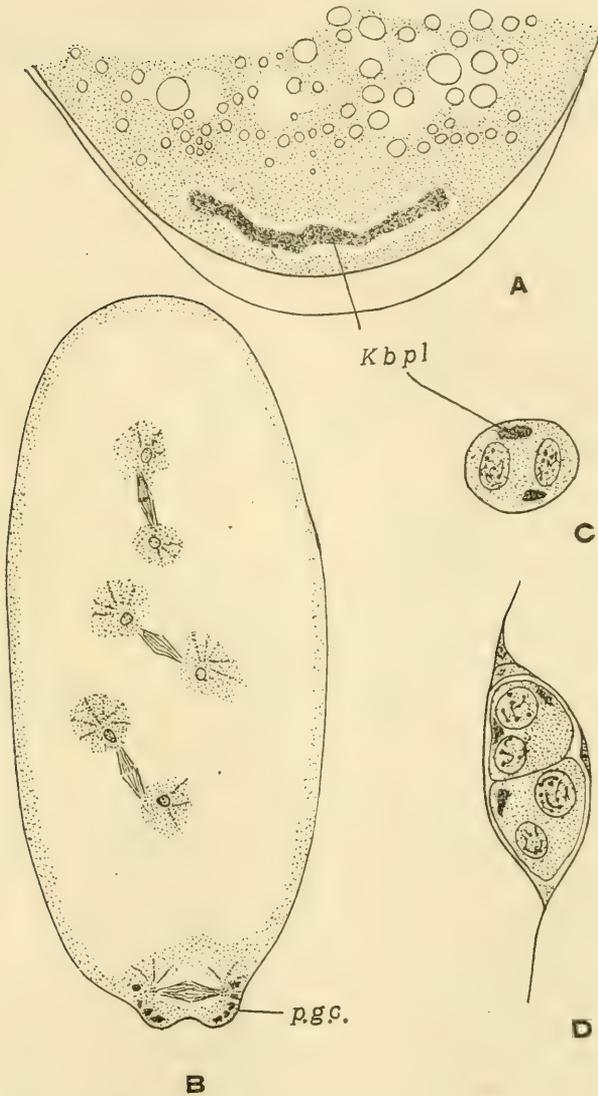


Fig. 1 Chironomus (redrawn from Hasper, '11). A, longitudinal section through the posterior end of a freshly laid egg. B, longitudinal section through egg during division of first four cleavage nuclei; at posterior end primordial germ cell is just being formed. C, one of primordial germ cells containing two nuclei and remains of 'Keimbahnplasma.' D, germ gland of the larva in which remains of 'Keimbahnplasma' still appear. *Kbpl*, 'Keimbahnplasma'; *p.g.c.*, primordial germ cell.

Calliphora. Noack ('01) found a dark granular layer, which he called the 'Dotterplatte' (fig. 2, *Dpl*) at the posterior end of the egg of *Calliphora erythrocephala* similar to the 'Keimwulst' discovered by Ritter in *Chironomus*. Each pole-cell took part of this layer of granules with it as it passed through the 'Keimhautblastem.' Concerning this process Noack says, "Im nächsten Stadium haben die Kerne eine runde Gestalt angenommen, die Platte hat sich in so viel Theile getrennt, als Kerne in ihren

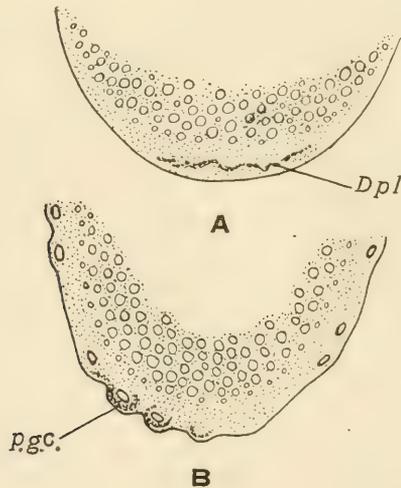


Fig. 2 *Calliphora* (redrawn from Noack, '01). A, longitudinal section through posterior end of freshly laid egg showing 'Dotterplatte' (*Dpl*). B, longitudinal section through posterior end of egg at time of blastoderm formation showing protrusion of primordial germ cells (*p.g.c.*).

Bereich eingetreten sind, und bildet nun um jeden dieser Kerne einen peripher gelegenen feinkörnigen Halbmond. Hiermit ist die erste Zelldifferenzierung eingeleitet." Those cells which now contain granules from the "Dotterplatte" are recognized as pole-cells, while the remaining cells which have reached the periphery of the egg constitute the blastoderm. The 'Halbmond' of granules which surrounds the nucleus of each pole-cell now

. schliesst sich allmählich zu einem Kreise, welcher um so mehr auffällt, weil die von ihm eingeschlossene und den Kern einbet-

tende Protoplasmamasse fast farblos erscheint (fig. 2, B, *p.g.c.*). Bei der Fortentwicklung der Polzellen schwindet allmählich die scharfe Grenze zwischen Zellprotoplasma und Polplatte. Letztere löst sich auf und es entsteht eine gleichmässige Pigmentirung, welche den Polzellen noch auf lange Zeit ein ganz charakteristisches Aussehen verleiht.

Concerning the nature of this 'Dotterplatte' Noack says:

Dass die Platte am hinteren Pole des Musciden-Eies sich aus Dotterelementen zusammensetzt. Sie scheint den Zweck zu haben, das Wachsthum am hinteren Pol zu beschleunigen, ferner durch Eintritt in die Polzellen es diesen zu ermöglichen, sich auch weiterhin lebhaft zu vermehren, obgleich sie vom Dotter her keine Nahrung mehr erhalten. Schliesslich verursacht sie die charakteristische Pigmentirung dieser Zellen.

B. The Keimbahn in Miastor americana Felt

The paedogenetic flies of the genus *Miastor* furnish especially favorable material for the study of the germ-cell cycle. The process of paedogenesis was discovered by Wagner, and a short statement was published by him in 1862; three years later a more detailed account appeared by Wagner ('65) whose extraordinary discovery was confirmed by Meinert ('64), Pagenstecher ('64) and Ganin ('65); but of the early authors Leuckart ('65) and Metschinkoff ('65, '66) have given the best descriptions of the developmental stages. In 1870 Grimm announced the occurrence of paedogenesis in a species of *Chironomus*. From that date until 1907 nothing new concerning this peculiar method of reproduction in insects was learned. Zavrel ('07) then reported paedogenesis in the genus *Tanytarsus*, and this has been confirmed for *T. dissimilis* by Johannsen ('10). In the meantime Kahle ('08) published perhaps the best account that has ever appeared on the 'Keimbahn' of any animal, using *Miastor metraloas* for this purpose. He was able to trace the germ cells from one generation to the next with remarkable clearness. Many of Kahle's results have been confirmed (Hegner, '12) for *Miastor americana* Felt and reference will be made to them more in detail in the following pages. Finally a short study of the life history of *Miastor metraloas* has been made by G. W. Müller ('12).

Metschnikoff's ('66) studies on *Miastor* indicated that the germ-cells of this fly are set aside very early in embryonic development, and led me in 1907 to attempt to obtain material of this genus. I was informed at that time by Prof. Samuel W. Williston that no paedogenetic Diptera were known to occur in this country. On October 5, 1910, however, Dr. E. P. Felt discovered great numbers of the larvae of *Miastor americana* Felt under the partially decayed inner bark and in the sapwood of a chestnut rail near Highland, New York, and kindly sent me an abundant supply of material.

Habitat and life history. Dr. Felt found the larvae of *Miastor americana* living

. . . . in the moist, partly rotten inner bark and punky sapwood which has not been invaded to any considerable extent by other Dipterous larvae or Coleopterous borers. They exhibit a tendency to occur in segregated masses, frequently between loose flakes of bark or in rather broad crevices. These colonies contain in autumn old empty skins of mother larvae; a number of yellowish mother larvae with approximately five to fifteen young within; very numerous, small yellowish larvae showing no trace of embryos; a number of white, various sized larvae, frequently white, sometimes semi-transparent; and a few quiescent white larvae containing young embryos. The mouth parts of the larvae, though the anterior portion of the head is strongly chitinized, appear to be comparatively weak. The alimentary canal contains little that can be discerned with the aid of a compound microscope, and we are inclined to believe that a considerable portion of their nourishment is absorbed by osmosis after escaping from the mother larva, as well as before. It would appear as though the several types of larvae occurring in a colony are possibly only modifications due to the relative amount of nourishment obtained by the individual.

Normally, reproduction by paedogenesis occurs throughout the warm months of the year and even into late fall, and commences in early spring, the cold weather of winter simply causing a suspension of activities. The adults of *Miastor* and *Oligarces* occur in midsummer, a season when midges of most of these forms are probably abroad (Felt, '11).

Methods. A number of fixing and staining methods have been employed in an endeavor to determine the origin and history of the 'polares Plasma' which plays such an important rôle in the differentiation of the germ cells of *Miastor*. Perhaps the easiest

and most successful methods for studying the Keimbahn are fixing in Gilson and staining in Mayer's acid hemalum followed by Bordeaux red. Entire larvae may be fixed, sectioned and stained in this way. To bring out cytoplasmic details other methods were resorted to. The anterior and posterior ends of larvae were cut off and the middle part of the body containing the ovaries, and eggs and young were fixed in Meves' fluid. In other cases the eggs and young were dissected out and fixed in Meves' fluid. Still other larvae were fixed in Carnoy's solution. The best fixation was obtained with Gilson's Mercurio-nitric fluid. Besides acid hemalum the following stains were used: Heidenhain's iron hematoxylin followed by Bordeaux red or eosin, the iron hematoxylin method used by Rubaschkin ('12) in his studies of the mitochondria in the embryonic cells of the guinea pig, safranin followed by light green, Altmann's acid fuchsin differentiated in picric acid, Benda's method for the study of mitochondria, and the Erhlich-Biondi triple stain.

The morphological continuity of the germ cells. The ovaries of *Miastor* lie on either side of the body in the tenth or eleventh segment. They appear yellowish green in living white larvae, but are whitish transparent in the young yellowish larvae. Each ovary, when in the stage shown in figure 27, is surrounded by a thin cellular envelope (*en*) and contains typically thirty-two oocytes (*ooc.n*) each with an accompanying group of mesodermal nurse cells (*n.c.*), which are enclosed with it in the follicular epithelium (*f.ep*). The oocytes grow at the expense of the nurse cells, separate from the ovary, and are distributed throughout the body of the larva. Usually from five to seventeen embryos develop in one larva, but sometimes only one or two larvae are produced by a single mother-larva.

The nucleus of the oocyte (fig. 27, *ooc.n*) is large and clear, and the chromatin within it forms slender threads, rather evenly scattered about in the nuclear sap.

Figure 28 is a longitudinal section of an oocyte just before the maturation division. The germinal vesicle (*g.v.*) is large and clear and contains a great number of small scattered chromatin granules. It lies near one side of the oocyte in preparation for

the formation of the first polar spindle. The nurse chamber (*n.c.*) contains a syncytium with about eighteen large nuclei, each of which possesses a large, centrally placed nucleolus surrounded by irregular chromatin granules. At the posterior pole of the egg is an accumulation of material (*pPl*) which stains deeply, and, as will be shown later, is intimately associated with the origin of the primordial germ-cell. This mass of material has been termed by Kahle 'polares Plasma'—a term adopted in the following description. A discussion of the origin and significance of the 'polares Plasma' will be reserved until later (p. 396).

There is nothing unusual in the process of maturation. The egg nucleus preparing for division is shown in figure 29, *m.s.* One polar body is formed in *Miastor metraloas* and the number of chromosomes could not be determined by Kahle but is probably from twenty to twenty-four. The number of chromosomes appears to be similar in *M. americana* but an accurate count could not be made. The first polar body divides by mitosis (fig. 30, *p.b.*) and the pronucleus (*f.n.*) moves over into the mass of cytoplasm (*c*), which is apparently elaborated by the nuclei in the nurse chamber, and becomes the cleavage nucleus. Here in this mass of cytoplasm the first cleavage takes place resulting in two apparently similar daughter nuclei (fig. 31, *c.n.*).

Reference must be made to Kahle's figures and description for most of the events of early cleavage. The two nuclei of the two-cell stage divide by mitosis and the four daughter nuclei are apparently similar. They have been numbered I, II, III, IV, beginning at the anterior pole (fig. 3). The succeeding nuclear division is important, since the advance from the four-cell to the eight-cell stage witnesses the origin of the primordial germ-cell as well as a casting out (diminution) of chromatin by nuclei I, II, and III. In figure 3 nuclei I, III and IV are shown in mitosis. Spindle IV is undergoing the ordinary process of mitosis, but spindles I and III (and also spindle II, which is not shown in the figure) are long and slender and a large portion of their chromatin (fig. 3, *cMp*) does not take part in the formation of the daughter nuclei but remains behind in the cytoplasm as a 'Chromosomen-mittellplatte.' These masses of cast-out chromatin are never

found in stages earlier than the four-cell stage, but are present in many of the later stages (figs. 32, 33, 34, 35, 36, *cR*) and are called by Kahle 'Chromatinreste.' One daughter nucleus resulting from the division of nucleus IV (fig. 3) becomes imbedded in the 'polares Plasma' and, with this substance, is cut off from the rest of the egg as the primordial germ-cell (fig. 32, *p.g.c.*). The other daughter nucleus of spindle IV remains in the egg. These two nuclei are the only ones at this stage which contain a complete amount of chromatin.

During the next stage (VIII-XV) the daughter nucleus of cleavage cell IV, which remains in the egg, undergoes a diminution process whereby it loses part of its chromatin, and the other six nuclei within the egg pass through a second diminution process during which a second 'Chromosomenmittelplatte' is formed (figs. 32 and 4, *cMp*). At the fifteen-cell stage, therefore, one nucleus (that of the primordial germ-cell, fig. 32, *p.g.c.*) contains the full amount of chromatin; whereas all of the others (somatic nuclei) have lost a large portion of their chromatin. After the second diminution process, according to Kahle, the somatic nuclei possess only half the number of chromosomes present in the germ cells, that is "der Diminutionsprozess und der Reductionsprozess in derselben Karyokinese vereinigt sind." My material did not contain enough of the early cleavage stages to enable me to confirm in detail Kahle's investigations, but one egg contained well marked mitotic figures which represent stages in the second diminution process (fig. 32) and a large number of sections were obtained which contained chromatin masses ('Chromatinreste,' figs. 32, 33, 34, 35, 36, *cR*). The details of the second diminution process are shown in figure 4.

The history of the germ-cells, from the time of the formation of the primordial germ-cell to the production of the sixty-four oocytes contained in the two ovaries, thirty-two in each, will now be described briefly.

The somatic nuclei divide rapidly, forming the blastoderm as shown in figures 33 and 34. Chromatin masses (*cR.*) representing chromatin cast out during the diminution processes are present in these early stages. The primordial germ-cell (fig. 32,

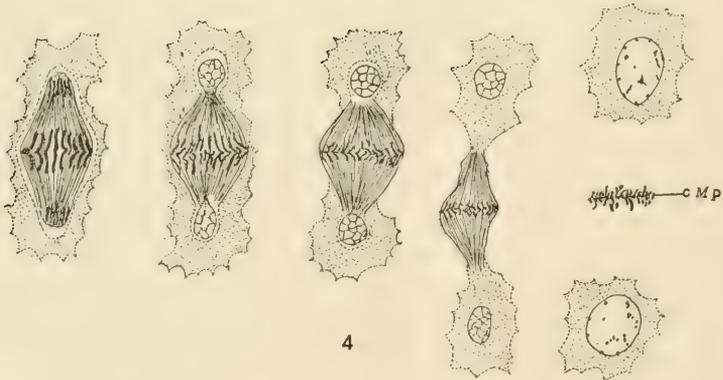
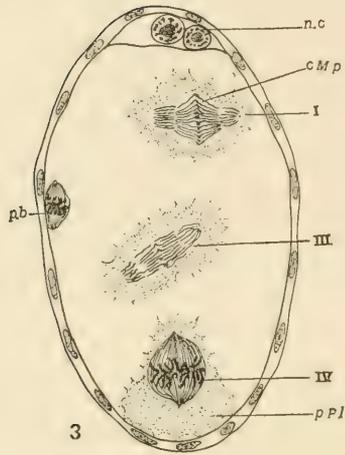


Fig. 3 *Miastor metraloos* (redrawn from Kahle, '08). A section through an egg showing a dividing polar body (*p.b*); cleavage nuclei I and III undergoing the chromatin-diminution process; and cleavage nucleus IV dividing normally. The daughter nucleus of the latter which enters the 'polares Plasma' (*p. Pl*) becomes the nucleus of the primordial germ cell.

Fig. 4 *Miastor metraloos* (redrawn from Kahle, '08). Five stages in the second chromatin-diminution process. *cMp*, 'Chromosomenmittelplatte.'

p.g.c.) divides by mitosis resulting in two oogonia (fig. 33, *oog*₁) which lie at the posterior end of the egg. Each of these divides again about the time when the blastoderm cells are cut off by cell walls. Four oogonia of the second order (fig. 34, *oog*₂) are formed in this way. A third division results in the production of eight oogonia of the third order (fig. 35, *oog*₃). The germ-band then forms and segments, and the eight oogonia are passively carried around by the growth of the tail fold as shown in figure 36, *oog*₃). The embryo then grows broader and shorter until it entirely surrounds the yolk and the end of the tail fold coincides with the posterior end of the egg. During these developmental stages the oogonia remain undivided, but become separated into two groups of four each, which lie in two rows, one on either side of the embryo in the region of the eleventh segment (fig. 37, *oog*₃). Soon each row of four oogonia becomes enclosed by mesoderm cells, forming an ovary. The germ-glands then become almost spherical and soon the oogonia undergo a division by mitosis, thus forming eight oogonia of the fourth order in each germ-gland (fig. 38, *oog*₄). These divide again by mitosis (fig. 38, *a*) producing sixteen oogonia of the fifth order (fig. 39, *oog*₅) in each germ-gland. The final division of the oogonia takes place shortly before the larva hatches.

Typically, there are then thirty-two oogonia of the sixth order in each germ-gland, but in some cases certain of the oogonia of the fifth order are prevented from dividing. All of the oogonia do not produce embryos, since, as a rule, only from five to seventeen larvae are produced by a single mother-larva. The oogonia of the sixth order grow into oocytes (fig. 40, *ooc.*); each of these, together with a syncytium containing about twenty-four nurse-cells of mesodermal origin (fig. 27, *n.c.*), becomes surrounded by follicular epithelium (fig. 27, *f.ep.*) also of mesoderm cells. During this process the nucleolus of the germ-cells disappears and the chromatin forms long slender threads (fig. 27, *ooc.n.*). This completes the history of the germ-cells from one generation to the next. The accompanying diagram (fig. 5) shows graphically the germ-cell cycle in this animal.

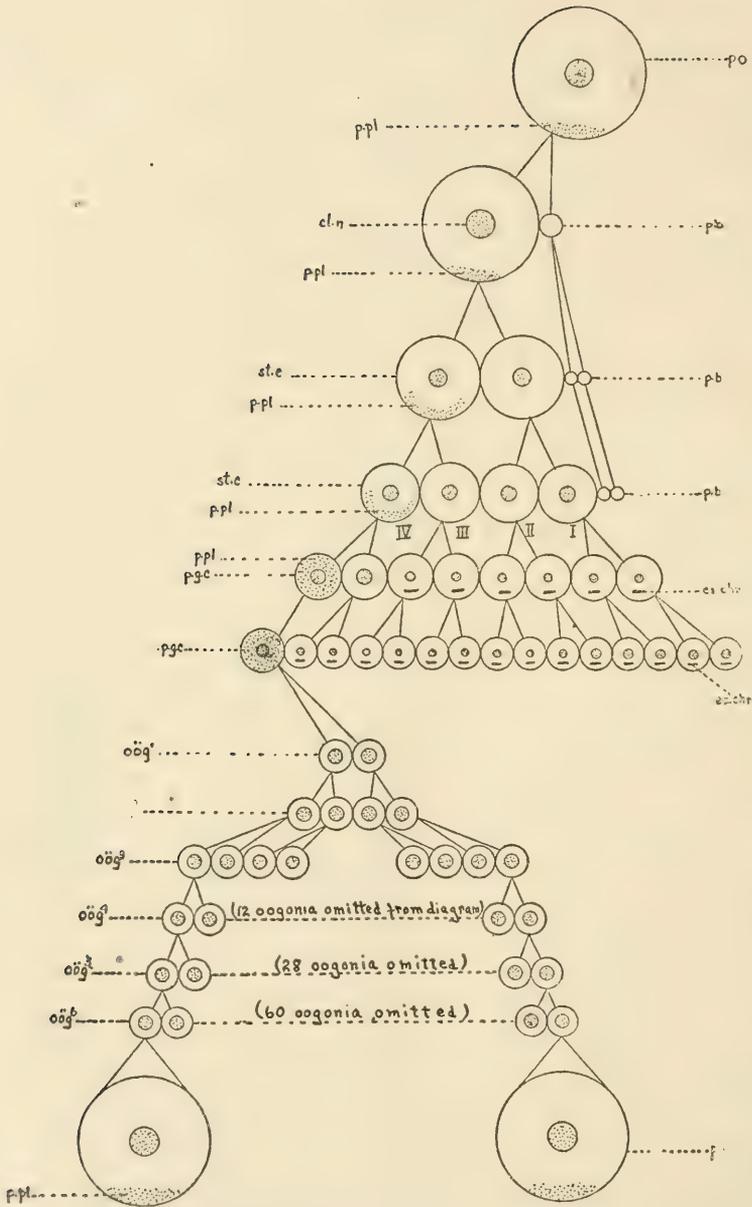


Fig. 5 *Miastor americana*; diagram showing the entire germ cell cycle. *cl.n.*, cleavage nucleus; *ex.chr.*, extruded chromatin; *oog*, oogonium; *p.b.*, polar body; *p.g.c.*, primordial germ cell; *p.o.*, primary oocyte; *p.pl.*, 'polares Plasma'; *st.c.*, stem-cell.

Summary. The principal points that should be emphasized are as follows:

1. *Miastor americana* Felt is truly paedogenetic and agrees with *M. metraloas* in regard to its reproduction as described by Kahle ('08).

2. One polar body, which divides by mitosis, is produced (fig. 30).

3. A diminution process takes place during the division of the first four cleavage nuclei in which a large part of the chromatin of three of these cleavage nuclei is cast out into the cytoplasm (fig. 3).

4. One daughter nucleus of the fourth cleavage nucleus, which does not lose any of its chromatin, passes into a deeply staining mass of material ('polares Plasma') situated at the posterior end of the egg, and is cut off from the egg by a cell wall. This cell which thus contains the 'polares Plasma' and a nucleus with the full amount of chromatin is the primordial germ cell (figs. 32, *p.g.c.*).

5. A second diminution process takes place, during which each of the seven somatic nuclei loses part of its chromatin and emerges with one-half of the number of chromosomes. The primordial germ-cell does not undergo a diminution process (fig. 32).

6. The primordial germ-cell divides by mitosis until eight oogonia are produced. These separate to form two rows of four oogonia each. After a long period of rest further divisions result in the production of thirty-two oogonia in each germ gland.

7. The nurse cells are of mesodermal origin.

8. We have here for the first time a definite number of oogonial divisions, namely six, a definite and equal number of oogonia in each germ-gland, and a definite number of oocytes (sixty-four) produced by the primordial germ-cell. It is no longer necessary, therefore, to express our ignorance by saying that there are n divisions during the period of multiplication of the oogonia, since in *Miastor* the number (n) is known positively to be six.

The differentiation of the germ cells. We have seen from the foregoing account that in *Miastor* there are two features which distinguish the germ cells from the somatic cells; (1) the nucleus of the primordial germ cell is the only one in the egg which retains the full amount of chromatin and the complete number of chromosomes; and (2) the primordial germ cell contains, in addition to this nucleus, *all* of the 'polares Plasma' and apparently no other kind of material. In considering the differentiation of the germ cells we must therefore examine more in detail these two features.

Kahle does not discuss the origin of the 'polares Plasma.' In describing this structure in an oocyte just before the formation of the polar body, he says, "Ganz auffällig ist eine Ansammlung von Protoplasma am hinterer Eipol. Sie wird durch Anilin- und Karminfarben tiefer tingiert als das übrige Plasma und macht den Eindruck einer ausserordentlich verdichteten Substanz" (p. 12). Further on the following statement is made:

Wie wir sahen, stammt der Kern der Urgeschlechtszelle in direkter Folge vom Furchungskern ab, das Protoplasma der Urgeschlechtszelle aber war schon lange vor ihm da. Es ist dasselbe, das ich als polares Plasma bezeichnet habe, das sich durch besonders intensive Färbung auszeichnet, dessen Vorhandensein in unveränderter Lage in allen aufeinanderfolgenden Stadien nachweisbar ist das im Fortgang der Entwicklung ein immer stärkeres Wachstum zeigt und sich bis in die ungeriffte Eizelle zurückverfolgen lässt. Das polare Plasma ist infolge dessen also Keimplasma aufzufassen, das wahrscheinlich besondere Qualitäten enthält und bereits in der ungerifften Eizelle präformiert wird, um später seine Aufgabe als Geschlechtsplasma zu erfüllen. Erscheint uns also die Bildung der Urgeschlechtskerns schon als eine sehr frühe, so ist die Differenzierung des Urgeschlechtsplasmas auf ein noch viel jüngeres Stadium verlegt. Dieses Plasma erwartet gewissermassen seinen Kern, um sich dann sofort mit ihm als Urgeschlechtszelle zu isolieren (p. 21).

If, as Kahle says, the 'polares Plasma' represents the 'Keimplasma,' it is of the greatest importance to determine its origin and fate. For this reason hundreds of young were preserved, sectioned and stained by the methods most likely to enable one to trace the history of this substance (p. 388). It must be confessed, however, that notwithstanding the efforts made with

this end in view, the problem is still unsolved. It is evident from the preparations that the oocyte is nourished by and grows at the expense of the nurse cells (figs. 28-32). It is also absolutely certain that these nurse cells are not derived from the oogonia, as is true in so many insects, but, are modified mesoderm cells (figs. 38, 39, 27). At first, the growth of the oocyte takes place so slowly as to make almost no perceptible difference in the character of the cytoplasm contained within it. The oogonia are remarkably easy to distinguish during the embryonic development because (1) of their comparatively enormous nuclei, filled with large chromatin granules; and (2) the deeply staining quality of their cytoplasm, consisting of the corresponding deeply staining substance of the 'polares Plasma' of the mature egg (figs. 31-32). As the oocyte grows its cytoplasm becomes less deeply colored and presents a uniform appearance not distinguishably different from the cytoplasm of the other cells (fig. 40). Sometime before the oocyte is ready for maturation, however, deeply staining cytoplasm appears in the neighborhood of the nurse chamber and a substance begins to accumulate at the posterior pole which has a strong affinity for various dyes (fig. 28). The former is evidently elaborated under the influence of the nurse cells; the latter, which represents the 'polares Plasma,' may be derived from the nurse cells, but if it is, the process is so slow and its mass compared with the mass of the remaining egg contents so small that its passage from the nurse chamber to the posterior end of the oocyte is indistinguishable. We must conclude, therefore, that the 'polares Plasma' may originate from or under the influence of the nurse cells, but that this has not been demonstrated and probably never can be established.

A second hypothesis which may account for the presence of the 'polares Plasma' in succeeding generations is that of continuity and growth. Each oogonium is supplied with a portion (typically one sixty-fourth) of the 'polares Plasma' of the mature egg. Hence a certain amount of this substance, as well as a like amount of nuclear material, is passed on from one generation to the next. What is more probable than that this part, although

minute when compared with the enormous contents of the mature egg, may become segregated at the posterior end of the egg and there bring about the development of a greater volume of similar substance, either by the division or budding of preexisting particles, or from the yolk or cytoplasm under its influence. A full discussion of this subject will be reserved until the Keimbahn-determinants of other animals have been described (p. 460).

C. The Keimbahn in Compsilura concinnata Meig

Compsilura is a tachinid fly, introduced into this country in 1906 for the purpose of destroying gypsy and brown-tail moths. "Its eggs hatch in the uterus of the mother, and the tiny maggots are deposited beneath the skin of the host caterpillar by means of a sharp, curved 'larvipositor,' which is situated beneath the abdomen" (Howard and Fiske, 1912, p. 219). The maggot is ready for pupation in about two weeks; the pupal period is about one week; and the females require only about three or four days after their emergence to become sexually mature. I wish here to acknowledge my indebtedness to Dr. John N. Summers of the Gypsy Moth Parasite Laboratory, Melrose Highlands, Massachusetts, for an abundance of material.

The internal reproductive organs of a sexually mature female are shown in figure 6. Oocytes of various sizes are present within the ovarian tubules (*o.*). At a point near the union of the two oviducts (*od*), the uterus is connected with two accessory glands (*a.g.*), and three seminal receptacles (*s.r.*). The mature eggs, which make their way down the oviduct and into the uterus, are here fertilized. They then gradually move down the uterus and are present to the number of about one hundred in a sexually mature individual. All stages from the maturation of the egg to the condition when the larva is ready to be deposited are passed through within the uterus of the mother, and most of these may be observed in a single specimen. Those eggs nearest the ovaries are of course the youngest. An attempt to trace the origin of the pole-disc granules in this species was unsuccessful, so only two illustrations are presented here to show that in this species

there is a primary cellular differentiation similar to that already described in other Diptera. Figures 41 and 42 represent two stages in the formation of the primordial germ cells at the posterior end of the egg. The granules of the pole-disc are encountered by the cleavage nuclei which chance to reach the posterior pole; they surround these and are distributed about

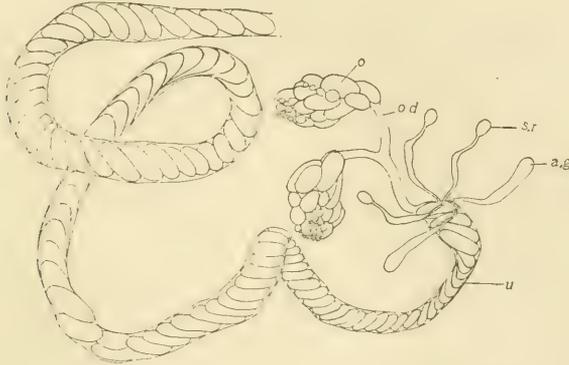


Fig. 6 *Compsilura concinnata*; reproductive organs of a female. *a.g.*, accessory gland; *o*, ovary; *od*, eviduct; *s.r.*, seminal receptacle; *u*, uterus full of eggs in various stages of development.

within the cytoplasm of the germ cells when they are cut off by cell walls. The further history of the germ cells does not seem to differ in any way from that described in other Diptera.

Cecidomyia strobiloides. A pole-disc was also found in the eggs of the willow-cone gall just before deposition (fig. 43), and an attempt was made, as in *Miastor*, to trace this structure to its place of origin. The growth of the egg of this species resembles that of *Miastor* and all efforts to connect the pole-disc with substances within the oocyte previous to its appearance at the posterior end were futile.

3. COLEOPTERA

A. *Historical account*

The primordial germ cells of beetles have not received as much attention from investigators as have those of the Diptera, probably because they are not so conspicuous. The germ glands have been described in the embryo of *Hydrophilus piceus* by Heider ('89), in *Leptinotarsa* (*Doryphora*) *decemlineata* by Wheeler ('89), in *Melolontha vulgaris* by Voeltzkow ('89b), in *Hydrophilus piceus*, *Melolontha vulgaris*, and *Lina tremulae* by Graber ('91), in a number of Chrysomelid beetles by Lecaillon ('98), and by Friederichs ('06), in *Tenebrio molitor* by Saling ('07), and in Chrysomelid beetles by Hegner ('08, '09, '09b, '11a, '11b) and Wieman (10a', 10b', '10c). Of these only the work of Wheeler, Lecaillon, Hegner, and Wieman needs to be considered. Although Wheeler ('89) failed to find the pole-cells in the very early stages of *Leptinotarsa*, he figures several of them (his fig. 82) in a sagittal section of an egg carrying a segmented germ-band. Here are shown three cells "which are on the surface of the embryo in the amniotic cavity. They are very large and clear and the more anterior is apparently creeping in the manner of an Amoeba, along the surface of the abdominal ectoderm. These cells, the ultimate fate of which I have been unable to determine, probably escape from the anal orifice of the gastrula before it closes." This author also shows in transverse section (his fig. 87) a cell which, he says, is "about to wander through the blastopore into the amniotic cavity." He suggests that this may be the homologue of the 'Polzellen.' That the cells thus described by Wheeler are really pole-cells was proved by my investigations on the same species.

The embryological development of the following species of Chrysomelidae was studied by Lecaillon ('98); *Clytra laeviuscula*, *Gastrophysa raphani*, *Chrysomela menthastri*, *Lina populi*, *L. tremulae*, *Agelastica alni*. In *Clytra*, the principal form examined, Lecaillon found that the first nuclei to arrive at the posterior pole of the egg became the centers of the primitive germ-cells; these could be distinguished from neighboring cells by their

large size, larger nuclei, and more deeply staining cytoplasm. The germ-cells did not stop when they reached the surface of the egg, but passed outside and became separated from it; their number increased . . . peu à peu par suite de l'arrivée de nouvelles cellules périphériques et aussi sans doute de la division des premières cellules détachées du pôle de l'oeuf." The germ-cells then started to re-enter the egg, retarding, by this migration, the formation of the blastoderm at this point. "Finalement, le blastoderme achève de se former au pôle postérieur de l'oeuf, et alors les cellules sexuelles se trouvent groupées. . . . entre le vitellus et l'enveloppe blastodermique."

Several species of Chrysomelid beetles were also studied by Friederichs ('06), who discovered that the cleavage nuclei in *Donacia crassipes* reach the posterior later than the anterior end of the egg; the reverse is the rule in species of allied genera. After the blastoderm is formed "an der Ventralseite unmittelbar seitlich vor dem Pol, findet eine besonders lebhaftige Zellvermehrung statt, so dass einzelne Zellen aus dem Blastodermverband heraus und ins Innere gedrängt werden." These, the primitive germ-cells, were not very different in *Donacia* from blastoderm-cells, but in *Timarcha nicoeensis* and *Chrysomela marginata* they could be distinguished by the larger size and darker color of their nuclei.

In a series of papers published within the last six years, the writer has given the results of morphological and experimental studies on the primordial germ cells of Chrysomelid beetles, particularly *Calligrapha bigsbyana*, *C. multipunctata*, *C. lunata*, and *Leptinotarsa decemlineata*. It has been possible to trace the entire Keimbahn in these insects, and to carry on experiments with the eggs and embryos without preventing further development. The reader is referred to the original papers for details, but a general account will be given here as an introduction to the original work to be presented in the succeeding pages.

At the time of deposition, the eggs of the Chrysomelid beetles studied are not always in the same stage of development, although usually polar body formation is taking place. The egg

figured (fig. 7, A) was fixed four hours after deposition. The polar bodies have already been produced and the male and female nuclei are in the act of conjugation. The egg consists of a large central mass of yolk and a comparatively thin peripheral layer of cytoplasm, the 'Keimhautblastem' of Weismann. The interdeutoplasmic spaces are filled with cytoplasm which is connected with the 'Keimhautblastem' by delicate strands of the same material. The enormous amount of yolk contained in these eggs makes the identification of other substances extremely difficult. The yolk-globules range in size from large deutoplasmic spheres to small granules, and, as the dissolution of some of them is continually taking place, one is unable to determine where yolk ends and cytoplasm begins. The only accumulations of cytoplasm large enough for examination are those surrounding the nuclei within the yolk mass, and the peripheral layer, the 'Keimhautblastem.' No differences in composition or staining qualities were observed between the cytoplasm of these two regions. The 'Keimhautblastem' consists of a fluid ground substance in which are suspended very fine granules. It is a homogeneous layer of cytoplasm everywhere except at the posterior end of the egg. At this point there is a disc-shaped mass of larger granules imbedded within the inner portion of it. These granules stain deeply with haematoxylin. They are easily seen, not only in sections but also in eggs that have been properly stained in toto. Because of their ultimate fate I have called these granules the germ-cell determinants (fig. 7, A, *g.c.d.*).

The first cleavage divisions take place where the pronuclei fuse. The daughter nuclei move away from each other and as cleavage progresses a separation of the nuclei into two sections occurs. The nuclei of one group form a more or less regular layer equidistant from the periphery; these preblastodermic nuclei (fig. 7, B, *pbl.n*) move outward and fuse with the Keimhautblastem. Cell walls now appear for the first time and a blastoderm is formed of a single layer of regularly arranged cells.

The genesis of the pole-cell is as follows: (1) four nuclei lying near the posterior end of the egg are recognized by their

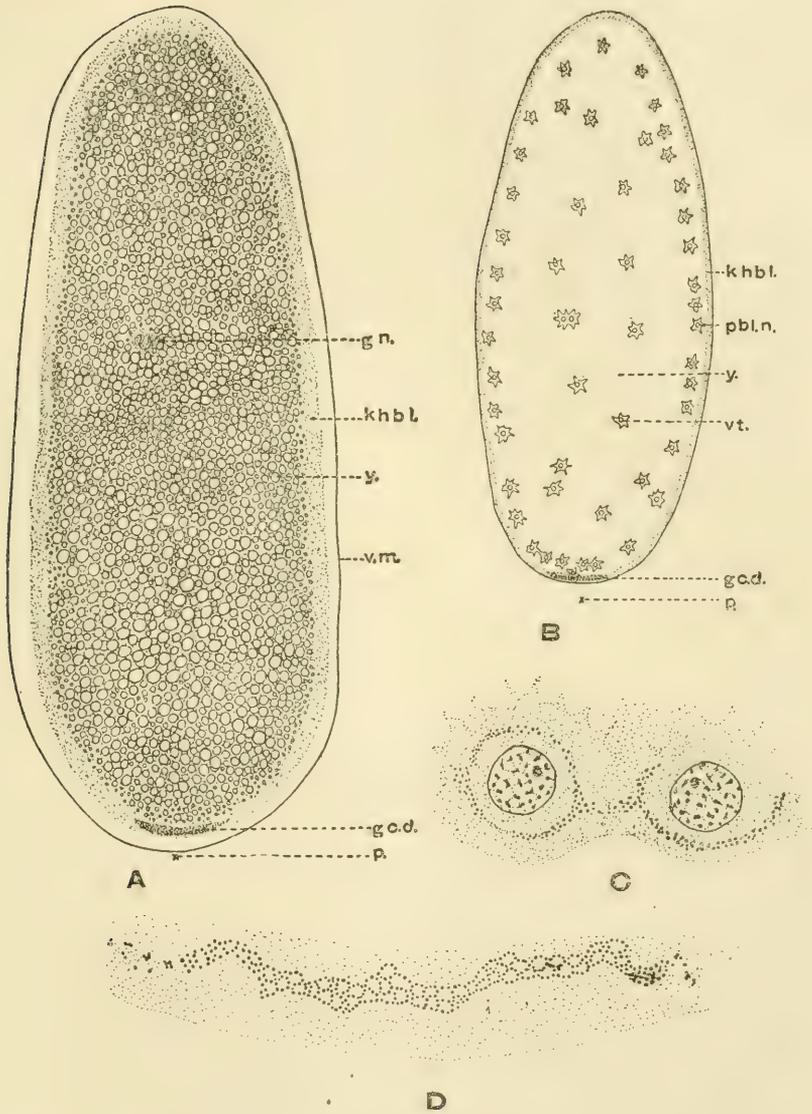


Fig. 7 Calligrapha (from Hegner, '09a and '09b). A, longitudinal section through an egg of *C. bigsbyana*, four hours after deposition. B, longitudinal section through an egg of *C. bigsbyana* 14 hours after deposition. C, two germ cells just protruding from posterior end of egg of *C. multipunctata*. D, the pole-disc in an egg of *C. multipunctata*. *g.c.d.*, pole-disc; *g.n.*, germ nuclei fusing; *khbl*, keimhautblastem; *p*, posterior end of egg; *pbl.n.*, preblastodermic nuclei; *v.m.*, vitelline membrane; *vt.*, vitellophags; *y*, yolk.

position as pole-cell antecedents; (2) these four nuclei divide producing eight daughter nuclei which move closer to the periphery of the egg; (3) these in turn divide resulting in sixteen nuclei, arranged in pairs, each of which separates entirely from the egg, carrying with it a portion of the Keimhautblastem containing pole-disc granules (fig. 7, *C*); (4) the sixteen primary pole-cells divide to form thirty-two secondary pole-cells; these divide resulting in sixty-four tertiary pole-cells which do not increase in number until a late period of embryonic life; (5) in mitosis the pole-disc granules are approximately equally distributed between the two daughter cells (fig. 8, *B*). After separation from the egg the pole-cells are (1) carried slightly forward on the ventral surface of the egg by the contraction of the ventral plate; (2) they sink into the posterior depression of the ventral groove, which is the beginning of the posterior amniotic cavity; (3) they are carried along by the developing tail-fold, which penetrates dorso-anteriorly into the yolk; (4) they migrate through a pole-cell canal into the embryo by means of amoeboid movements; (5) upon reaching the interior of the embryo they separate into two groups, which come to lie, one on either side of the body, in the last two abdominal segments; (6) these two strands become shorter by a crowding together of the germ-cells; (7) each of the two germ-glands thus produced acquires an epithelial covering of mesoderm-cells; (8) the germ-glands, situated as before in the last two abdominal segments, are carried, by the shortening of the embryo, to a ventral position on either side of the body; (9) by its lateral growth around the yolk, the embryo carries the germ-glands to a point near the dorsal surface on either side of the mid-gut; (10) the sexes can be distinguished at this time by the shape of the germ-glands, that of the male being dumb-bell shaped, while the female reproductive organ is pear-shaped, and shows the development of terminal filaments.

In all stages the germ cells may be distinguished easily from the surrounding somatic cells. Figure 8, *A* shows a pole cell shortly after separation from the egg. The pole-disc granules are quite conspicuous, and pseudopodia-like projections are

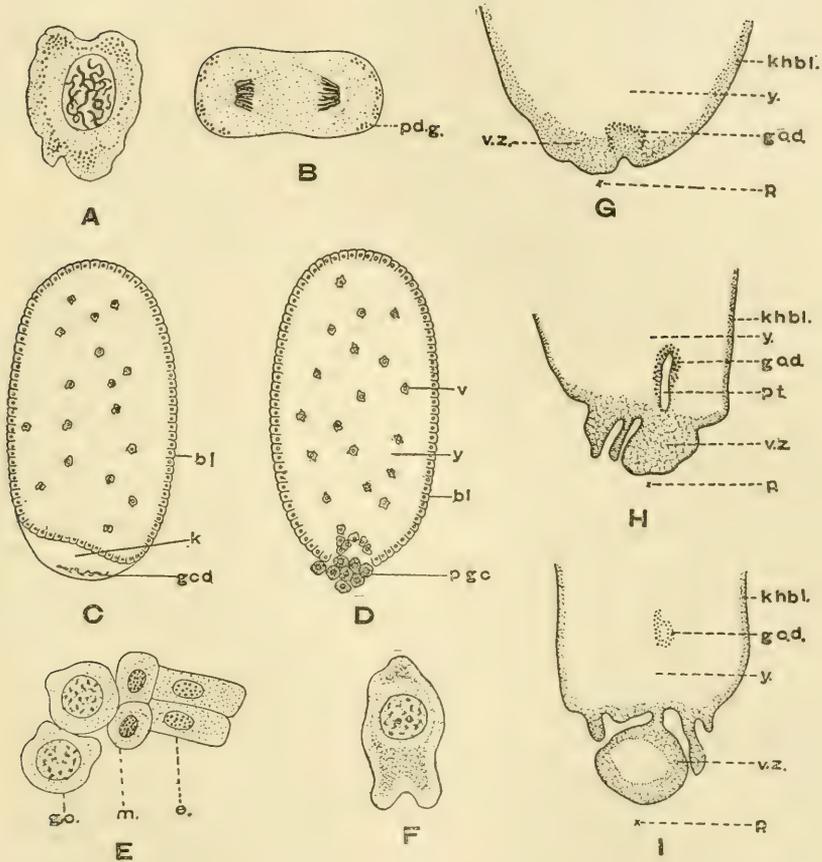


Fig. 8 *Calligrapha* (from Hegner, '09a, '09b, '11a). A, a germ cell of *C. multipunctata* shortly after being cut off from the egg. B, division of a primordial germ cell. C, longitudinal section through egg of *C. bigsbyana* at blastoderm stage; the posterior end was killed with a hot needle just after deposition. D, longitudinal section through uninjured egg at same stage. E, two ectoderm cells (*e*), two mesoderm cells (*m*), and two germ cells (*g.c.*) from an egg three days old. F, germ cell during migration into the embryo (three days old). G.H.I, longitudinal sections through eggs centrifuged for one hour, two hours and four hours respectively. *bl*, blastoderm; *g.c.d.*, granules of pole-disc; *k*, killed portion of egg; *khbl*, keimhautblastem; *p*, posterior; *p.g.c.*, primordial germ cells; *v*, vitellophags; *v.z.*, vesicular zone; *y*, yolk.

plainly evident. Sixteen pole-cells were present at this time. After a mitotic division, during which the pole-disc granules are apparently approximately equally divided between the daughter cells (fig. 8, *B*), the pole cells are smaller, but, although no larger than the neighboring blastoderm cells, they may still be distinguished by the presence of the pole-disc granules and also by the larger nucleus containing a lesser number of chromatin granules. Pole-disc granules are still faintly visible at a later period when the germ cells are migrating into the embryo through the pole-cell canal, and still later, as figure 8, *E* shows, the germ cells can be distinguished easily from the ectoderm and mesoderm cells although the pole-disc granules have entirely disappeared.

The pole-disc. The pole-disc varies somewhat in compactness but in most cases appears in section as shown in figure 7, *D*. Nothing resembling it occurs in other parts of the egg. Its granules are very susceptible to stains and can be made visible by means of a number of different dyes. The 'Keimwulst' of *Chironomus* (Ritter '90), the 'Dotterplatte' of *Calliphora* (Noack, '01; fig. 2) and the 'Keimbahnplasma' of *Chironomus* (Hasper '11; fig. 1) all present a similar appearance. In these forms, as well as in the Chrysomelid beetles I have studied, all or nearly all of the granules (fig. 7, *C*) are taken out of the egg by the pole-cells. Wieman ('10a) however, gives a figure showing a section of the posterior end of the egg of *Leptinotarsa signaticollis* after the protrusion of the pole cells, in which there is still represented what he calls the pole-disc. The fact that the mass of granules described by Wieman does not resemble the pole-disc as I have found it, nor other similar accumulations in insect eggs (Keimwulst, Dotterplatte, Keimbahnplasma) and the statement that "the granules are not all taken up by the cells in their migration and the greater part of them remains behind after the cells have passed through" (Wieman, '10a, p. 186), a condition contrary to that described by every one of the writers cited above, lead to the conclusion that Wieman has confused something else for the pole-disc. This seems all the more probable, since the species studied by Wieman, namely *Leptinotarsa signaticollis*, is very

closely allied to one of the species that I investigated (*L. decemlineata*) in which a typical pole-disc like that shown in figure 7, *D* occurs. Furthermore the cells which Wieman designates as pole cells have none of the characteristics of the pole cells described by other writers.

Several important results have been obtained by experiments that I have performed with the object of determining the character and significance of the pole-disc. When the freshly laid eggs of *Leptinotarsa decemlineata* are centrifuged with the posterior end toward the center of revolution the pole-disc is moved gradually toward the outer anterior end as shown in figure 8, *G,H,I, g.c.d.* The movement *en masse* of the pole-disc granules proves that they are heavier than the oil globules of the vesicular zone (*v.z.*) and indicates that they do not form an adventitious accumulation but constitute a definite structure of sufficient rigidity to withstand the dispersing effects of a strong pull exerted during a period of at least four hours. It was hoped that by means of centrifugal force the pole-disc could be located in a part of the egg different from that normally occupied and that experimental proof of the necessity of their presence for the formation of germ cells might thus be obtained, but the abnormal development of the eggs prevented an accurate determination of the various cells in sections of these centrifuged eggs.

Two sets of experiments were undertaken in an attempt to deprive the eggs of the pole-disc. If the embryo developing from an egg without the pole disc failed to possess germ cells the obvious conclusion would be that the pole-disc consists of real germ cell determinants, necessary for the differentiation of germ cells. In the first set of experiments the freshly laid eggs were oriented and then pricked with a sharp needle in the center of the posterior end. Since the egg is turgid a small drop of the contents was forced out. Eggs treated in this manner continue to develop producing embryos and larvae apparently normal. Sections of these seemed to show that less than the characteristic number of germ cells were present. My inability, however, to determine whether all of the pole-disc had been

removed constituted a source of error which made the results uncertain.

A second set of experiments (Hegner '11a) were therefore devised and a method employed which made it absolutely certain that the pole-disc could not take part in the development. In these experiments the posterior end of the egg was touched with a hot needle and that portion containing the pole-disc was killed. In every instance the development continued and the blastoderm formed normally over all of the surface except at the posterior end; here it was built at the end of the living substance as shown in figure 8, *C, bl.* No germ cells were produced, as in the normally developed egg at this stage (fig. 8, *D*). I conclude from this that the pole-disc granules or the substances in which they are imbedded *are* necessary for the formation of germ cells.

B. Nuclear division and differentiation in the eggs of Chrysomelid beetles

This work was undertaken in order (1) to determine whether or not a chromatin diminution process takes place in the cleavage nuclei of Chrysomelid eggs similar to that described in *Ascaris* by Boveri ('92) and in *Miastor* by Kahle ('08); (2) to study the differentiation of the nuclei which take part in the formation of the primordial germ cells, blastoderm cells, and vitellophags; and (3) to record what appears to be amitotic nuclear division among the vitellophags.

General account of early cleavage. Eggs of *Calligrapha multipunctata*, *C. bigsbyana*, and *Leptinotarsa decemlineata* have been used for this purpose. Beetles were kept in the laboratory in Stender dishes and closely watched, so that the exact interval between the time of deposition and time of fixation could be determined. Eggs were killed in Tower's second solution heated to the boiling point. The chorion was dissected off after a few days and no difficulty was encountered in imbedding in paraffin and sectioning. Because of the abundance of yolk, Mayer's acid hemalum was used principally for staining pur-

poses although iron hemotoxylin was also employed. The hemalum does not stain the yolk if properly used. It is frequently difficult to stain the early cleavage nuclei, but several hundred preparations have furnished all the necessary stages required. Division figures are comparatively rare and one can only conclude that mitosis takes place very rapidly and is followed by a long period of rest.

The stage of development is not the same in all eggs at the time of deposition, but usually the maturation divisions are in progress or the first cleavage has begun. As shown in figure 44, the polar bodies are produced about half way between the anterior and posterior ends of the egg. The nucleus of the egg lies in an accumulation of cytoplasm at the periphery. Two polar bodies are formed; then the female nucleus with its surrounding cytoplasm moves out toward the center of the egg and copulates with the male nucleus (figs. 7, A, and 45). The first cleavage occurs at this point. The cleavage nuclei, each with a small portion of cytoplasm, then move apart and divide almost synchronously. Figures 44 to 51 were made from longitudinal sections of eggs of various stages and all of the nuclei are here represented in a single plane, but their relative positions are indicated as determined by superimposing sketches made with a camera lucida. It will be seen that the nuclei are more numerous near the anterior end of the egg and that they become rather evenly scattered throughout the yolk. There are of course no cell walls and the thin strands of cytoplasm between the yolk globules connect the cleavage nuclei. Each nucleus lies in a sort of ameboid island of cytoplasm, forming a body equivalent to a cell, but probably better designated by the term energid (Sachs, '92).

In figure 46 two energids are shown just after the first cleavage division. Figure 47 represents the division of the first four energids to form eight; those lettered *a* and *c* are in the anaphase of mitosis, whereas *b* and *d* have reached the telophase. The relative positions of the first four energids is indicated in figure 48 which was reconstructed from a series of transverse sections. Here a condition exists quite similar to that shown in figure 47;

the nuclei *c* and *d* are in the anaphase and *a* and *b* in the telophase stages of mitosis. The egg from which figure 49 was made contained eight energids; that represented by figure 50 possessed sixteen. So far as could be determined the nuclei of these energids are all alike. One of them is shown in figure 52. One hundred and thirty-three nuclei were counted in the egg shown in figure 51; these are still alike. Soon after this stage is reached part of the energids move out to the periphery and fuse with the 'Keimhautblastem' to form the blastoderm (fig. 7, *B*), whereas the rest remain behind among the yolk globules and become vitellophags. Those energids which encounter the pole-disc do not take part in the blastoderm formation but become the primordial germ cells.

Does a chromatin-diminution process occur in Chrysomelid eggs? The fact that part of each chromosome is cast out into the cytoplasm in all except the 'stem-cell' during the early cleavage of *Ascaris* is well known (fig. 19, p. 442). A similar process was described by Kahle ('08) in *Miastor metraloas* and confirmed by me (Hegner, '12) in *Miastor americana* (fig. 3, p. 392). This chromatin-diminution process results in the formation of a single primordial germ cell containing the complete amount of chromatin and a number of somatic cells with a reduced amount of chromatin. The origin of the germ cells has been carefully studied in a number of forms which in other respects resemble *Ascaris* and *Miastor*, but in none of them has such a process been discovered. Hasper ('11) was unable to establish it for *Chironomus* which is very similar to *Miastor* in early development, nor has such a phenomenon been found in *Sagitta* (Elpatiewsky, '09, '10; Stevens, '10; Buchner, '10a, '10b) and the copepods (Haecker, '97, Amma, '11) and *Cladocera* (Kuhn, '11, '13) which undergo total cleavage and are otherwise similar to *Ascaris*.

The nuclear divisions in the eggs of Chrysomelid beetles have been examined by the writer with considerable care, but nothing resembling a diminution process was found. Furthermore, there are no evidences of chromatin bodies in the cytoplasm or yolk as in *Ascaris* (fig. 19) and *Miastor* (figs. 33, 34, *cR*) where the cast-out chromatin does not disintegrate immediately, but can

be distinguished for a considerable period during early embryonic development. It seems necessary to conclude therefore that in Chrysomelid eggs both germ cells and somatic cells possess the full amount of chromatin, or else the elimination of this substance takes place in some other way. This point will be more fully considered later (p. 465).

The differentiation of the nuclei of the blastoderm cells, primordial germ cells, and vitellophags. The conclusion that no chromatin-diminution process occurs during the early cleavage divisions in the eggs of Chrysomelid beetles necessitates the search for some other method of differentiation among the cleavage nuclei. The insect egg is particularly advantageous for testing Roux's hypothesis of qualitative nuclear divisions, since we have here the production of an enormous number of nuclei before any cell walls are formed, and an egg that is remarkably definitely organized, as indicated by my experiments (Hegner, '09b, '11a), before the blastoderm is formed.

I have been unable to find any differences in the nuclei before the energids fuse with the keimhautblastem, but as soon as this does occur, a gradual change takes place, and at the time when the blastoderm is completed three sorts of nuclei are distinguishable: (1) The nuclei of the primordial germ cells (fig. 53) are larger than the others and contain comparatively few spherical chromatin granules evenly distributed. The cytoplasm of these cells is distinguishable from that of all other cells because of the presence of granules from the pole-disc; (2) The nuclei of the blastoderm cells (fig. 54) are small and completely filled with large spherical chromatin granules; (3) The nuclei of the vitellophags (fig. 55) resemble the early cleavage nuclei; they are midway between the other two kinds in size, and their chromatin is more diffuse.

Whether these three kinds of nuclei were all potentially alike before their differentiation is an important question. Visibly they are all similar until they become localized in definite regions of the egg, and associated with particular cytoplasmic elements. One can but conclude that they were all potentially alike and that their differentiation was brought about through the influence

of the cytoplasm in which they happened to become imbedded. The writer has shown (Hegner, '11a) that if the posterior end of a freshly laid egg of *Leptinotarsa decemlineata* is killed with a hot needle, thus preventing the pole-disc granules and surrounding cytoplasm from taking part in development, no primordial germ cells will be produced. A large series of similar experiments have also proved that at the time of deposition, "The areas of the peripheral layer of cytoplasm (fig. 7, A, *khbl.*) are already set aside for the production of particular parts of the embryo, and if the areas are killed, the parts of the embryo to which they were destined to give rise will not appear. Likewise, areas of the blastoderm are destined to produce certain particular parts of the embryo" (Hegner '11a, p. 251). What becomes of the nuclei which are prevented from entering the injured region of the egg? No evidence has been discovered to indicate that they disintegrate, so they probably take part in development after becoming associated with some other part of the egg. If these nuclei were qualitatively different they should produce germ cells and other varieties of cells in whatever region they chance to reach. It is evident that they are not potentially different and that their 'prospective potency' and 'prospective significance' do not coincide. The cytoplasm is, therefore, the controlling factor at this stage in the germ-cell cycle, although cytoplasmic differentiations are for the most part invisible and probably the result of nuclear activity during earlier stages.

Amitotic nuclear division in vitellophags. The cleavage nuclei in Chrysomelid eggs all divide by mitosis, but, after the blastoderm has become established, the vitellophag nuclei, which remain behind in the yolk, show evidences of amitotic division. At this stage of development the vitellophags are more or less evenly distributed within the yolk mass. Here and there groups of from two to four or more nuclei are present, indicating that several divisions have occurred in quick succession. All of the stages characteristic of amitotic nuclear division are abundant. Three of them are shown in figure 56; nucleus *a* is just becoming dumb-bell-shaped; nucleus *b* has almost become constricted into

two; and nucleus *c* is apparently undergoing a second amitotic division before the first division is actually completed—the result would probably have been a row of four nuclei. As recorded by Child ('07), Patterson ('08), Maximow ('08), Wieman ('10b) and others, amitosis here likewise occurs in rapidly dividing nuclei and is probably due to some physiological condition.

A type of amitosis differing from that just described was discovered in eggs that had been subjected to the action of centrifugal force. The three nuclei shown in figure 57 are from vitellophags of an egg of *Leptinotarsa decemlineata* which was centrifuged for sixteen hours after deposition and was then fixed immediately. The chromatin in nuclei *a* and *b* forms rather condensed clumps in the center of the nucleus and the nuclear membrane appears to force its way through the center of this clump thus bringing about the formation of two daughter nuclei. This membrane cannot be a cell wall since vitellophags do not possess cell walls, and this appearance cannot be due to poor fixation because other nuclei in this egg were perfectly preserved. This sort of division must therefore be a normal process or else due to some unknown influence of the centrifugal force. Various authors have contended that there are within the nuclear membrane all the elements necessary for an equal division of chromatin. This view is supported by the discovery of part or all of the mitotic figure within the nuclear membrane in certain cells, e.g., during the maturation division of *Canthocamptus* (Hegner, '08). The case just described and figured in this paper contributes to the support of this hypothesis.

C. The growth of the oocytes and development of testicular cysts in Chrysomelid beetles

The investigations on Chrysomelid beetles described in the succeeding pages were undertaken for the following purposes: (1) To study the differentiation of the nurse cells and oocytes from the oogonia; (2) to determine the origin of the pole-disc granules; (3) to discover, if possible, stages in the cycle of the

male germ cells corresponding to the formation of nurse cells in the cycle of the female germ cells; and (4) to test Wieman's ('10b) statements regarding the occurrence of amitosis in the oogonia and nurse cells, and in the spermatogonia during the formation of testicular cysts.

(1) *The differentiation of nurse cells and oocytes in the ovary of Leptinotarsa decemlineata.* So far as we know, in the majority of cases the cells which are segregated as primordial germ cells in the embryo do not produce eggs or spermatozoa. Many of them degenerate, although they probably once possessed the potentiality of true germ cells; others undergo modifications, becoming nurse cells, Sertoli cells, etc.

Among the most interesting cases of the differentiation of nurse cells from germ cells is that of *Dytiscus marginalis*. We owe detailed accounts of the process in this species to Giardina ('01), Debaisieux ('09) and Günthert ('10), but as long ago as 1886 Korschelt figured what was evidently one stage in this differentiation. Giardina ('01) established the fact for *Dytiscus* that the mitoses which result in the formation of nurse cells are differentiating, as theoretically postulated by Pauleke ('00). During the four divisions preceding the formation of the oocyte, a single oogonium gives rise to one oocyte and fifteen nurse cells. A differentiation takes place in the chromatin of the oogonial nucleus, one half consisting of a condensed mass, the other half of large granules which correspond to the forty chromosomes of the oogonium. During mitosis the chromosomes become arranged as an equatorial plate, and the chromatic mass forms a ring about it—the 'anello cromatico.' This ring passes intact to one of the daughter cells, whereas the chromosomes are equally divided. During the succeeding mitoses similar differential divisions occur, resulting in one oocyte containing the chromatic ring, and fifteen nurse cells lacking this nuclear substance. Thus, as Pauleke's theory demands, the difference between the nurse cells and the oocytes is the result of internal and not external causes.

Giardina ('01) considered the formation of the chromatic ring as a sort of synapsis, and later ('02) distinguished between

a complete synapsis, such as ordinarily occurs in the germ-cell cycle, and a partial synapsis as exhibited by *Dytiscus*. Regarding the significance of this differential mitosis, he maintains that this phenomenon is the cause of the differentiation into nurse cells and oocytes, resulting in a complete amount of chromatin in the keimbahn cells and perhaps also an unequal distribution of cytoplasmic substances. As in the cases of *Ascaris* and *Miastor*, it might better be regarded as a means of depriving the nurse cells (somatic cells or abortive germ cells?) of part of their chromatin. Moreover, Boveri ('04) has compared the chromatin diminution in *Ascaris* with Giardina's differential mitoses. Debaisieux ('09) and Günthert ('10) have confirmed Giardina's results, and the latter studied two other *Dytiscidae* (*Acilius* and *Colymbetes*) which also exhibit differential mitoses similar except in certain details. Günthert found that the chromatic ring is composed of fine granules which may split off from the surface of the chromosomes (compare with *Ascaris* and *Miastor*) and stain like cytoplasm. He interprets this as 'Zerfallsprodukte' of the chromosomes. Debaisieux, on the other hand, claims that this cast-out nuclear material is nucleolar rather than chromatic in nature.

It seems highly probable that the 'anello cromatico' of Giardina consists of chromatin, and Goldschmidt ('04) and others do not hesitate to class it as an example of a 'Chromidialapparat.' Furthermore it is apparently the result of a chromatin-diminution, as Boveri ('04) maintains, differing from the similar process in *Ascaris* and *Miastor* in details, but not in the ultimate result. Finally, the discovery of this peculiar body in *Dytiscus* adds one more argument to the hypothesis that the chromatin content of the germ cells differs from that of the somatic cells quantitatively, at least in some cases, and perhaps also qualitatively.

Many are the bodies that have been homologized with the 'anello cromatico' of *Dytiscus*. Buchner ('09, '10) claims that the nucleolar-like structure in the oogonia and young oocytes of *Gryllus* is homologous to both the accessory chromosomes of spermatogenesis and to this chromatin ring in *Dytiscus*. This 'accessorische Körper' passes intact into one half of the oocytes

where it disintegrates into granules of a 'trophische Natur.' Foot and Strobell ('11) have also compared it with the chromatin nucleolus in the oogonia of *Protenor* with which it has certain characteristics in common, but no such differential divisions occur as in *Dytiscus*.

Govaerts ('13) has recently reported upon the differentiation of the oocytes in *Carabus*, *Cicindela*, and *Trichiosoma*. He was unable to find anything resembling the chromatic ring of *Giardina*, and concludes

que la formation d'une masse chromatique, extériorisant la différenciation entre l'ovocyte et les cellules nourricières, est jusqu'à présent un fait isolé, observé uniquement chez les *Dystiscides*. Mes recherches démontrent que ce phénomène n'est pas applicable à tous les Insectes, et qu'il faut chercher au fait de la différenciation, de ces éléments une cause plus large que la répartition inégale de certains éléments chromatiques.

If no differential divisions are present, as in *Dytiscus*, what is the cause of the formation of oocytes and nurse cells? Govaerts decides that, since the ultimate oogonium possesses a definite polarity marked by the localization of the 'residu fusorial,' and the two kinds of daughter cells arise from opposite ends of the mother cell, the cause of the differentiation resides in the polarization of the oogonium. He does not, however, account for this 'polarité pre-différentielle.'

Ovaries of *Leptinotarsa decemlineata* were dissected out in Ringer's solution and placed immediately in the fixing solution. The fluids of Gilson, Altmann, Meves, and Carnoy were most frequently employed. Sections were stained by Benda's method, acid fuchsin, iron hemataxylin, and Mayer's acid hemalum. The best results were obtained with material fixed in Meves' modification of Flemming's solution and stained by the method of Benda.

The general arrangement of the cells in the ovary of an adult beetle is shown in figure 9. The terminal chamber of the ovarian tubule contains three kinds of cells, (1) nurse cells (*n.c.*), (2) young oocytes (*y.o.*) and growing oocytes, and (3) epithelial cells. The nurse cells and oocytes are both derived from the

oogonia; the epithelial cells are of mesodermal origin. As noted above, the investigations of Giardina ('01) and many others upon the genesis of the nurse cells in the ovaries of insects have established the fact that in some species a single oocyte and a definite number of nurse cells arise from a single ultimate oogonium. Wieman ('10b) has followed the history of the oogonia in *Leptinotarsa signaticollis* through the larval and adult stages, but was unable to find any evidence that the nuclei inaugurate differentiation as in *Dytiscus* (Giardina, '01; et al.). He concludes that "the process seems to be the result of several distinct cell elements which operate together as a whole" (p. 148) and that the semi-fluid matrix which results from the liquefaction of cells at the base of the terminal chamber may exert a "specific effect on those germ cells coming under its influence, enabling them to develop into ova, while the more distant germ cells become nurse cells" (p. 147). My observations agree with those of Wieman; no definite numerical relations nor nuclear evidence were discovered during the differentiation of the oogonia into oocytes and nurse cells. The data available do not suggest any method of differentiation not already proposed, and still leave the question whether the nurse cells should be regarded as abortive germ cells or true somatic cells, one of personal opinion.

(2) *The origin of the pole-disc granules in Leptinotarsa decemlineata.* The pole-disc in Chrysomelid eggs has already been described and figured, and comparisons with similar structures in the eggs of other animals will be made later (p. 461). Previous to the publication of my results ('08, '09) no granules resembling those of the pole-disc had been discovered in the eggs of Chrysomelid beetles, although Wheeler ('89), Lecaillon ('98) and others had studied various species belonging to this family. Wieman ('10a) has attempted to determine the origin of these granules, using the germ cells of *Leptinotarsa signaticollis* for this purpose. His conclusion, which was arrived at from circumstantial evidence, is "that the granules of the pole-disc consist of particles derived from the food stream of the ovum that form an accumulation in the protoplasm in its posterior part" (p. 187). In a previous paper (Hegner, '09a) I have

described and figured the pole-disc in the egg of *Leptinotarsa decemlineata* which was preserved shortly before deposition. Since that time a complete series of oocytes have been prepared and examined for the purpose of tracing the history of these granules.

The positions of the stages to be described are indicated in the diagram (fig. 9) and the nuclear and cytoplasmic structures are shown in figures 58 to 67. Two oocytes and a neighboring epithelial cell from position 58 in figure 9 are shown in figure 58. The nuclei of the oocytes are large and contain a distinct spireme; the cytoplasm is small in amount and apparently homogeneous. After a short period of growth, the oocytes form a linear series in the ovarian tubule and become connected with the spaces between the nurse cells by means of eggs strings (fig. 9, *e.s.*) through which the nutritive streams flow into the oocytes. One of the youngest of these oocytes is represented in figure 59 (position 59 in fig. 9). The nucleus is not larger than in those of the earlier stage; its chromatin forms a reticulum, and a distinct nucleolus is present. The cytoplasm, on the other hand, has trebled in amount and within it are imbedded a number of spherical bodies which stain with crystal violet after Benda's method, and appear to be mitochondrial in nature. At a slightly later stage (fig. 60, position 60 in fig. 9) the nucleus is larger and contains several small spherical chromatic bodies besides the nucleolus. The cytoplasm has increased more rapidly in volume and a corresponding increase in the number of mitochondrial granules has also taken place. Further growth results in an increase in the volume of both nucleus and cytoplasm (fig. 61, position 61 in fig. 9), and a slight increase in the number of mitochondria. Whether these bodies developed *de novo* or by division of the preexisting granules could not be determined.

In succeeding stages growth is very rapid. The cytoplasm (fig. 62, position 62 in fig. 9) still remains homogeneous except for the mitochondria which increase slightly in size and become situated as a rule near the periphery. The nucleus at this time contains a large number of chromatin granules and a diffuse reticulum. Part of an older oocyte is shown in figure 63 (posi-

tion 63 in fig. 9); the cytoplasm has taken a reticular appearance; the mitochondrial granules are present in greater numbers, and the nucleus is larger, oval in shape and contains a distinct reticulum with many chromatin bodies of various sizes. A still older oocyte (fig. 64, position 64 in fig. 9) is interesting, particularly because of the rapid increase in the mitochondria and the localization of these near the periphery. From this stage on the character of the contents changes until, as shown in figure 9, the central part of the oocyte (*ooc*) consists of homogeneous cytoplasm (*cy*), and the outer region of cytoplasm is crowded with granules and spherical bodies of various sizes. Apparently the mitochondria lying near the periphery (figs. 65 and 66) increase in size, gradually losing their affinity for the crystal violet stain and swelling up until they constitute the large yolk globules so numerous in the mature egg. All stages in the evolution of these bodies are illustrated at this time as represented in figure 66. In the meantime material is brought into the egg through the egg string from the nurse cells, thus probably adding several sorts of granules to the contents of the oocyte.

To determine the origin of the pole-disc granules it is necessary to trace the various bodies in the oocytes and the nurse stream up to the time when the pole-disc appears. An egg just before this structure becomes visible the posterior end, as shown in figure 66, consists of cytoplasm, more or less reticular, containing yolk globules of various sizes and a number of small granules of a mitochondrial nature. It is impossible at present to state definitely, however, that these granules increase in number by division to form the pole-disc, or are added to from neighboring regions, since no intermediate stages between the condition here represented and that of the completely formed pole-disc were discovered. Wieman ('10a) claims that the pole-disc granules come from the nutritive stream. I admit that this may be true, but it seems more probable from my preparations that they evolve from granules of mitochondrial nature which, as we have seen, may be traced from the young oocytes.

The origin of the pole-disc granules has not, therefore, been definitely determined and, as in previous communications, it

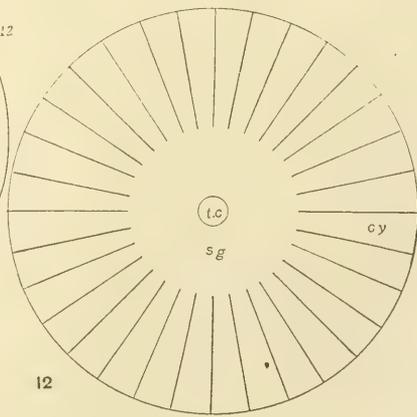
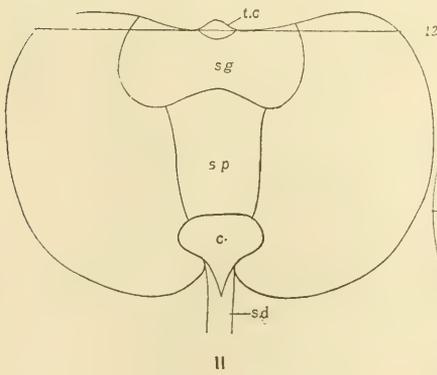
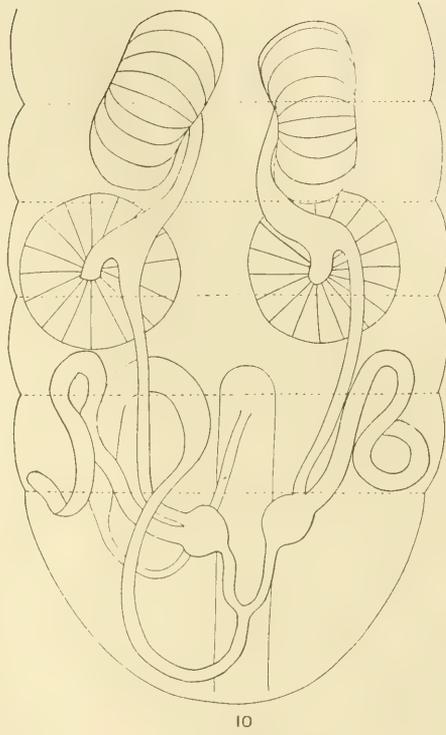
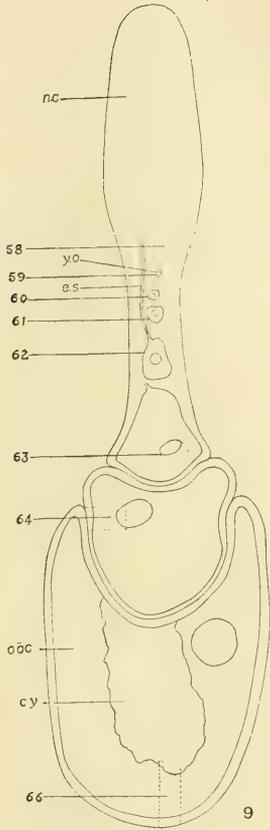
is only possible to suggest that they may be derived from (1) the cytoplasm of the egg, (2) the cytoplasm of the nurse cells, (3) chromatin from the germinal vesicle, (4) chromatin from the nurse cells, (5) nucleolar substance from either germinal vesicle or nurse cells or both, and (6) bodies of mitochondrial nature.

(3) *The spermatogonial divisions and formation of cysts in the testes of Leptinotarsa decemlineata.* The material on which this section is based consists of a complete series of stages from the time the male germ gland can be recognized in the half grown embryo to the adult condition. Embryos and young larvae were fixed in toto in hot Tower's fluid and sectioned entire. The germ glands of older larvae, pupae, and adults were dissected in Ringer's solution and transferred at once to the desired fixing fluid. Meves', Gilson's, and Carnoy's solutions were chiefly used for this purpose. Sections were stained with iron hematoxylin, Mayer's acid hemalum, and safranin and light green. All of the figures were drawn from preparations made from material fixed in Carnoy and stained with iron hematoxylin.

The form and position in the abdomen of the adult male reproductive organs are shown in figure 10. There are two pairs of testes, one pair on either side of the body. Each testis consists of a large number of follicles radiating out from near the center. Figure 11 is a diagram of a longitudinal section made from the testis of an old larva. At the lower end is attached the sperm duct (*s.d.*) which is connected with a cavity (*c.*) within the testis. Just above this cavity is a region containing spermatozoa; above this region is a mass of spermatogonia (*sg.*) not yet within cysts; and this mass is capped by a small group of epithelial cells (*t.c.*). The major part of the testis is composed

Fig. 9 *Leptinotarsa decemlineata*; diagram of an ovarian tubule showing various stages in the development of the oocyte. The figures refer to the positions of cells shown in the figures in plate 9.

Figs. 10 to 12 *Leptinotarsa decemlineata*. 10, Reproductive organs of male. 11, Longitudinal section through testis of full grown larva. 12, Transverse section through testis of full grown larva in region indicated by line labelled 12 in fig. 11. *c.*, cavity of testis; *cv.*, region of cysts; *s.d.*, sperm duct; *sg.*, spermatogonia; *sp.*, spermatozoa; *t.c.*, terminal cap.



of radiating follicles containing cysts of spermatogonia, spermatocytes, or spermatozoa (*cy*). A transverse section through the distal end of a testis is shown in figure 12 (position 12 in fig. 11). In the center is the terminal cap (*t.c*); surrounding this is a mass of spermatogonia not yet formed into cysts (*sg*); and from this the testicular follicles radiate out to the periphery. Wieman ('10b, '10c) has traced the development of the testis through the larval and pupal stages and I have nothing to add to his account. I wish, however, to describe certain stages in the divisions of the spermatogonia during cyst formation, since Wieman has reported the occurrence of amitotic nuclear division at this time, and I have discovered some cellular connections which makes it possible to compare certain processes in the testis with those known to take place during the differentiation of nurse cells and oocytes in the ovaries of several insects.

In that region of the testis surrounding and underlying the terminal cap (figs. 11, 12, *t.c*) there are a large number of spermatogonia not yet contained in cysts. All stages in cyst formation may be observed here, not only in larval testes but also in those of pupae and adults. The youngest spermatogonia are those lying near the terminal cap. Figure 68 shows a few cells of the terminal cap (*t.c.*) some of the neighboring spermatogonia (*spg*), and several of the epithelial cells (*ep*) which are scattered about among the spermatogonia. Cysts are formed toward the edge of the spermatogonial mass away from the terminal cap and figures 69 to 74 represent certain of the stages observed. The spermatogonia divide apparently exclusively by mitosis. A well developed spindle is formed and this persists after the cell wall has separated the two daughter cells. The spindle fibers which are at first perfectly distinct (fig. 69) unite into a compact strand (fig. 70) which stains dense black in iron hematoxylin after fixation in Carnoy's fluid. In many cases it was impossible to distinguish an intervening cell wall between the daughter nuclei (fig. 71). In either case, however, the spindle remains persist, forming a basic-staining strand with enlarged ends connecting the two nuclei. Since at this time and in all later stages the two or more spermatogonia may be found surrounded

by an envelope of epithelial cells it seems certain that, as Wieman ('10b) maintains, the spermatozoa in a single cyst are derived from a single spermatogonium.

A cyst containing four spermatogonia is represented in figure 72. Here again appear the strongly basic-staining spindle remains connecting the nuclei. These black strands persist until the succeeding mitotic division occurs as figure 73, which was drawn from a section of a cyst containing eight spermatogonia, shows. Spindle remains are still evident in later stages, as in figure 74, which represents a cyst containing thirty-two spermatogonia, but were not observed in cysts containing more than sixty-four cells.

Many investigators have figured spermatogonial divisions which result in rosette-like groups of cells similar to that represented in figure 73. Apparently, however, the remains of the spindle, if present, did not possess such a strong affinity for basic stains. Furthermore, only those of my preparations which were fixed in Carnoy's fluid and stained in iron hematoxylin exhibited these black strands. Similar spindle remains have been observed in *Dytiscus* (especially by Günthert, '10) and *Carabus* (Govaerts, '13) during the differentiation of nurse cells and oocytes from oogonia, and there can be little doubt but that the process of cyst formation in the male, as described above, is similar to the differential divisions in the female and may also be compared with the differentiation of spermatocytes and Sertoli cells in mammals (Montgomery, '11; von Winiwarter '12).

In *Dytiscus* Günthert found that the chromatic mass eliminated from the nucleus always passed to the pole of the oogonium containing the 'Spindelreste.' Govaerts ('13) has pointed out that, although in *Carabus* and *Cicindela* no such chromatic mass is demonstrable, still a distinct 'residu fusorial' exists, and that during the differential divisions one cell differs from its sister in the possession of these spindle remains. Polarity is held responsible for the localization of the 'residu fusorial' and the cause of the differential divisions is therefore considered to be a 'polarité pre-differentielle.'

The discovery of these distinct spindle remains in the spermatogonial divisions enables us to homologize one more period in the cycle of the male germ cells with a corresponding period in the cycle of the female germ cells.

Thus the ultimate spermatogonium passes through a certain number of divisions—probably five or six—which correspond to the differential divisions so clearly exhibited by the ultimate oogonia of *Dytiscus*. Just as in the maturation processes, however, where only one female cell but all of the male cells are functional, so these earlier divisions result in the female in the production of a single oocyte and a number of nurse cells which may be considered abortive eggs, whereas in the male every daughter cell is functional. The limited period of division in the cycle of the male germ cells in man (Montgomery, '11; von Winiwarter, '12) is also similar to those in *Dytiscus* and *Leptinotarsa*.

(4) *Amitosis in the germ cells of Leptinotarsa*. We have already described what appears to be amitotic nuclear division in the vitellophags of Chrysomelid eggs, and shall now examine certain stages in the germ cell cycle where amitosis has been reported.

Wilson ('00) defines amitosis as "mass-division of the nuclear substance without the formation of chromosomes and amphiaster" (p. 437), and concludes from a review of the literature up to the year 1900, "that in the vast majority of cases amitosis is a secondary process which does not fall in the generative series of cell-divisions" (p. 119). During the past ten years interest in direct nuclear division has been maintained, principally because of the claims of certain investigators that germ cells may multiply in this way and still give rise to functional eggs or spermatozoa.

During amitosis the chromatin remains scattered within the nucleus and does not form a spireme nor chromosomes, and therefore its individual elements, the chromatin granules, do not divide. As a result of this *mass*-division there can be no accurate segregation of chromatin granules in the daughter nuclei as is demanded by the theory that the nucleus, and partic-

ularly the chromatin, contains the determiners of hereditary characteristics. Furthermore, nuclear division without the formation of chromosomes obviously condemns the hypothesis of the genetic continuity of the chromosomes, and hence seriously interferes with current ideas regarding the significance of the accessory chromosomes in the determination of sex. Among the animals in whose germ cells amitosis has been reported are certain amphibia, coelenterates, cestodes, and insects.

Amphibia. Vom Rath ('91, '93), Meves ('91, '95) and McGregor ('99) have recorded amitosis in the germ cells of Amphibia. Meves claims that the spermatogonia of *Salamandra* divide amitotically in the autumn but return to the mitotic method in the spring, later giving rise to functional spermatogonia. Vom Rath finds amitosis but contends that the cells which divide in this way do not become spermatozoa but are degenerating, being used as nutritive material by the other spermatogonia. The amitotic divisions described by McGregor ('99) in *Amphiuma* differ in certain respects from those of both Meves and vom Rath. In this species the primary spermatogonia divide by amitosis, their products later divide by mitosis and produce functional spermatozoa. Our knowledge concerning amitosis in the spermatogonia of Amphibia is therefore in an unsatisfactory state, although the observations of Meves and McGregor argue strongly in favor of this method.

Coelenterata. While no direct nuclear divisions were recorded by Hargitt ('06) in the germ cells of *Clava leptostyla* the absence of mitotic figures in the early cleavage stages of the egg led him to the conclusion that the 'nuclear activity differs greatly from the ordinary forms of mitosis, and appears to involve direct or amitotic division' (p. 229). If this were true the germ cells which are derived from these cleavage cells must be descended from cells which once divided amitotically. This case of supposed amitosis has been cleared up by the subsequent studies of Beckwith ('09) who collected material of *Clava* very early in the morning and found typical mitotic divisions during the maturation and early cleavage of the egg and no evidence of amitosis.

Cestoda. Child concluded ('04) from a study of the cestode *Moniezia expansa* that the amitotic method of cell division occurs in the antecedents of both the eggs and the spermatozoa. This writer has published a series of papers upon this subject using *Moniezia expansa* and *M. planissima* for his material ('04, '06, '07, '10), and his principal conclusion is that in these species the division of the cells destined to become eggs and spermatozoa is predominantly amitotic. Mitotic division also occurs but comparatively rarely. Cells which have divided amitotically then divide mitotically during maturation and form typical ova.

The nature of the nuclear division in the cestodes was later investigated by Richards ('09, '11) who studied the female sex organs of the same species employed by Child as well as material obtained from *Taenia serrata*. Richards finds that mitosis unquestionably occurs in the young germ cells but was unable to demonstrate amitosis. Richards claims that amitosis cannot be demonstrated except by the observation of the process in the living material and the subsequent study of this material by cytological methods. Child ('11) agrees with Richards that amitosis cannot be demonstrated in fixed material but nevertheless concludes after an examination of Richards' preparations "that direct division plays an important part in the developmental cycle of *Moniezia*, in the germ cells as well as in the soma" (Child, '11, p. 295).

Finally Harman ('13) was unable to find any evidence of amitotic divisions in the sex cells of either *Taenia teniaeformis* or *Moniezia* and concludes that the conditions which suggest amitosis can just as well or better be explained by mitosis. Experiments with living cells of *Taenia* were without results, since the cells did not divide when placed in Ringer's solution, although they continued to live outside the body of the host for forty-eight hours. Morse ('11) likewise failed to observe divisions in living cells of *Calliobothrium* and *Crossobothrium* which were kept in the plasma of the host.

Insecta. In the Hemiptera amitosis was described by Preusse ('95) in the ovarian cells of *Nepa cinerea* and similar conditions were reported by Gross ('01) in insects of the same order. Gross,

however, claims that the cells which divide amitotically do not produce ova but are degenerating or secretory.

Foot and Strobell ('11) described in ovaries of *Protenor*, the amitotic division of certain cells which later produce ova. There is, however, considerable difference of opinion among investigators as to the origin of the ova from the various regions of the insecta ovary and, since Payne ('12) has shown that in *Gelastocoris* the cells which apparently multiply amitotically do not produce ova, it seems safe to conclude that in *Protenor* the ova are not descended from cells that divide amitotically.

Amitotic division of germ cells followed by mitotic division has been described by Wieman ('10b, '10c) in the ovaries and testes as well as in the nurse cells of *Leptinotarsa signaticollis*. Germ cells in both ovary and testis taken from full grown larvae were found in stages of division recognized by Wieman as amitotic. It was difficult to demonstrate actual division of the cytoplasm but that such a division really occurs was inferred because binucleated cells apparently gave rise to spermatocytes with single nuclei. Rapid cell division is assumed by Wieman to account for amitosis. This is brought about by fluctuations in the nutritive supply, or in the case of the testis, by the rapid proliferation of cells during the formation of cysts.

I have studied my preparations of Chrysomelid beetles carefully with the aim of detecting amitotic division and have observed what appears to be direct nuclear division among the nurse cells, but could not demonstrate with certainty this kind of division among the oogonia, or spermatogonia. Three stages in the direct division of nurse cell nuclei in *Leptinotarsa decemlineata* are shown in figure 67. Oogonia and spermatogonia, however, do not exhibit such clearly defined stages, and after examining my preparations and several slides kindly sent me by Doctor Wieman I am forced to conclude that amitosis has not been demonstrated. It is true that frequently dumb-bell shaped nucleoli occur in certain of the nuclei and frequently two nucleoli are present at opposite ends. Also two nuclei may be surrounded by a single cell wall, but no stages were present which could not be attributed as well or better to mitotic phenomena.

Conclusion. From the evidence at present available we must conclude that amitotic division of the germ cells has not been demonstrated, and that not until such a process is actually observed in living cells will any other conclusion be possible.

4. HYMENOPTERA

A number of papers have appeared which contain references to the germ glands of Hymenoptera (Hegner, '09a, pp. 245-248). The most important of these from the standpoint of the present discussion are (1) Silvestri ('06, '08) on some parasitic species, and (2) Petrunkevitch ('01, '03), Dickel ('04), and Nachtsheim ('13) on the honey-bee.

In an endeavor to test the 'Dzierzon theory,' that the eggs which produce drone bees are normally unfertilized, Petrunkevitch ('01-'03) discovered some unusual maturation divisions. In 'drone eggs' the first polar body passes through an equatorial division, each of its daughter nuclei containing one-half of the somatic number of chromosomes. The inner one of these daughter nuclei fuses with the second polar body, which also contains one-half of the somatic number of chromosomes; the resultant nucleus with sixteen chromosomes, the 'Richtungscopulationskern', passes through three divisions giving rise to eight 'doppelkernige Zellen.' After the blastoderm is completed, the products of these eight cells lie in the middle line, near the dorsal surface of the egg, where the formation of the amnion begins; the nuclei of these cells are small, and lie imbedded in dark staining cytoplasm. Later they are found just* beneath the dorsal surface near the point of union of the amnion with the head-fold of the embryonic rudiment. They are next located between the epithelium of the mid-gut and the ectoderm; from here they migrate into the coelomic cavities, and finally, at the time of hatching, form a 'wellenartigen' strand, the germ-gland, extending through the third, fourth, fifth and sixth abdominal segments. The fertilized eggs of the bee were also examined by Petrunkevitch, but no 'Richtungscopulationskern' was discovered. In these eggs "entstehen die Genitaldrüsen

aus Mesodermzellen, die in die Mesodermröhren von der Bauchseite herindringen." Doubt was immediately cast on these results, although Weismann ('04) vouched for their accuracy. Thus Wheeler ('04) says:

Even in his first paper there is no satisfactory evidence to show that the cells regarded as derivatives of the polar bodies in the figures on plate 4 are really such, and not dividing cleavage cells or possibly vitellophags When we take up the second paper we wonder how anybody could regard the figures there presented as even an adumbration of proof that the testes of the drone are developed from the polar bodies.

Dickel ('04) could find no connection between the polar bodies and the cells Petrunkevitch claims originate from the 'Richtungskopulationskern,' but considers these 'Dotterzellen.' Nachtsheim ('13) agrees with Dickel. "Die im Blastodermstadium am Blastoporus liegenden Syncytien sind Dotterzellen, stehen also zu den Richtungskörpern in keiner Beziehung. Sie finden sich in den befruchteten und unbefruchteten Eiern in gleicher Weise, nicht, wie Petrunkevitch angegeben hat, nur in den letzteren" (p. 198).

The investigations of Silvestri ('06, '08) on parasitic Hymenoptera are of particular interest, since in both the polyembryonic species and those whose eggs produce a single individual, the keimbahn-determinant is a plasmosome which escapes from the germinal vesicle. Silvestri ('06) first studied *Copidosoma (Litomastix) truncatellus*, a polyembryonic species which lays its eggs in the eggs of the moths of the genus *Plusia*. In the germinal vesicle of this species are two nucleoli, one chromatic the other plasmatic (fig. 13, *A*). Just before maturation the plasmosome escapes and becomes situated near the posterior end. Maturation occurs near the anterior pole (fig. 13, *B*). First and second polar bodies are formed, and the first divides, thus making three in all (fig. 13, *C*); these remain near the anterior end, whereas the female nucleus comes to lie near the nucleolus at the opposite pole (fig. 13, *C*). In both fertilized and parthenogenetic eggs the maturation processes, the behavior of the nucleolus, and segmentation are similar. The nucleolus is segregated in

one cleavage cell (fig. 13, *D*) during the first and second divisions, and the cell containing it in the four-cell stage (fig. 13, *E*) is situated dorsally. Then the nucleolus becomes vacuolated and its substance slowly surrounds the nucleus, occupying a large

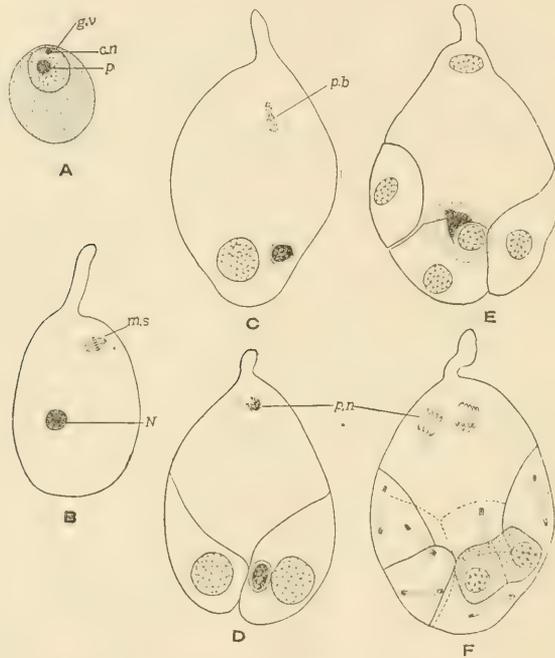


Fig. 13 *Copidosoma* (*Litomastix*) *truncatellus* (redrawn from Silvestri, '06). A, oocyte showing germinal vesicle (*g.v.*) containing a chromatin-nucleolus (*c.n.*) and a plasmosome (*p.*). B, egg, a few minutes after deposition, showing first maturation spindle (*m.s.*) and 'nucleolo' (*N*). C, egg about one hour after deposition, showing three polar bodies (*p.b.*), the first cleavage nucleus (*c.n.*), and the 'nucleolo.' D, egg in two-cell stage, about one and one-half hours old. *p.n.*, polar nucleus. E, egg about one and one-half hours old; in four-cell stage. F, egg about four and one-half hours old showing two polar nuclei dividing, two embryonic cells containing nucleolar substance, and six embryonic cells (dividing) without nucleolar substance.

part of the cytoplasm. Division of this cell is not synchronous with that of the other cleavage cells but is slightly slower. When it does divide each daughter cell receives a share of the nucleolar substance (fig. 13, *F*). Silvestri did not trace the cells contain-

ing the substance after the fourth cleavage division when there were four of them present, but he thinks this can be done and expresses his ideas regarding their history and potency. Two embryonic regions are formed in *Copidosoma* (1) an anterior 'massa germinigena' which produces normal larvae, and (2) a posterior 'massa monembrionale' which produces larvae without genital, respiratory, circulatory, and excretory systems; these he calls sexless larvae. He believes that the cells provided with nucleolar material are germ cells, whereas those lacking this substance are somatic cells, and that the 'massa monembrionale' contains only somatic cells, hence the larvae derived from this region are sexless.

Further studies were made by Silvestri ('08) on other species of parasitic Hymenoptera, and several interesting variations in the behavior of the nucleolus were observed. In *Ageniaspis* (*Encyrtus*) *fuscicollis*, and *A. fuscicollis praysincola* the structure of the egg is similar to that of *Copidosoma* and the nucleolus becomes situated in one of the first two blastomeres. The cell with the nucleolus divides more slowly than the other, and, before its cleavage, the nucleolus breaks up into granules which are distributed between the daughter cells. The cleavage stages thus are as follows: (1) A two-cell stage, one cell with the nucleolus; (2) a three-cell stage, one cell with and two without nucleolar material; (3) a four-cell stage, two cells with and two without nucleolar material; (4) a six-cell stage, two with and four without nucleolar material; and (5) a twelve-cell stage, four with and eight without nucleolar material. The further history of the cells containing nucleolar material was not determined.

In *Encyrtus aphidivorus*, which is not polyembryonic, the nucleolus remains at the posterior end of the egg until a late period of cleavage; then its substance becomes distributed among the primordial germ cells, which, as in *Copidosoma* and *Ageniaspis*, divide more slowly than the somatic cells. In this case there seems to be no doubt that the cells containing nucleolar material become germ-cells, whereas all of the rest become somatic cells.

The structure of the egg, formation of polar bodies, segmentation, and distribution of the nucleolar substance was found to be similar in *Oophthora semblidis* to these processes in *Encyrtus aphidivorus* (fig. 14).

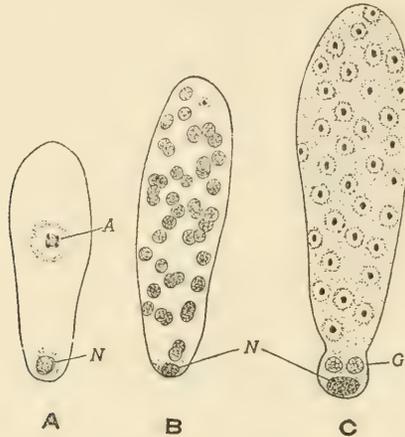


Fig. 14 *Oophthora* (redrawn from Silvestri, '08). A, egg with germinal vesicle (A) and 'nucleolo' (N). B, egg containing many cleavage nuclei. C, formation of primordial germ cells (G) at posterior end of an egg.

II. THE ORIGIN AND SIGNIFICANCE OF THE KEIMBAHN-DETERMINANTS IN ANIMALS

In the following pages the writer has attempted to describe briefly the history of the Keimbahn-determinants in animals (except the insects), and to determine the genesis, localization, distribution, and fate of these substances. The literature of this subject is rather large and widely scattered, so it was considered advisable to provide selected figures from original sources wherever they would aid in making the discussion more clear. The data regarding the insects have been set forth in the first paper of this series; they will of course be included in the general considerations in this contribution.

1. THE KEIMBAHN IN THE CRUSTACEA

The Keimbahn in the Crustacea is best known in certain Cladocera and Copepoda. Of special interest are the investigations of Grobben ('79), Weismann and Ischikawa ('89), Haecker ('97), Amma ('11), and Kühn ('11, '13).

Grobben ('79) studied the embryology of *Moina rectirostris* and gives a remarkably fine account of early cleavage stages considering the early date when the work was done. He figures stages showing a foreign body which he considered a polar body, segregated in one of the early blastomeres, the segregation and characteristics of the primordial germ cell and the first entoderm cell, and the division and later history of the germ cells. His results have been, in the main, confirmed by Kühn ('11, '13) and, since the work of the latter has been done with the aid of better methods, an account of Grobben's observations is not necessary here.

The following description quoted from Weismann and Ischikawa ('89) is a brief but adequate account of the discoveries of these authors (fig. 15):

Die Thatsachen sind, kurz zusammengefasst, die folgenden. In dem befruchtungsbedürftigen Winterei von sechs Arten von Daphniden, welche vier Gattungen angehören, bildet sich während der Ovarialentwicklung des Eies eine Zelle in der Eizelle, an Volumen viel kleiner als diese und wie ein fremder Eindringling langsam in ihr sich umherbewegend. Sie entsteht, indem in dem noch jungen und dotterlosen Ei (*Moina*) ein Theil der Kernsubstanz activ aus dem Keimbläschen in die umgebende Protoplasmamasse austritt, sich zu einem wirklichen Kern (Paranucleus) organisirt und zugleich sich mit einem Zellkörper umbüllt.

Bei der Eiablage gleitet die 'Copulationszelle,' in der Masse des Eikörpers gelegen, mit in den Brutraum und verhält sich zunächst ganz passiv. Nachdem aber die Befruchtung durch eine inzwischen eingedrungene Samenzelle stattgefunden, der Furchungsprocess seinen Anfang genommen und sich mehr oder weniger weit fortgesetzt hat, bewegt sich die Copulationszelle auf eine der im Innern des Dotters versenkten Furchungszellen los, streckt kurze Fortsätze aus und verschmilzt mit ihr in einem förmlichen Copulationsact, indem zuerst die Zellkörper, dann die Kerne der beiden Zellen zusammenschmelzen. Bei zwei Arten geschieht dies schon im Stadium von 2 Furchungszellen, bei den vier andern erst im Stadium von 8 Furchungszellen.....

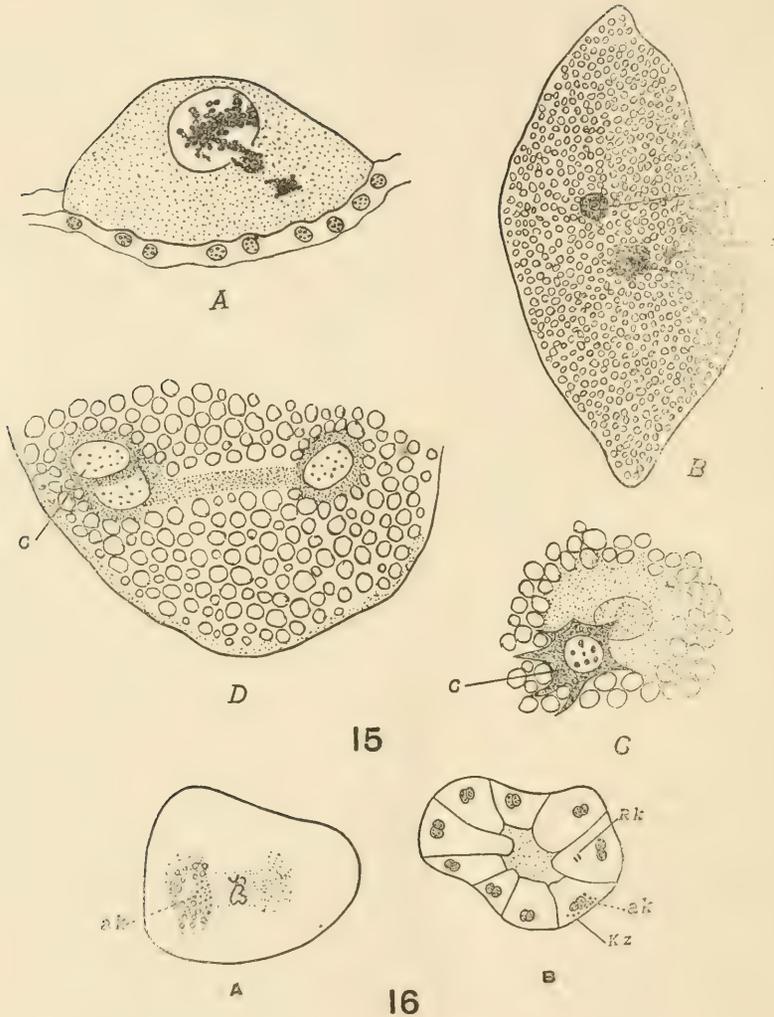


Fig. 15 *Moina paradoxa* (redrawn from Weismann and Ishikawa, '89). A, egg with substance escaping from germinal vesicle. B, egg containing egg nucleus (e), sperm nucleus (s) and 'Copulationszelle' (C). C, cleavage cell and 'Copulationszelle' (c) fusing. D, cleavage cell and 'Copulationszelle' fusing.

Fig. 16 *Cyclops* (redrawn from Haecker, '97). A, egg showing 'Aussenkörnchen' (ak) at one end of first cleavage spindle. B, thirty-two cell stage showing 'Aussenkörnchen' (ak) in the primordial germ cell (Kz). Rk, polar bodies.

Die erstere Thatsache lässt freilich vermuthen, dass es immer dieselbe Furchungszelle sei, mit welcher die Copulationszelle sich verbindet, die letztere deutet darauf hin, dass der Vorgang mit der geschlechtlichen Fortpflanzung etwas zu thun hat (pp. 182-183).

The Keimbahn of Cyclops and some closely allied forms has been very carefully investigated by Haecker ('97) and Amma ('11), with results which are of particular interest so far as germ cell determinants are concerned. In Cyclops, according to Haecker, 'Aussenkörnchen' arise at one pole of the first cleavage spindle (fig. 16, A, *ak*); these are derived from disintegrated nucleolar material and are attracted to one pole of the spindle by a dissimilar influence of the centrosomes. During the first four cleavage divisions the granules are segregated always in one cell (fig. 16, B, *kz*); at the end of the fourth division these 'Aussenkörnchen' disappear, but the cell which contained them can be traced by its delayed mitotic phase, and is shown to be the primordial germ cell.

The most recent and complete account of the Keimbahn in Copepoda is that of Amma ('11). This author studied the early cleavage stages of eleven species of Cyclops, three species of Diaptomus, one species of Canthocamptus, and one species of Heterocope. Cyclops fuscus var. distinctus is made the basis for the most detailed study, but short descriptions and figures are presented of the others. In all of the sixteen species examined, the stem-cell, which gives rise to the primordial germ-cell, may be recognized, as Haecker ('97) discovered in Cyclops, first by the presence of granules which do not occur in the other cleavage cells, and later by a delayed mitotic division. The process is essentially as described by Haecker.

The following summary of the Keimbahn in Cyclops fuscus var. distinctus is given by Amma:

1. Während der ersten Furchungsteilungen ist eine bestimmte Folge von Zellen, die Keimbahn, durch das Auftreten von Körnchen, die sich bei der Teilung jeweils um einen Spindelpol der Teilungsfigur ansammeln, gekennzeichnet (fig. 17, A).

2. Die Körnchen oder Ectosomen entstehen immer erstmals während des Stadiums der Diakinese, vermehren sich während der nächstfolgenden Phasen noch bedeutend und verschmelzen gegen das Ende

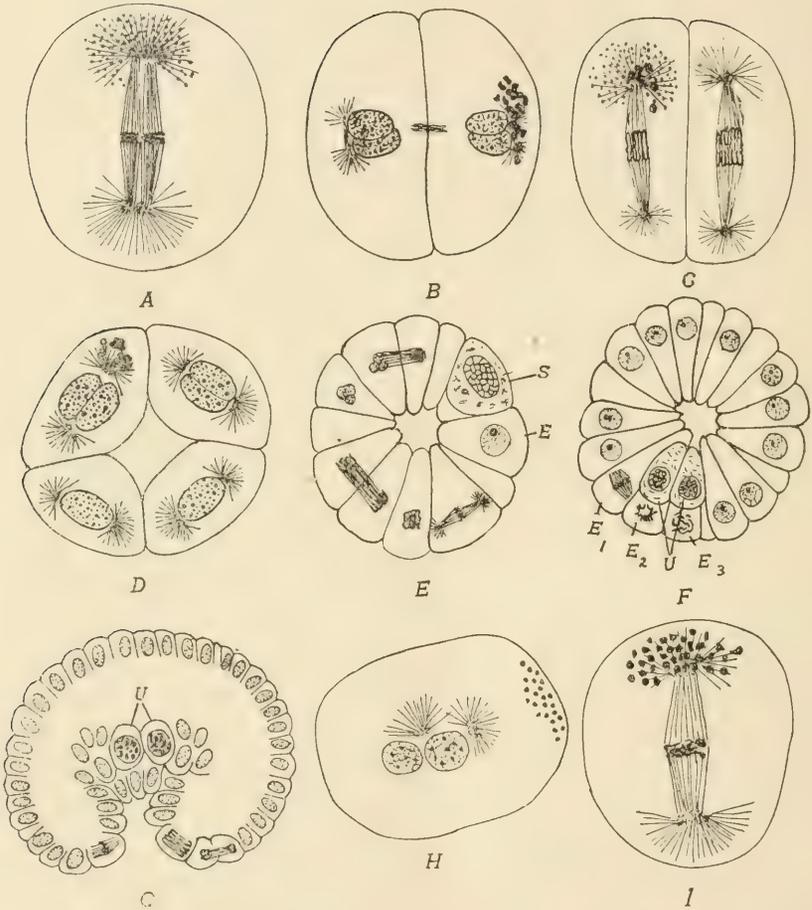


Fig. 17 *Cyclops fuscus* var. *distinctus* (A-G); *Diaptomus coeruleus* (H), *Cyclops vitidis* (I); (redrawn from Amma, '11). A, ectosomen at end of first cleavage spindle. B, two-cell stage; ectosomes dissolving. C, old and newly formed ectosomes at end of one of second cleavage spindles. D, eight-cell stage; ectosomes dissolving in stem-cell. E, sixteen- to twenty-eight-cell stage. S, cell with, E, cell without granules. F, one hundred and twelve-cell stage, with two primordial germ cells (U) and three ectoderm cells (E). G, two hundred and forty-cell stage. U, primordial germ cells. H, appearance of ectosomes before cleavage spindle forms. I, increased production due to carbonic acid gas.

der Teilung zu grösseren, unförmigen Brocken, welche allmählich während des Ruheperiode der Zelle aufgelöst werden. (fig. 17, *B*)

3. Die neue Körnchenzelle geht stets vom körnchenführenden Produkte der alten Körnchenzelle hervor, was direkt dadurch bewiesen werden kann, dass sich in der neuen Körnchenzelle immer noch aufgelöste Überreste der Ectosomen der alten Körnchenzelle vorfinden; alle Körnchenzellen stammen somit in direkter Linie von einander ab. (fig. 17, *C*).

4. Vom II—Zellenstadium an bleibt die Körnchenzelle immer in der Teilung hinter den andern Furchungszellen zurück; es ergibt sich eine Phasendifferenz, welche in immer stärkeren Masse in den höheren Furchungsteilungen zunimmt (fig. 17, *D.E*).

5. Aus dem körnchenführenden Produkte der Körnchenzelle des vierten Teilungsakts, der Stammzelle *S*, gehen, nachdem diese sich an dem fünften Furchungsschritte nicht beteiligte, gegen Ende des sechsten, im LX—Zellenstadium, die beiden definitiven Urgeschlechtszellen hervor; bei dieser Teilung der *S*-Zelle erscheinen die Ectosomen in ganzen Zellräume. (fig. 17, *F*).

6. In Ausnahmefällen beginnt die *S*-Zelle sich etwas früher zu teilen, nämlich schon während des Übergangs des XXX—zum LX—Zellenstadium.

7. Die Urgeschlechtszellen verlieren den Verband mit dem Blastoderm, sie werden allmählich in die Tiefe gedrängt (fig. 17, *G*), (pp. 529–530).

An important departure from the usual method of origin of the 'Ectosomen' is recorded from *Diaptomus coeruleus*. Amma says concerning the process in this species, that whereas "bei andern Formen die Ectosomen bei der ersten Furchungsteilung gewöhnlich erst im Stadium der Diakinese oder der Äquatorialplatte zum Vorschein kommen, treten sie hier schon vor dem Stadium der Copulation der Geschlechtskerne auf" (fig. 17, *H*).

The origin and nature of the Ectosomen are considered by Amma at some length. The hypothesis that these granules arise by the splitting of particles of chromatin from the chromosomes as occurs in *Ascaris* is rejected (1) because in one species, *Diaptomus coeruleus* (fig. 17, *H*), the Ectosomen appear before the nuclear membrane breaks down in preparation for the formation of the first cleavage spindle, and (2) because the Ectosomen do not stain as deeply as chromatin but only slightly darker than the cytoplasm. The origin of the Aussenkörnchen (Ectosomen) from the nucleolus, as considered probable by Haecker ('97), could not be confirmed. The condition in *Diaptomus coeruleus*

is also a serious objection to this theory. The Ectosomen are different from chromidia since chromidia arise from the nucleus and

. . . . man gewinnt im Gegenteil entscheiden den Eindruck, dass die Körnchen ganz unabhängig von den Kernsubstanzen, völlig autogen, im Zellplasma entstehen (p. 553).

Wir haben also offenbar in den Chondriosomen und Ectosomen zwei wesentlich voneinander verschiedene Arten von Gebilden vor uns, denen nicht dieselbe Entstehungsursache und dieselbe Bedeutung zukommt (p. 555).

Aus dem ganzen Verlaufe der Körnchenentwicklung geht nun soviel mit Sicherheit hervor, dass man es bei den Ectosomen mit vergänglichen Gebilden zu tun hat, denen keine weiteren Funktionen zukommen, die im Leben der Zelle nicht weiter verwendet werden. In den Prophasen der Kernteilung entstehen die Körnchen zunächst als feine Tröpfchen im Zellplasma; im weiteren Verlauf der Teilung erfahren sie dann noch eine Zunahme, bis sie ungefähr im Stadium des Dyasters ihre höchste Entwicklung erreicht haben. Von hier ab beginnt der regressive Prozess der Körnchen: sie fliessen zu grösseren, unförmigen Klumpen zusammen, welche vom Zellplasma allmählich vollständig resorbiert und aufgelöst werden. Bei der nächsten Teilung der Keimbahnzelle erscheinen dann die Ectosomen wieder von neuem. Um ein einfaches Unsichtbarwerden während der Zellenruhe, wie es, z.B. vom Centrosoma von vielen Forschern angenommen wird, kann es sich bei den Ectosomen nicht handeln, denn vielfach konnten ja neben den neuen, frisch entstandenen Ectosomen noch die Überreste der Ectosomen der letzten Körnchenzelle nachgewiesen werden. Es erfolgt also bei jedem neuen Teilungsschritte tatsächlich eine *Neubildung und Wiederauflösung* der Körnchen.

Gestützt auf diese Tatsachen, möchte ich nun die Ansicht vertreten, dass die Ectosomen als *Abscheidungen, Endprodukte des Kern-Zelle-Stoffwechsels* aufzufassen sind, welche zu bestimmten Zeiten im Plasma der Zelle zur Abscheidung gelangen und wieder aufgelöst werden (p. 557).

According to Amma, if the above hypothesis be correct, a greater amount of Ectosomen would be present if an egg were allowed to develop in carbonic acid gas. The results of a number of experiments with oxygen and carbonic acid gas indicate that a greater amount of Ectosomen occur when the egg is developed in the latter as shown in figure 17, *I*, of an egg of *Cyclops viridis* placed one hour after deposition in carbonic acid gas for one hour.

When various stains were used it was found that the Ectosomen became colored much like the cytoplasm. For example, when stained in methylen blue followed by eosin, the chromosomes were blue and the Ectosomen and cytoplasm red, and when stained by the methy lgreen-fuchsin-orange G method of Heidenhain the chromosomes were green and the cytoplasm and Ectosomen red.

Amma also attempts to explain the fact that the Ectosomen appear at only one end of the first cleavage spindle and in only one of the cleavage cells until the two primordial germ cells are formed. He rejects Haecker's hypothesis that the centrosomes possess an unequal influence upon the Ectosomen and that one centrosome attracts all of them because it is stronger than the other, and is inclined to favor the idea that the Ectosomen are the visible evidence of an organ-forming substance which is thus distinguished from the rest of the cytoplasm as 'Körnchenplasma.' Amma's statement is, "*dass im Zellplasma des noch ungefurchten Copepodeneies ein vom übrigen Eiplasma qualitativ verschiedenes Körnchenplasma existiert, welches die organbildende Substanz, die Anlagesubstanz für die Geschlechtsorgane darstellt*" (p. 564).

Kühn ('13) has studied the Keimbahn in the summer egg of a Cladoceran, *Polyphemus pediculus*, and has confirmed certain parts and corrected other portions of the work done by earlier investigators—Grobber ('79), Samassa ('93), and Weismann and Ischikawa ('89). In this species usually one (but sometimes two or three) of the nurse cells (fig. 18, *A*) passes into the egg before cleavage. This cell (or cells) becomes imbedded near the periphery at the vegetative pole (fig. 18, *B, n*). During each of the early cleavage divisions this nurse cell is confined to one cell (fig. 18, *C-E*) which gives rise during the third cleavage (8 to 16-cell stage) to the primordial germ cell, containing the remains of the nurse cell (fig. 18 *E, K*) and to the primordial entoderm cell which does not receive any part of the nurse cell (fig. 18, *E, e*). The primordial germ cell and primordial entoderm cell do not divide as quickly as the other blastomeres during the succeeding cleavage stages, a fact that aids in their identification. While the egg is undergoing cleavage, the nurse cell is gradually chang-

ing so that when the sixteen-cell stage is reached it has become disintegrated into dark staining granules and fragments of various forms and sizes (fig. 18, *E*). During the division of the 'Keimbahnzelle' (From 16-32 cell stage) these granules and fragments are about equally distributed between the daughter cells (fig. 18, *F*). A similar distribution takes place in succeeding divisions of the primordial germ cells and this is accompanied by a further decrease in the size of the dark staining granules.

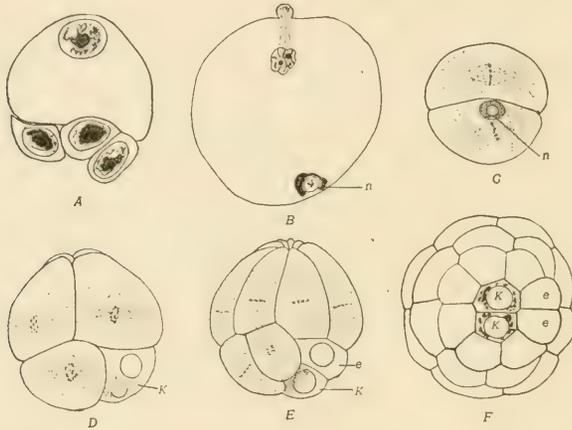


Fig. 18 *Polyphemus pediculus* (redrawn from Kühn, '11, '13). A, egg with three nurse cells. B, egg at close of maturation. *n*, 'Nahrzellenkern.' C, two-cell stage; view of vegetative pole. D, eight to sixteen-cell stage. *K*, 'Keimbahnzelle.' E, sixteen- to thirty-cell stage; *e*, entoderm cell. F, thirty-two-cell stage from vegetative pole. *K*, primordial germ cells; *e*, entoderm cells.

A blastula of 236 cells is figured by Kühn which shows at the vegetative pole four primordial germ cells lying next to eight entoderm cells and bordered by twelve mesoderm cells. During gastrulation this group of twenty-four cells becomes surrounded by the ectoderm cells, and the primordial germ cells may then be recognized as the anlage of the reproductive organs.

Kühn discusses the origin and significance of the 'Nahrzellenkern,' and compares this structure with similar bodies which have been found in the primordial germ cells of other animals, but is unable to arrive at any final conclusion.

In certain Cladocera and Copepoda, as we have seen, there are visible substances within the cytoplasm of the egg which becomes segregated in, and render distinguishable, the primordial germ cells. Some species belonging to these and other groups of Crustacea have been studied in which such a visible substance peculiar to the primordial germ cells is absent.

Samassa ('93) not only failed to find the primordial germ cell during the cleavage stages of *Moina rectirostris*, but claims that the germ cells arise from four mesoderm cells. Kühn ('08), from a study of the parthenogenetic generation of *Daphnia pulex* and *Polyphemus pediculus*, also derives the germ cells from the mesoderm. Vollmer ('12) could not distinguish the germ cells of *Daphnia magna* and *D. pulex* in the developing winter eggs until the blastoderm was almost completed and Muller-Calé ('13) could not find these cells in *Cypris incongruens* until the germ layers were fully formed. McClendon ('06) has shown that in two parasitic copepods, *Pandarus sinuatus* and an unnamed species, the primordial germ cell is established at the end of the fifth cleavage (32-cell stage) instead of at the end of the fourth as Haecker ('97) found in *Cyclops*. It is suggested that this delay may be due to the large amount of yolk present. The stem cell from which it arises is, however, not made visibly different from the rest of the blastoderm by peculiar granules as is the case in *Cyclops*.

Bigelow ('02) has described in *Lepas anatifera* and *L. fascicularis* certain stages which may bring the forms in which no early segregation of the germ cells has been discovered into line with the apparently more determinate species. In *Lepas* the yolk, which at first is evenly distributed within the egg, passes to the vegetative pole and becomes segregated in one of the first two cleavage cells (cd^2). At the 16-cell stage the yolk lies within the single entoblast cell ($d^{5 \cdot 1}$), which occupies a position corresponding to that of the primordial germ cell in *Moina*. In this connection may be mentioned the fact that in many animals the germ cells are supposed to come from the entoderm and are characterized by the possession of much yolk.

2. THE KEIMBAHN IN THE NEMATODA

The classical example of the Keimbahn in animals is that of *Ascaris megalocephala* as described by Boveri ('87, '92). The first cleavage division of the egg of *Ascaris* results in two daughter cells, each containing two long chromosomes (fig. 19, *A*). In the second division the chromosomes of one cell divide normally

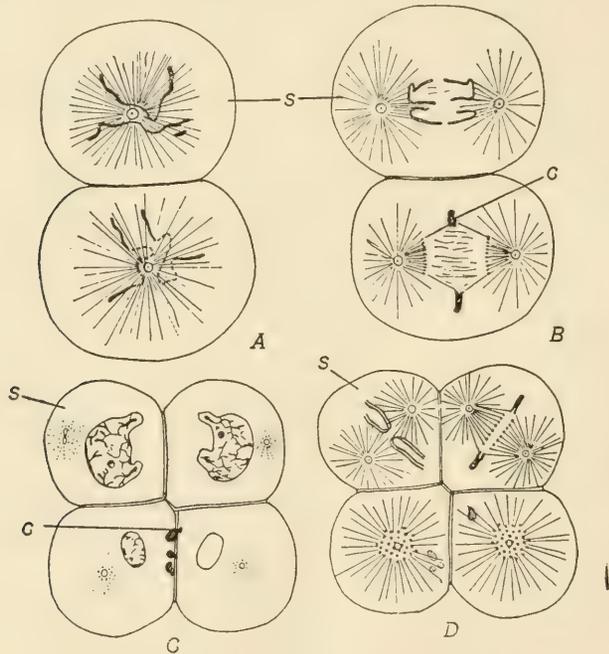


Fig. 19 *Ascaris* (redrawn from Boveri, '92). A-D, stages showing chromatin-diminution in all cells except the stem-cell (*s*).

and each daughter cell receives one half of each (fig. 19, *B*, \bar{s}). The chromosomes of the other cell behave differently; the thin middle portion of each breaks up into granules (fig. 19, *A*), which split, half going to each daughter cell, but the swollen ends (fig. 19, *B*, *C*) are cast off into the cytoplasm. In the four-cell stage there are consequently two cells with the full amount of chromatin and two with a reduced amount. This inequality in the amount

of chromatin results in different sized nuclei (fig. 19, *C*); those with entire chromosomes (*s*) are larger than those that have lost the swollen ends (*c*). In the third division one of the two cells with the two entire chromosomes loses the swollen ends of each; the other (fig. 19, *D*, *S*) retains its chromosomes intact. A similar reduction in the amount of chromatin takes place in the fourth and fifth divisions and then ceases. The single cell in the 32-cell stage which contains the full amount of chromatin has a larger nucleus than the other thirty-one cells and gives rise to all of the germ cells, whereas the other cells are for the production of somatic cells only. The cell lineage of *Ascaris* is shown in the accompanying diagram (fig. 21).

Meyer ('95) extended the study of chromatin diminution to other species of *Ascaris*. In *A. lumbricoides* no diminution takes place until the four-cell stage; then three of the nuclei become deprived of part of their chromatin. A diminution of this sort had been described by Boveri as a variation in the process observed in *A. megalocephala*. In *A. rubicunda* the differentiation of the cleavage cells seems to resemble *A. megalocephala* more than it does *A. lumbricoides*. Only late cleavage stages of *A. labiata* were obtained by Meyer, but there is no doubt that a similar process occurs here. The general conclusion is reached that the cleavage cells of all *Ascaridae* undergo a chromatin diminution.

Bonnevie ('01), however, while able to confirm Meyer's results so far as *A. lumbricoides* is concerned, could discover no process of diminution in *Strongylus paradoxus*, and *Rhabdonema nigrovenosa*.

The elimination of chromatin from all of the somatic cells of *Ascaris* and not from the germ cells led to the conclusion that the germ plasm must reside in the chromatin of the nucleus. The more recent experimental investigations of Boveri ('10a, '10b), indicate, however, that it is not the chromatin alone that determines the initiation of the diminution process, but that the cytoplasm plays a very important rôle. Dispermic eggs were found to segment so as to produce three types as follows (fig. 20, *A*, *B*):

Type I, with one stem-cell (P) and three primordial somatic cells (AB);

Type II, with two stem cells and two primordial somatic cells; and

Type III with three stem cells and one primordial somatic cell.

Figure 20, B shows a cleavage stage of Type II. Here are represented two stem cells (P) with the complete amount of chromatin, both of which are preparing to divide to form the

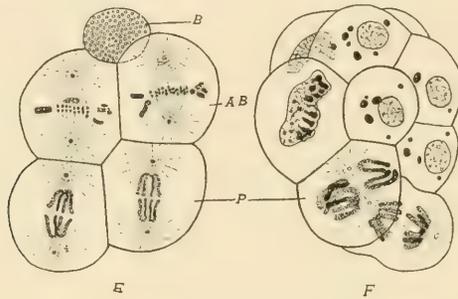


Fig. 20 *Ascaris* (redrawn from Boveri '10b, F, chromatin-diminution in a centrifuged egg. E, chromatin-diminution in a dispermic egg. AB, somatic cells; p , stem-cells.

stem cells (P_2) of the next generation. From the study of these dispermic eggs Boveri ('10b) draws the following conclusions:

Durch die simultane Vierteilung eines dispermen *Ascaris*-Eies entstehen (vielleicht mit ganz seltenen Ausnahmen) Zellen, welche die gleiche Wertigkeit besitzen, wie diejenigen, die durch Zweiteilung eines normal-befruchteten Eies gebildet werden, nämlich die Wertigkeit AB oder P_1 . Es können drei Zellen die Qualität AB besitzen oder zwei oder eine; dem jeweiligen Rest kommt die Qualität P_1 zu. Schon beim Uebergang vom vierzelligen zum achtzelligen Stadium lässt sich aus der Teilungsrichtung mit Sicherheit diagnostizieren, welche der vier primären Blastomeren als AB, welche als P_1 aufzufassen sind; und diese Wertbestimmung wird durch die weiteren Schicksale der vier Zellfamilien in jeder Hinsicht bestätigt (p. 157).

The opinion is expressed that it is "die einrichtigen plasmatischen Qualitäten des sich entwickelnden Zellenkomplexes" which cause the injurious results of dispermy, and that if, of the

three types of dispermic eggs described, the cells could be isolated in pairs, one AB-cell paired with one P_1 -cell, an embryo, normal except in size, would result from each pair.

Eggs which were strongly centrifuged cut off at the beginning of the first cleavage a granular ball at the heavy pole. This phenomenon was previously reported by Hogue ('10) and such

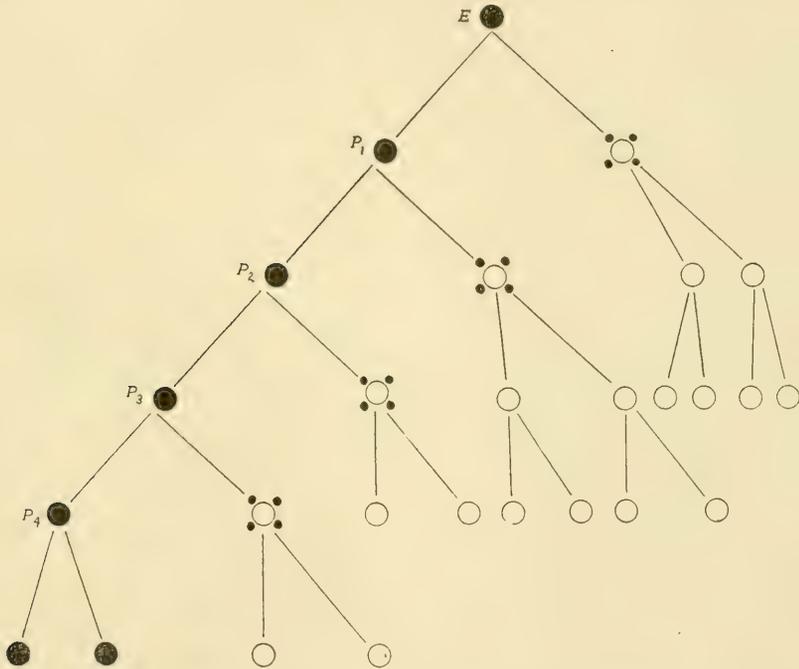


Fig. 21 *Ascaris* (from Boveri, '10b); diagram showing segregation of primordial germ cells. E , egg; P_1 , P_2 , P_3 , stem-cells; P_4 , primordial germ cell; circles represent somatic cells.

eggs were termed 'Balleier.' In these eggs the two cells of the four-cell stage which are adjacent to the 'Ball' undergo the diminution process; the remaining two are stem-cells which give rise to the germ cells. Thus there are two 'Keimbahnen' proceeding side by side in a single egg and four primordial germ cells are produced instead of two as in normal eggs (see fig. 21).

Miss Hogue's experiments with centrifuged force led her to conclude that there must be an 'unsichtbare Polarität' or 'Protoplasmaachse' in the egg of the *Ascaris*. Boveri agrees with this and considers further that the initiation of the diminution process is not determined by the chromatin but by the cytoplasm of the egg. He states that

Was aber auch hier durch weitere Untersuchungen noch erreicht werden mag, Eines halte ich für sicher, dass sich alles, was über die Wertigkeit der primären Blastomeren bei abnormer Furchung ermittelt worden ist, durch die Annahme sehr einfacher Plasmadifferenzen erklären lässt, wogegen die Hypothese einer differenz erendenden Wirkung des Kerns in jeder Form auf unüberwindliche Schwierigkeiten stösst (p. 206).

3. THE KEIMBAHN IN SAGITTA

Sagitta has proved to be of considerable importance to those interested in the Keimbahn of animals. Hertwig ('80) figures the four primitive germ cells in the gastrula and later stages, proving that these cells are early set aside in embryonic development. Recently the work of Elpatiewsky ('09, '10) has given Sagitta a new importance, since this writer has found within the fertilized egg a cytoplasmic inclusion which is intimately associated with the segregation of the germ cells. The presence of this inclusion has been confirmed by Buchner ('10a, '10b) and Stevens ('10) and several ideas have been expressed regarding its origin, fate and significance.

Elpatiewsky ('09) found in Sagitta, at the time when the male and female nuclei were lying side by side in the middle of the egg, a body situated near the periphery at the vegetative pole (fig. 22, B, x). This body, which he called the 'besondere Körper,' consists at first of 'grobkörnigen' plasma which stains like chromatin but not so intensely; later it condenses into a round homogeneous body with a sharp contour. During the first five cleavage divisions the 'besondere Körper' is always confined to a single cell. At the completion of the fifth cleavage (32-cell stage), the blastomere containing this cytoplasmic inclusion is recognizable as the first 'Urgeschlechtszelle' (fig. 22, C, G), and

its larger sister cell as the first 'Urentodermzelle' (fig. 22, *C*, *E*). The primordial germ cell is the last to divide during the sixth cleavage and the 'besondere Körper' does not, as before, pass entire into one of the daughter cells, but breaks up into a number of pieces, part of which are included in each of the two daughter cells (fig. 22, *D*, *E*, *X*). One of these daughter cells apparently

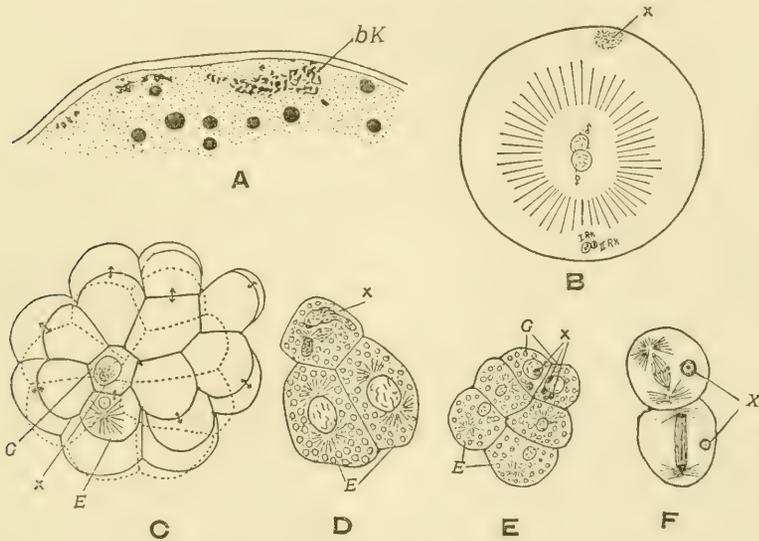


Fig. 22 Sagitta (redrawn from Elpatiewsky, '09, '10). A, first appearance of the 'besondere Körper' (*bK*) in the egg. B, egg with germ nuclei fusing. *X*, 'besondere Körper.' C, thirty-two cell stage; the primordial germ cell (*G*) contains the 'besondere Körper' (*X*). D, division of 'besondere Körper' (*X*) during division of primordial germ cell. E, two primordial germ cells showing unequal distribution of 'besondere Körper' (*X*). F, division of first two primordial germ cells; one dividing more rapidly than the other.

acquires more of the 'besondere Körper' than the other. This division appears to Elpatiewsky to be differential, separating the primordial oogonium from the primordial spermatogonium, the latter being the cell which receives the larger portion of the 'besondere Körper' and which during the next (seventh) division is slightly delayed (fig. 22, *F*). Subsequent to the seventh cleavage the remains of the 'besondere Körper' become pale and

gradually disappear, apparently dissolving, and, in the four germ cells resulting from the next division, only occasionally can stained granules from this body be distinguished.

Buchner ('10a, '10b) had no difficulty in finding the 'besondere Körper' of Elpatiewsky and in tracing it during the cleavage stages. He claims that it originates from the 'accessory fertilization cell' described by Stevens ('04) as degenerating after the egg breaks away from the oviducal wall, and that it is chromidial in nature and should therefore be called 'Keimbahnchromidien.' Stevens ('10), however, has carefully examined abundant material from *Sagitta elegans* and *S. bipunctata* and could trace no connection between the 'accessory fertilization cell' and the 'besondere Körper,' the latter appearing for the first time at the stage when the egg and sperm nuclei lie side by side in the middle of the egg, thus confirming Elpatiewsky's conclusions. She admits the possibility of the origin of the 'besondere Körper' from granules of the accessory fertilization cell, provided this material loses its staining capacity for a period, and suggests also that the granules of chromatin-like material extruded from the nucleus of the egg during maturation may take part in its formation. Miss Stevens also believes with Elpatiewsky that the 'besondere Körper' divides unequally between the two daughter cells of the primordial germ cell and that this is a differential division. She was unable, however, to detect any constant difference between either the cytoplasm or the nuclei of oogonia and spermatogonia. It is worthy of mention that Elpatiewsky ('10) believes that the 'besondere Körper' may originate "aus dem achromatischen Kernkörper."

The differentiation of oogonia and spermatogonia from indifferent germ cells during the development of hermaphrodites is a subject of great interest and importance. Attempts have been made, especially with molluscs (Ancel '02, '03; Buresch, '11; Boveri, '11; Schleip, '11), but without as definite results as are desirable. The early differentiation of germ cells into oogonia and spermatogonia in *Sagitta*, which was pointed out by Hertwig ('80), has been employed by Pedaschenko ('99) as a basis for a theory of sex determination in a copepod, *Lernaea branchialis*.

In this crustacean there are at one cleavage stage four primordial germ cells which resemble the quartette of cells described by Hertwig in *Sagitta*, two of which were proved by him to be oogonia and the other two spermatogonia. Pedaschenko supposes that the four primordial germ cells form two pairs, a pair lying on either side of the median line. Each pair becomes a single cell by fusion and the probable disintegration of one nucleus. One cell of each pair is believed by Pedaschenko to be male, the other female; and the sex of the cell whose nucleus does not degenerate determines the sex of the resulting individual, since a persisting female cell would form an ovary and a persisting male cell a testis. Thus is a potentially hermaphroditic organism changed to a dioecious organism.

4. THE KEIMBAHN IN VERTEBRATES

Animals from all classes of vertebrates have been employed for determining the origin of the germ cells, but in no case have these cells been traced back to cleavage stages. Early authors, and even many writers at the present time, believed in the germinal epithelium theory of Waldeyer ('70). This investigator first distinguished germ cells in the epithelium covering the genital ridge and thought that they evolved from these epithelial cells. The gonotome theory of Rückert ('88) and Van Wijhe ('89) holds that germ cells arise in the embryo from a certain part of the mesoblastic segments called by the latter the 'gonotome;' from here they are carried into the peritoneum. There can be no doubt from the most recent investigations that the germinal epithelium and gonotome theories are incorrect, and that the germ cells of vertebrates are formed at a much earlier period, giving good basis for the idea that these cells arise from cleavage cells as has been abundantly proved for many invertebrates. Some of those who have advocated such an early origin of germ cells are Nussbaum ('80) in the trout and frog, Eigenmann ('92, '96a, '96b) in *Cymatogaster*, Wheeler ('99) in the lamprey, Beard ('00, '02) in *Raja* and *Pristiurus*, Nussbaum ('01) in the chick, Woods ('02) in *Squalus*, Allen ('06, '07, '09) in *Chry-*

semys, Rana, Amia and Lepidosteus, Rubaschkin ('07, '09, '10, '12) in the chick, cat, rabbit and guinea-pig, Kuschakewitsch ('08) in Rana, Jarvis ('08) in Phrynosoma, Tschaschin ('10) in the chick, von Berenberg-Gossler ('12) in the chick, Schapitz ('12) in Amblystoma, and Fuss ('12) in the pig and man. This is by no means a complete list, but indicates the range of forms studied and the current interest in this subject. No attempt will be made here to review this mass of literature, but interesting facts will be selected from several papers in the list.

In the first place, the vertebrates do not furnish as favorable material for germ cell studies as do many of the invertebrates, and a large number of the contributions have not added anything particularly important to our knowledge of the subject, but have simply demonstrated that similar conditions prevail among members of different classes, orders, etc. One author (Eigenmann, '91, '96) believes that the germ cells in *Cymatogaster* are differentiated as such in the thirty-two cell stage. This, however, was not proved and no confirmatory data have since been furnished. Within the past three years, however, several communications have been published which give us hope of really tracing the Keimbahn back into the cleavage stages. Before this time some of the characteristics by means of which germ cells could be distinguished in vertebrate embryos were as follows: (1) the presence of yolk, (2) an amoeboid shape, (3) large size, and (4) slight staining capacity. Of the more recent investigations, I shall mention those carried on by Dodds ('10), Rubaschkin ('10, '12), Tschaschin ('10), and von Berenberg-Gossler ('12).

One fact discovered by Dodds ('10) in the teleost, *Lophius*, is of special interest, namely that the germ cells in the embryos of this fish cannot be definitely distinguished previous to the appearance in their cytoplasm of a body which stains like a plasmosome (fig. 23, C). Germ cells are undoubtedly segregated before this period, but they exhibited no characteristics with the methods employed which rendered them distinguishable. Dodds believes that this cytoplasmic body is extruded plasmosome material, probably part of one of the two plasmosomes possessed by many of the cells at this period. Thus far *Lophius*

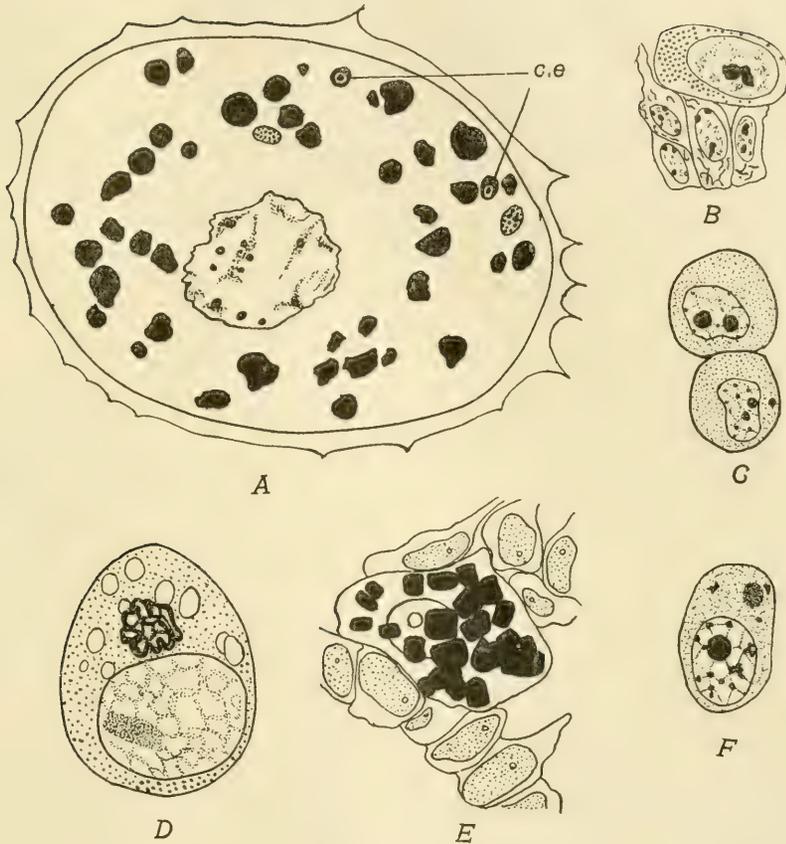


Fig. 23 A, oocyte of the cat, showing the 'corps enigmatique' (*c.e.*); (redrawn from van der Stricht, '11). B, one germ cell and several somatic cells from a guinea-pig embryo; (redrawn from Rubaschkin, '12). C, two germ cells from the embryo of *Lophius* with plasmosome extruded into cytoplasm; (redrawn from Dodds, '10). D, germ cell of chick, showing 'Netzapparat'; (redrawn from von Berenberg-Gossler, '12). E, a germ cell of *Raja batis*, filled with yolk material and surrounded by somatic cells; (redrawn from Beard, '02). F, oogonium of a sponge, (redrawn from Jörgensen, '09).

is the only vertebrate in whose primordial germ cells an extra-nuclear body has been found.

Rubaschkin, in 1910, announced the results obtained with the eggs of the guinea-pig by certain methods designed to bring

into view the chondriosomes. He shows that the chondriosomes of the undifferentiated cells are granular, and that as differentiation proceeds, these granules unite to form chains and threads (fig. 23, *B*). "Zwischen den somatischen und Urgeschlechtszellen existiert ein Unterschied in der Structur, welcher sich dadurch offenbart, dass die Urgeschlechtszellen primitive körnige Chondriosomen besitzen, während die somatischen Zellen mit veränderten, d.h. fadenförmigen Chondriosomen ausgestattet sind" (p. 428). The germ cells are those which remain in an undifferentiated condition situated in the posterior part of the embryo among the entoderm cells. Tschaschkin ('10) in the same year, came to a similar conclusion from studies made with chick embryos. Rubaschkin ('12) has also extended his investigations on guinea-pig embryos. The accompanying diagram (fig. 24) shows the fertilized egg and the early cleavage cells all alike (in black); some of their descendants become differentiated into the somatic cells of the germ layers (circles), but others (in black) remain in a primitive condition and are recognizable as the primordial germ cells (*p.g.c.*); these remain at rest for a considerable period, but finally multiply and become part of the germinal epithelium (*g.ep.*).

Von Berenberg-Gossler ('12) considers the 'Netzapparat' in the primitive germ cells of the chick of particular importance (fig. 23, *D*) comparing it with the 'wurstförmige Körper' described by Hasper ('11) in *Chironomus* (p. 385, fig. 1). The appearance of this structure in the 'Keimbahnzellen' is thought to be due to the long period during which these cells do not divide.

Certain events take place during the spermatogenesis of mammals which are concerned with the differentiation of germ cells. I refer to the formation of the Sertoli cells of man, as reported by Montgomery ('11), and in part confirmed by von Winiwarter ('12). The Sertoli cells are intimately connected with the germ cells in the mammalian testis and probably perform three functions: (1) they nourish the spermatocytes; (2) they provide the spermatic fluid; and (3) they exert some chemico-tactic stimulus which serves to orient the spermatozoa into bundles. The origin of the Sertoli cells has been for many

years in doubt. Many investigators claim that they arise from cells other than germ cells; these writers have been called by Waldeyer ('06) 'dualists.' An equal number of authorities believe that both Sertoli cells and spermatogonia originate from primordial germ cells; these are the 'monists.'



Fig. 24 Diagram to show the history of the germ cells in the embryo of the guinea-pig. *g.ep.*, germinal epithelium; (from Rubaschkin, '12).

The researches of Montgomery and von Winiwarter have decided the question, at least so far as man is concerned, in favor of the monists. Montgomery's results are as follows: Of thirty antepenultimate spermatogonia examined, twenty-three contained each a rod-shaped structure (fig. 25, *B, R*) and it seems probable that this peculiar body, which is identified by von Winiwarter with the 'cristalloide de Lubarsch' (Lubarsch, '96), is present in every cell of this generation. This rod is considered

by Montgomery to be of cytoplasmic origin and is termed by him a 'Sertoli cell determinant.' During the division of the antepenultimate spermatogonia the rod passes undivided into one of the daughter cells; thus one-half of the penultimate spermatogonia possess a rod, the other half do not. Of the forty-nine

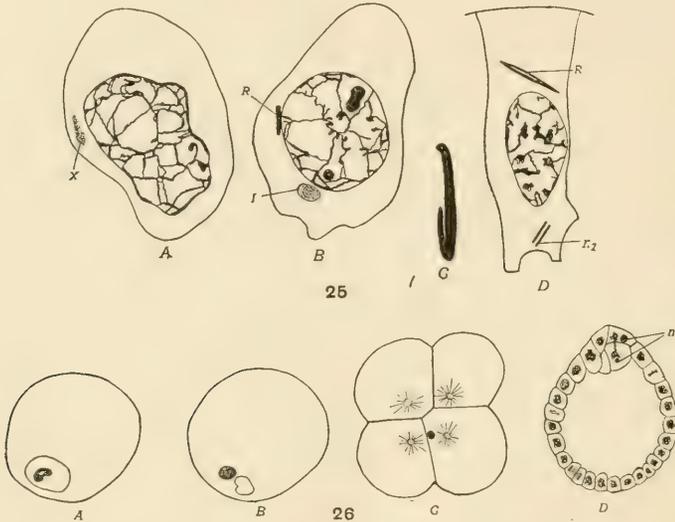


Fig. 25 Stages in the formation of the Sertoli cell in man; (redrawn from Montgomery, '11). A, spermatogonia containing granular inclusion (X) from which 'Sertoli cell determinant' may arise. B, antepenultimate spermatogonium showing rod (*R*) and idiozome (*I*). C, division of rod. D, a Sertoli cell containing a divided rod (*R*) and two rodlets (*r*₂).

Fig. 26 *Aequorea forskalea* (redrawn from Haecker, '92). A, freshly laid egg with germinal vesicle containing nucleolus. B, egg one-half hour after laying; nucleolus has escaped from nucleus. C, four-cell stage; nucleolus in one blastomere. D, blastula; certain cells contain nucleolar-like inclusions (*n*).

penultimate spermatogonia examined, twenty-four exhibited a rod and twenty-five did not. This result has been confirmed by von Winiwarter. When the rod-containing penultimate spermatogonia divide there is a similar segregation of the rod in one of the daughter cells, hence only one-fourth of the cells resulting from the divisions of the antepenultimate spermatogonia possess a rod. Of one hundred and forty-two cells of this genera-

tion studied by Montgomery, twenty-five were found with a rod and one hundred and seventeen without. That this ratio is less than one to three (1:3) is explained by the fact that some of the spermatogonia with rods may already have become Sertoli cells. The further history of the rod in the Sertoli cell is as follows: A primary rodlet is produced by a splitting of the rod (fig. 25, *C*) after which the rod either disappears at once or else persists for a time, in which case it may split longitudinally as shown in figure 25, *D*, *R*. However, in four-fifths of the cells examined (one hundred in number) the large rod disappeared before the growth of the Sertoli cell had begun. Each primary rodlet splits longitudinally into two approximately equal parts, called secondary rodlets (fig. 25, *D*, *r*₂), which persist until the end of the cycle of the Sertoli cell.

Neither Montgomery nor von Winiwarter was able to determine the origin of the rod. They do not consider it mitochondrial in nature, although it may arise from granules lying in the cytoplasm. Montgomery found in one cell a mass of granules from which the rod may have developed (fig. 25, *A*, *X*), and von Winiwarter noted that the rod had a granular appearance in the earliest stages he examined. It is also perfectly distinct from the idiozome (see fig. 25, *B*, *I*) and is apparently not directly derived from the nucleus. Von Winiwarter is not as certain as Montgomery regarding the history of the spermatogonia, the 'cristalloide de Lubarsch,' and the 'gâstonnets accessoires,' as he calls the rodlets. He was unable to decide regarding the number of spermatogonial divisions and believes it to be indeterminate. He finds, contrary to Montgomery, the rod persisting in fully developed Sertoli cells, and considers the fragmentation or fission of the rod to form the primary rodlets as doubtful. Further investigations with more favorable material are very desirable, but notwithstanding certain differences of opinion between the two writers whose results have been briefly stated above, it seems certain that Sertoli cells and germ cells are both derived from primordial germ cells, and that the Sertoli cells differ from the ultimate spermatogonia in the possession of a peculiar rod probably of cytoplasmic origin. Montgomery considers this a sort

of secondary somatic differentiation (the Sertoli cells representing the soma of the testis); the first somatic differentiation occurring when the tissue cells become differentiated from the germ cells in the embryo.

It may be worth mentioning at this place that among the invertebrates, instances of changes which take place in the germ cells of the male are known which may be brought into line with the facts in the history of the Sertoli cells of man. For example, in the spermatogenesis of the parasitic copepod, *Laemargus muricatus*, McClendon ('06) found that the cells in some of the groups of four spermatids became filled with an achromatic substance which resembled yolk—a substance called 'Austreibestoff' by Heider ('79). The origin of this substance could not be determined, but it probably came from the nucleus. The cells thus affected serve as nurse cells for the spermatozoa.

5. THE KEIMBAHN IN OTHER ANIMALS

It is not possible in this place to give either a general account or detailed account of the Keimbahn as it has been described in groups such as the Porifera, Coelenterata, etc., since we are here interested especially in the peculiar substances which apparently determine the Keimbahn, and thus far no results of importance have been obtained from studies of these animals. Under the above heading, however, I wish to mention a few stages in the development of certain forms, widely separated in the animal kingdom, which have either been compared or can be compared with conditions such as we have described in the preceding portions of this paper.

Metanucleoli. Haecker's ('97) belief that the 'Aussenkörnchen' in Cyclops are of nucleolar origin and Silvestri's ('06, '08) discoveries in parasite Hymenoptera indicate that in certain instances the nucleoli may play some rôle in the differentiation of the primordial germ cells.

The large nucleolus in the germinal vesicle of the medusa, *Aequorea forskalea* (fig. 26, *A*), according to Haecker ('92), disappears from the germinal vesicle about half an hour after the egg is laid, and a similar body becomes evident near the egg

nucleus which has in the meantime become smaller (fig. 26, *B*). These two bodies are considered by Haecker to be identical, and the term 'Metanucleolus' has been applied to them. The metanucleolus is, in each division up to the sixty-four cell stage, segregated intact in one cell. Its further history was not traced, but in the blastula, when the cells at the posterior pole begin to differentiate, nucleolar-like bodies appear in some of them which are absent from the undifferentiated blastula elements (fig. 26, *D, n*). These may be the descendants of the metanucleolus.

A body similar to the metanucleolus was also discovered by Haecker near the copulating germ nuclei in the egg of *Aurelia aurita*, but its history could not be determined because of the large amount of yolk present. Haecker identifies the metanucleolus of *Aequorea* with the spherical body described by Metschnikoff ('86) near the egg nucleus of the *Mitrocoma annae*, and considered by him as a sperm nucleus. A similar interpretation is given by Haecker for the cytoplasmic inclusion ('Spermakern') found by Boveri ('90) in *Tiara*. Similarly the 'Kleinkern' which Chun ('91) discovered in the egg cells of the *Stephanophyes superba*, and the bodies described by Hertwig ('78) near the maturation spindles of *Mytilus* and *Sagitta*, resemble very closely the metanucleolus of *Aequorea*.

Furthermore, the metanucleolus is considered by Haecker homologous to the 'Paracopulationzelle' described by Weismann and Ischikawa in the winter eggs of certain *Daphindae* (p. 434, fig. 15) and in both cases it is considered probable that these peculiar bodies are restricted to the 'Keimbahnzellen' of the embryo.

In the eggs of *Myzostoma*, Wheeler ('97) found that the nucleolus of the germinal vesicle does not dissolve soon after it is cast out into the cytoplasm during the formation of the first maturation spindle, but remains visible at least until the eight-cell stage, at which time it lies in the large posterior macromere, a cell which "very probably gives rise to the entoderm of the embryo." Later embryonic stages were not studied. According to Wheeler "the nucleoli are relegated to the entoderm cells as the place where they would be least liable to interfere in the

further course of development and where they may perhaps be utilized as food material after their disintegration" (p. 49).

McClendon ('06) has likewise described a body embedded in the cytoplasm of the egg of *Myzostoma clarki* which he derives from the 'accessory cells,' which, as Wheeler ('96) has shown, attach themselves to either pole of the oocytes. These 'accessory cells' are really the 'Nahrzellen' of other authors. The cleavage of the egg was not studied. Buchner ('10b) suggests that this body described by McClendon and the 'nucleolus' of Wheeler are identical and that through them the Keimbahn may be determined.

A metanucleolus has also been described by Hartmann ('02) in *Asterias glacialis*. The germinal vesicle of the ovarian egg possesses a 'Keimfleck' which contains all of the plastin and the chromatin of the nucleolus. During the maturation the germinal vesicle breaks down, the chromosomes escape from the 'Keimfleck' and the rest of this body becomes imbedded in the cytoplasm near the maturation spindle.

Granules. Granules of various sorts which are segregated in particular blastomeres have been noted in the eggs of various animals and may have some relation to the Keimbahn. For example, among the molluses, Blochmann ('81) has described the appearance of a group of granules in the early cleavage cells of *Neritina* which finally reach the velar cells. It is also probable that Fol ('80) observed similar granules in the 16-cell stage of *Planorbis*. In the same category, no doubt, belong the bodies figured by Fujitas ('04) in the 4-cell to the 16-cell stages of *Siphonaria* lying at the vegetative pole, and the 'Ectosomen' described and figured by Wierzejski ('06) in *Physa*. These granules appear at the vegetal pole in the blastomeres of *Physa* during the second cleavage; are at first imbedded in the entoderm mother cells, but finally become localized in the ectoderm cells. They periodically appear and disappear, and may, as suggested by Wierzejski, represent only "eine besondere Erscheinung des Stoffwechsels" (p. 536).

Similarly in the rotifer, *Asplanchna*, Jennings ('96) has traced a cloud of 'granules' from the eight-cell stage until the seventh

cleavage, when this mass forms part of the smaller entodermal cell. In *Lepas* there has also been recorded (Bigelow, '02) a segregation of granules in one blastomere. Many other substances, granular in form, have been described in the eggs of animals, some of them at least having migrated there from the somatic tissue. Blockmann ('87) discovered a number of bacteria-like rods in the undeveloped eggs of *Blatta germanica*; these rods multiplied by division and were considered symbiotic bacteria. 'Bacterienartige Stäbchen' were also noted by Heymons ('95) in the eggs of *Periplanata orientalis* and *Ectobia livida*; these sink into the yolk and disappear. More recently a report of Buchner ('12) indicates that these bodies are really organisms which seem to be symbiotic and not parasitic, although it remains to be proved what advantage the host receives from their presence. Of a similar sort are the Zooxanthellae which Mangan ('09) has shown enter the developing ovum from the parental tissues. All of these organisms become in some way imbedded in the germ cells, but, so far as we know, never serve to distinguish the Keimbahn, although a more selective distribution within the developing animal would obviously be greatly to their advantage.

Vander Stricht ('11) has compared the 'besonderer Körper' found by Elpatiewsky ('09, '10) in the egg of *Sagitta* with several bodies, the 'corps enigmatique,' which he discovered in the oocyte of the cat (fig. 23, A). One or two of these 'corps enigmatique' are present in the young oocyte originating from a few (one to five) cytoplasmic safraninophile granules which are visible at the beginning of the growth period. At first they lie near the nucleus, but as the size of the oocyte increases, they become situated near the periphery. Usually three parts can be recognized in the 'corps enigmatique:' "granulation centrale, couche intermédiaire et couche corticale foncée." As the term applied to them indicates, the functions of these bodies were not determined. The following suggestion is, however, made: "il est possible que cet élément nous montre, des l'origine, la 'Keimbahn' ainsi que les premières cellules génitales constituées" (p. 425).

A body stained deeply by nuclear dyes which was found by O. Van der Stricht ('09) in the bat at the time of the first cleavage mitosis, may be similar to the 'corps enigmatique' of the cat. It is also worth recording that Jørgensen ('10) finds that the oogonium of the sponge at the earliest stage when it can be recognized, contains a granular body not present in the somatic cells (fig. 23, *F*).

6. THE GENESIS, LOCALIZATION, DISTRIBUTION AND FATE OF THE KEIMBAHN-DETERMINANTS

It is customary to be suspicious of any peculiar bodies revealed to us in fixed and stained material under high magnification. There can be no doubt, however, that most, if not all, of the cytoplasmic inclusions mentioned in this paper are realities and not artifacts. Some of them have been seen in the living eggs; most of them have been described by several investigators; they occur after being fixed and stained in many different solutions; and their presence is perfectly constant. The genesis, localization, and fate of these bodies are difficult to determine, and their significance is problematical; but the writer has attempted in the following pages to draw at least tentative conclusions from the evidence available and to indicate what still needs to be done.

A. The genesis of the Keimbahn-determinants

The writers who have discussed the origin of the Keimbahn-determinants have derived them from many different sources. In a few cases they are known to be nuclear in origin, consisting of nucleolar or chromatic materials; they are considered differentiated parts of the cytoplasm by some investigators; in some species they are extra-cellular bodies, such as nurse cells.

Table 1 indicates the number and diversity of the animals in which Keimbahn-determinants have been described, and shows the increasing interest given to this subject within recent years, over half of the papers listed having been published since 1908. Several cases have been referred to in the text, but omitted from the table because of insufficient evidence regarding

their connection with the primordial germ cells. The list as given includes representatives of the Coelenterata, Chaetognatha, Nematoda, Arthropoda, and Vertebrata. The terms applied to the various substances have been chosen evidently because of their genesis, position in the egg, or supposed function.

a. From nuclear substances: Nucleoli. It is certain that bodies of a nucleolar nature behave as Keimbahn-determinants. Three

TABLE I

Principal cases of visible substances concerned in differentiation of germ cells (in chronological order)

NAME OF SPECIES, GENUS, OR GROUP	NAME APPLIED TO SUBSTANCE	AUTHORITY	DATE
Chironomus nigro- viridis	Dotterkörnchen	Weismann	1863
Miastor	Dottermasse	Metschnikoff	1866
Moina rectirostris	Richtungskörper	Grobben	1879
Chironomus	Keimwulst	Ritter	1890
Daphnidae	Paracopulationszelle	Weismann and Ischi- kawa	1889
Aequorea	Metanucleolus	Haecker	1892
Ascaris megalcephala	Chromatin	Boveri	1892
A. lumbricoides	Chromatin	O. Meyer	1895
A. rubicunda			
A. labiata			
Cyclops	Aussenkörnchen, Ektosomen	Haecker	{ 1897 1903
Calliphora	Dotterplatte	Noack	1901
Dytiscus	Anello cromatico	Giardina	1901
Apis mellifica	Richtungskörper	Petrunkewitsch	1901
Parasitic Hymenoptera	Nucleolo	Silvestri	{ 1906 1908
Chrysomelidae	Pole-disc	Hegner	1908
Miastor metraloas	polares Plasma	Kahle	1908
Sagitta	besonderer Körper	Elpatiewsky	1909
Guinea-pig	Chondriosomes	Rubaschkin	1910
Chick	Chondriosomes	Tschaschkin	1910
Lophius	Extruded plasmosome	Dodds	1910
Ascaris	Plasmadifferenzen	Boveri	1910
Chironomus	Keimbahnplasma	Hasper	1911
Copepoda	Ectosomen	Amma	1911
Polyphemus	Nahrzellenkern	Kühn	{ 1911 1913
Sagitta	Keimbahn-chromidien	Buchner	1910
Man	Sertoli cell determinant	Montgomery	1911

or more kinds of bodies are spoken of as nucleoli. Of these may be mentioned (1) the true nucleoli or plasmosomes, (2) karyosomes or chromatin-nucleoli, and (3) double-nucleoli consisting of usually a single principal nucleolus (Hauptnucleolus of Flemming) and one or more accessory nucleoli (Nebennucleoli of Flemming). Many nucleoli have been described which may perhaps represent intermediate stages in the evolution of one of the types mentioned above into another.

The young ovarian egg of most animals contains a single spherical nucleolus ('Keimfleck,' or germinal spot) but the number may increase greatly during the growth period. Usually during the formation of the first maturation spindle the nucleolus escapes from the nucleus into the cytoplasm where it disappears, often after breaking up into fragments. Many theories have been advanced regarding the origin, function and fate of the nucleoli of the germinal vesicle. They are considered by some of chromatic origin, arising as an accumulation of the chromatin (Retzius, '81; Mertens, '93; Foot and Strobell, '11; Payne, '12, and many others), or from the chromatin by chemical transformation (Schneider, '91; Obst, '99). Others consider them extranuclear in origin (Montgomery, '99).

Many functions have been attributed to the nucleoli; of these the following may be mentioned: (1) They function as excretory organs (Balbiani, '64; Böhm, '88; Hodge, '94; Bambeke, '97). (2) Nucleoli play an active rôle in the cell, since they serve as store-houses of material which is contributed to the formation of the chromosomes (Flemming, '82; Korschelt, '95; Lubosch, '02; G. T. Hargitt, '09; Jordan, '10; Foot and Strobell, '11) and may give rise to kinoplasm (Strasburger, '95) or 'Kineto-chromidien' (Schaxel, '10). (3) Nucleoli are passive by-products of chromatic activity; they become absorbed by active substances (Haecker, '95, '99). (4) Nucleoli represent nutritive material used by the nucleus into which it is taken from the cytoplasm (Montgomery, '99).

Undoubtedly the various bodies known as nucleoli originate in different ways, have different histories and execute different functions.

In the particular cases to be discussed here the nucleoli are not temporary structures, as is usually true, but persist for a comparatively long interval after the germinal vesicle breaks down. The most important and convincing evidence of the functioning of a nucleolus as a Keimbahn-determinant is that furnished by Silvestri ('06, '08) in parasitic Hymenoptera. Here, as shown in figures 13 and 14 and described on page 429, the nucleolus escapes from the germinal vesicle, comes to lie a considerable distance away at the opposite (posterior) pole of the egg, and later is segregated in the cytoplasm of the germ cells, apparently playing some rôle in the determination of the latter. Several events in the history of this nucleolus are unusual. (1) The nucleolus leaves the germinal vesicle before the nuclear membrane dissolves, whereas usually this body is not cast out into the cytoplasm until after the spindle has begun to form; (2) The nucleolus does not become granular and disappear but persists intact for a considerable period; (3) It comes to occupy a definite position in the egg, i.e., at the posterior pole; (4) During cleavage the nucleolar material becomes distributed apparently equally among all of the primordial germ cells, and is absent from all of the somatic cells. Silvestri has given us no data regarding the escape of the nucleolus from the germinal vesicle and the writer is at present unable to account for this peculiar behavior, although he is now at work on the growing eggs of a polyembryonic, hymenopterous parasite which he hopes will enable him to determine this point, as well as to trace the history of the nucleolus back into early stages.²

As we have already noted, in a few instances the nucleolus does not disappear during the maturation divisions but persists for a time as a 'metanucleolus' (p. 457). The nucleoli of these parasitic Hymenoptera are of this sort. They are evidently of a different nature from the usual type and are hence saved from immediate disintegration in the cytoplasm. The localization of this nucleolus at the posterior end of the egg is the result,

²This has since been completed and published in the *Anat. Anz.*, Bd. 46, pp. 51-69, 1914.

either of its own activity, or of that of the surrounding cytoplasm, or a combination of these. Gravity can have no decided effect upon it (Herrick, '95) since its position is constant, whereas the posterior end of the egg with respect to gravity is not. It also seems hardly possible that oxygenotactic stimuli are the cause of its change of position as has been suggested by Herbst ('94, '95), for the migration of the blastoderm-forming cells from the center to the surface of the eggs of certain arthropods. Concerning the fate of this nucleolus, one of the most difficult phenomena to explain is its fragmentation at a definite developmental stage and the apparently equal distribution of its substance to the primordial germ cells during their multiplication.

Haecker ('97) has suggested that the 'Aussenkörnchen' which appear in the egg of *Cyclops* during the formation of the first cleavage spindle may be nucleolar in nature. Later ('03) this idea was withdrawn and more recently Amma ('11) has likewise been unable to sustain this hypothesis. The most convincing data furnished by Amma are that in an allied form, *Diaptomus coeruleus* (fig. 17, *H*), these granules appear before the cleavage spindle is formed and before the nucleoli of the pronuclei have disappeared.

The remaining forms in which nucleoli have been considered as Keimbahn-determinants are merely suggestive. In *Aequorea*, Haecker ('92) traced the metanucleolus, which arises from the germinal vesicle, into certain cells of the blastula. Similar bodies appear in *Mitrocoma* (Metschnikoff, '86), *Tiara* (Boveri, '90), *Stephanophyes* (Chun, '91), *Myzostoma* (Wheeler, '97), and *Asterias* (Hartmann, '02), but their fate has not been determined.

It seems probable that in all these cases the same influences may be at work regulating the time, the place, and the method of localization of the nucleoli. Silvestri has made no attempt to explain this behavior. The writer can only conclude (1) that the 'nucleolo' of Silvestri and the 'metanucleoli' of other authors differ in nature from ordinary plasmosomes, chromatin-nucleoli, and double-nucleoli; (2) that these bodies are definitely segregated in a certain part of the egg or in a certain blastomere, probably by protoplasmic movements; and (3) that their disinte-

gration and the distribution of the resulting fragments or granules are controlled by reactions between them and the substances in which they are imbedded.

Chromatin. In two genera of animals the differentiation of the primordial germ cells is accompanied by a diminution of the chromatin in the nuclei of the somatic cells, so that eventually the nucleus of every germ cell is provided with the full complement of chromatin, whereas the nucleus of every somatic cell lacks a considerable portion of this substance, which remains behind in the cytoplasm when the daughter nuclei are reconstituted. These two genera are *Ascaris* and *Miastor*. This diminution process was described by Boveri ('92) in the former and confirmed by O. Meyer ('95) and Bonnevie ('01), and by Kahle ('08) in *Miastor* and confirmed by Hegner ('12). For details of these processes reference should be made to figures 3, 4, and 32 and pp. 390 and 442. It may be pointed out here that, although the final results are similar, the process differs in the two genera. In *Ascaris* both ends of each chromosome are split off, whereas in *Miastor* approximately one-half of each daughter chromosome is left behind to form the 'Chromosomen-mittelplatte' (fig. 32, *cMp*) and later the 'Chromatinreste' (fig. 34, *cR*).

The elimination of chromatin during the maturation and early cleavage divisions of the egg, as well as during the mitotic divisions of other kinds of cells, has often been recorded. For example, Wilson ('95, p. 458) estimates that only about one-tenth of the chromatin in the germinal vesicle of the starfish is retained to form the chromosomes during the first maturation division, and Conklin ('02) finds that "in *Crepidula* the outflow of nuclear material occurs at each and every mitosis" (p. 51). Furthermore, Rhode ('11) argues that chromatin diminution is a normal histological process, and describes such phenomena in blood cells, nerve cells, and cleavage cells of several Amphibia, comparing conditions with the chromatin-diminution in *Ascaris* and *Dytiscus*. His conclusion is as follows:

In der Histogenese der allerverschiedensten Gewebe tritt uns also die Erscheinung entgegen, dass die sich entwickelnden Zellen, bzw.

Kerne einen Teil ihres Chromatins abstossen, d.h. also eine Chromatindiminution erfolgt, wenn auch die Befunde selbst im speziellen von den bisher beobachteten in der Einleitung beschriebenen Fällen der Chromatindiminution etwas abweichen.

Eine Chromatindiminution tritt also nicht nur am Anfang und Ende der Keimbahn, wie es bisher angegeben worden ist, sondern in den verschiedensten Entwicklungsstadien und bei den verschiedensten Geweben und Tieren ein, sie hat also offenbar eine allgemeine Bedeutung (pp. 24–25).

Diminution processes similar to those in *Ascaris* and *Miasstor* have not been discovered in other animals, although investigators have been on the watch for such phenomena and have studied allied species, e.g., the work of Hasper ('11) on *Chironomus* and my own work on the Chrysomelid beetles (pp. 410 to 411). If, therefore, there be a similar difference in chromatin content between the germ cells and somatic cells in all animals, the elimination of chromatin from the latter must take place by the transformation of the basichromatin of the chromosomes into oxychromatin which passes into the cytoplasm during mitosis, or else by the more direct method advocated by the believers in the chromidia hypothesis.

The causes of the diminution of chromatin in *Ascaris* and *Miasstor* are unknown. Recently Boveri ('10) has concluded from certain experiments on the eggs of *Ascaris* (p. 444) that in this form it is the cytoplasm in which the nuclei are imbedded which determines whether or not the latter shall undergo this process. Kahle ('08) does not explain the cause of the diminution in *Miasstor*. To the writer it seems more important to discover why the nuclei of the Keimbahn cells *do not* lose part of their chromatin, since the elimination of chromatin during mitosis is apparently such a universal phenomenon. I would attribute this failure of certain cells to undergo the diminution process, not to the contents of the nucleus alone, but to the reaction between the nucleus and the surrounding cytoplasm. As stated in a former paper (Hegner, '09):

In *Calligrapha* all the nuclei of the egg are apparently alike, potentially, until in their migration toward the surface they reach the 'Keimhautblastem;' then those which chance to encounter the granules of

the pole-disc are differentiated by their environment, i.e., the granules, into germ-cells. In other words, whether or not a cell will become a germ-cell depends on its position in the egg just previous to the formation of the blastoderm (pp. 287-288).

Similarly, in *Ascaris*, the cleavage nuclei are conceived as similar so far as their 'prospective potency' is concerned, their future depending upon the character of their environment, i.e., the cytoplasm. In the egg of *Miastor*, the cleavage nucleus IV (fig. 3) does not lose part of its chromatin because of the character of the reaction between it and the substance of the 'polares Plasma.' In Chrysomelid beetles (Hegner, '08, '09a) and *Chironomus* (Hasper, '11) however, although no diminution process has been discovered in the nuclei which encounter the pole-disc or 'Keimbahnplasma,' the other nuclei in the egg, so far as known, are similar in this respect. The nuclei of the primordial germ cells, however, may be distinguished easily from those of the blastoderm cells in Chrysomelid beetles (figs. 53, 54), proving conclusively that a differentiation has taken place, either in one or the other. This differentiation probably occurs in the nuclei which take part in the formation of the blastoderm since the nuclei of the germ cells (fig. 53) retain more nearly the characteristic features of the preblastodermic nuclei (fig. 55), whereas those of the blastoderm cells (fig. 54) change considerably.

In some cases the eliminated chromatin may have some influence upon the histological differentiation of the cell, since it is differentially distributed to the daughter cells, but in *Ascaris* and *Miastor* no mechanism exists for regulating the distribution of the cast out chromatin and there is consequently no ground for the hypothesis that "in *Ascaris* those cells which become body cells are the ones that include the cast-off chromosome ends in their cytoplasm, and it will probably be found that these ejected chromosome parts engender such cytoplasmic differentiations as characterize the body cells" (Montgomery, '11, p. 192).

Chromidia. To several of the bodies listed in table 1 on page 461 as Keimbahn-determinants has been ascribed an origin from the chromatin of the germinal vesicle. Many cases of the elimination of chromatin from the nuclei of growing oocytes are

to be found in the literature. Blochmann ('86) discovered a process of 'budding' in the oocytes of *Camponotus ligniperda* resulting in the formation of 'Nebenkerne.' These appear first as small vacuoles lying near the nucleus; later they contain small staining granules and acquire a membrane. The 'Nebenkerne' grow in size and increase in number, while the nucleus of the oocyte becomes smaller. Stuhlmann ('86) described a similar phenomenon in about a dozen different species of Hymenoptera. The oocyte nucleus in all species examined becomes localized near the anterior end; then the small nuclear-like bodies form around it at its expense. The time of their production varies in the different species; in some they appear in the very young eggs; in others not until a much later stage has been reached. Sometimes they fuse to form a large 'Dotterkern' lying at the posterior pole of the egg; or they may remain separate and later become scattered. Paulcke ('00) also noted nuclear-like bodies near the oocyte nucleus of the queen bee, and Marshall ('07) has likewise found them in *Polistes pallipes*. In this species the nuclear-like bodies form a single layer around the nucleus; later they come to lie near the periphery of the oocyte and finally disappear. Loewenthal ('88) has described what appears to be chromatin in the cytoplasm of the egg of the cat, and an elimination of chromatin was noted by van Bambeke ('93) in the ovarian egg of *Scorpaena scrofa*. In none of these species, however, have Keimbahn-determinants been discovered.

According to Buchner ('10b) the 'besonderer Körper' in the egg of *Sagitta* and, in fact, Keimbahn-determinants in most other animals are of a chromidial nature, representing the trophochromatin demanded by the binuclearity hypothesis. The term chromidia was introduced by R. Hertwig in 1902 and applied to certain chromatin strands and granules of nuclear origin in the cytoplasm of *Actinosphaerium*. Goldschmidt ('04) transferred the chromidia hypothesis to the tissue cells of *Ascaris*. Since then chromidia have been described in the cells of many animals, including both somatic and germ cells. Thus far the group of zoologists which favor the chromidia idea has not received very extensive backing, but the fact remains

that chromatin particles are in some cases cast out of the nuclei in the oocytes of certain animals and continue to exist as such in the cytoplasm for a considerable period. It is also possible that, as Buchner ('10b) maintains, the Keimbahn-determinants may be in reality 'Keimbahnchromidien.'

This view was suggested by the writer in 1909 (p. 274) to account for the origin of the pole-disc granules in the eggs of Chrysomelid beetles. It was thought that here, as in the Hymenoptera (Blochmann, '86; et al.), chromatin granules might be cast out of the nuclei of the oocytes, and that these granules might gather at the posterior end to form the pole-disc. It was also suggested that chromatin granules from the nurse-cell nuclei might make their way into the oocyte and later become the granules of the pole-disc. It should not be forgotten, moreover, that these granules stain like chromatin. Finally, mention should be made of the 'anello cromatico' of Giardina ('01) which is associated with the differentiation of the oocytes in *Dytiscus* (p. 414).

Conclusion. Certain Keimbahn-determinants consist of nucleolar material which is derived from the germinal vesicle and which persists until the primordial germ cells are established. In some cases the Keimbahn cells are characterized by the possession of the complete amount of chromatin, in contrast to the somatic cells which lose a part of this substance. Since, however, the chromatin-diminution process does not occur in many species, it is not a universal phenomenon, and consequently cannot be of fundamental importance. Most of the evidence, on the other hand, points toward the conclusion that all of the cleavage nuclei are qualitatively alike, and that the cytoplasm is the controlling factor.

b. From cytoplasmic or extracellular nutritive substances: Yolk and nurse cells. It was pointed out on page 450 that one of the characteristics used to distinguish primordial germ cells from other embryonic cells is the presence of yolk material within them. In many vertebrates the yolk globules persist in the primordial germ cells until a comparatively late stage, and indeed are often so numerous as practically to conceal the nuclei of these cells. A large

number of the Keimbahn-determinants which have been described are supposed to consist of nutritive substances. Some of the earliest investigators were aware of the yolk content of the primordial germ cells. For example, in *Chironomus* Weismann ('63) found four oval nuclei lying in the 'Keimhautblastem' at the posterior end of the egg; each of these, he says, "besaßen einen Kreisrunden, klaren, etwas röthlich schimmernden Kern, und in einigen Lagen ausserdem noch ein oder zwei Dotterkörnchen." These are the 'Polzellen.' In another dipteran (*Simula sp.*) Metschnikoff (1866) records four or five pole-cells which "bestehen ausser einem Kerne noch aus einer die feinsten Dotterkörnchen enthaltenden Zellsubstanz." The same author ('66) also states that when the pseudovum in the paedogenetic larva of *Miastor* contains twelve to fifteen nuclei, "Man bemerkt zunächst, dass der am spitzen Pole des Pseudovums liegende Keimkern von einer dicken dunkeln Dottermasse schärger umgeben wird und mit dieser zusammen bald in eine besondere, 0.017 mm. grosse, membranlose Zelle sich abschnürt." This gives rise to the pole-cells.

In certain Daphnidae, Weismann and Ischikawa ('89) describe a 'Paracopulationszelle' which is derived from the contents of the germinal vesicle (p. 433); but the recent work of Kühn ('11, '13) renders it probable that this body is nothing but the remains of a nurse cell. The 'Dotterplatte' discovered by Noack ('01) at the posterior end of the egg of *Calliphora* (fig. 2) is considered by this investigator to consist of yolk elements. In previous communications (Hegner, '08, '09a, '11b) the writer has discussed the probability that the pole-disc in Chrysomelid eggs consists of nutritive material, and Wieman ('10a) also has offered arguments for this view.

Kühn ('11, '13) has presented what appears to be certain evidence that the Keimbahn-determinants in the egg of the Cladocera, *Polyphemus pediculus*, arise from one or more nurse cells. The granules segregated in certain cleavage cells of *Neritina* (Blochmann, '82) *Asplanchna* (Jennings, '96), *Lepas* (Bigelow, '02), *Siphonaria* (Fujitas, '04), and *Physa* (Wierzejski, '05) may be of a nutritive nature and these cells may be the stem cells

from which the germ cells of these animals eventually arise. The hypothesis that the nucleoli consist of food substance also argues in favor of the idea that the Keimbahn-determinants are nutritive.

The importance of these nutritive substances to the primordial germ cells can be stated with some degree of certainty. According to some authorities the primordial germ cells remain in the primitive condition and do not undergo differentiation at the same time, or at least at the same rate, as do the other embryonic cells. On this account their yolk contents are not at first utilized, since their metabolic activities are so slight. This is more especially true of the vertebrates, in which it has been suggested (Hegner, '09a, p. 276), that the yolk contents of the germ cells are transformed into the energy of motion during the characteristic migration of these cells into the germinal epithelium. Why these nutritive substances are segregated in the primordial germ cells is more difficult to answer.

Finally, it is interesting to note that the differentiation of the indifferent germ cells of *Helix arbustorum* into spermatogonia or oogonia has been found to depend upon nutrition (Buresch, '11). "Ob aber eine indifferente Geschlechtszelle sich in männlicher oder weiblicher Richtung weiter entwickeln wird, das können wir schon sehr früh sagen, nämlich nach der Lage dieser Zelle näher oder weiter von einer Nährzelle" (p. 327).

Yolk nucleus. There are many bodies in the cytoplasm of growing oocytes which have been called yolk nuclei and which may be responsible for the origin of the Keimbahn-determinants. Some of these bodies have already been considered, but the term 'yolk nucleus' has been applied to so many different cytoplasmic inclusions (Munson, '12) that no attempt will be made here to describe them nor to trace their history.

Mitochondria. The condition of the chondriosomes in the primordial germ cells of certain vertebrates (Rubaschkin, '10, '12; Tschaschkin, '10) and the theories proposed regarding the rôle of these bodies in heredity make it necessary to refer to them briefly here. A review of the literature on mitochondria, chondriosomes, plastosomes, etc., would be superfluous since

this has been done by Benda ('03) Prenant ('10), Faure-Fremiet ('10), and especially Duesburg ('11). At the present time it is difficult to make any definite statement regarding the origin, nature, and significance of the various cytoplasmic inclusions that have been grouped under the general title of mitochondria. It seems probable that we are concerned with a number of different sorts of inclusions, and with various stages in their evolution.

Mitochondria appear to be present in practically all cells, at least at some period in their existence. They have been observed in plants as well as in animals, and in living as well as in fixed and stained cells. Many terms have been applied to them of which the most frequently employed are mitochondria (Benda, '03), chondriosome (Meves, '08) plastochondria (Meves, '10) and plastosomes (Meves, '10). The advocates of the chromidia believe that these granules include mitochondrial formations (Goldschmidt, '04) and that the latter are therefore of nuclear origin, i.e., chromatic (Popoff, '07; Wassilieff, '07; Buchner, '10; Jörgensen, '10; et al.). On the other hand, the majority of investigators consider the mitochondria as cytoplasmic bodies (Vejdovsky, '07; Meves, '08; Duesberg, '11; Wilke, '12; et al.). Various functions have been ascribed to the mitochondria. Benda considered them to be motile; Regaud ('09) thinks that they fix and concentrate various substances in the cell ('fonction électique' of Renaut); and Meves ('08) maintains that they represent an important heredity substance, with the same relation to the cytoplasm that the chromosomes have to the nucleus.

Of particular interest to us are the results of Rubaschkin ('10, '12) and Tschaschin ('10) on the germ cells of vertebrates. In the guinea-pig and chick the chondriosomes of the cleavage cells are spherical and all similar, but, as development proceeds, those of the cells which become differentiated to produce the germ layers unite to form chains and threads, whereas those of the primordial germ cells remain in a spherical and therefore primitive condition (figs. 23, *B*; 24). This distinction between the mitochondrial nature of the primordial germ cells and the surrounding somatic cells may enable us to trace the Keimbahn

in vertebrates back into cleavage stages—something that has not been accomplished as yet.

It seems too early to speculate as to the influence of the mitochondria upon the primordial germ cells. That they are bearers of hereditary qualities and that those brought into the egg by the spermatozoon fuse with those of the egg as described by Meves ('08, '11) is doubted by many observers. Montgomery ('12) has shown that in *Peripatus* the mitochondria are entirely cast out of the spermatozoon during its metamorphosis, and that, at least in this species, the male cell does not contribute any of these bodies to the egg during fertilization. The same writer (Montgomery, '11) proposes an hypothesis to account for the segregation of germ cells as follows: "Any cleavage cell which failed to receive mitochondria, or failed to receive particular ones or a particular amount of them, would be incapacitated from engendering such somatic specializations (fibrillar structures), it would thereby become a germ cell" (p. 791). This substitution hypothesis is also offered by Montgomery, that the mitochondria of the prospective germ cells remain unaltered or latent, while those of the other cells undergo developmental changes. As we have already noted, evidence of such a condition had already been supplied and a similar hypothesis proposed by Rubaschkin ('10) and Tschaschin ('10); but so far as I know there are no data which enable us to sustain the first hypothesis. The data given in a previous part of this paper show that the pole-disc in *Chrysomelid* eggs may arise from mitochondria, but this does not seem very probable.

An examination of the various Keimbahn-determinants listed in table 1 (p. 461) has led the writer to conclude that none of them is of a mitochondrial nature, but the results obtained by the special methods employed by students of mitochondria give us good reason to hope that other substances may be made visible which will help to clear up the problem of primary cellular differentiation.

Metabolic products. Among the most difficult cases to explain are those of *Sagitta* and certain copepods, since here the Keimbahn-determinants apparently arise *de novo* in the cytoplasm.

Buchner's ('10b) contention that the 'besonderer Körper' of Sagitta is the remains of the 'accessory fertilization cell' of Stevens ('04) is not sustained by either Stevens ('10) or Elpatiewsky ('10). The nucleolar nature of the 'Aussenkörnchen' in Cyclops ('97) was later discarded ('03) and the conclusion was reached "dass ich die Aussenkörnchen ähnlich wie die Nukleolen, für temporäre, nicht-strukturierte Abscheidungen oder Zwischenprodukte des Kern-Zelle-Stoffwechses halte, welche in ganz bestimmten Zuständen der Zelle zur Abscheidung gelangen bzw. wieder aufgelöst werden" (p. 308-309). Amma ('11) has considered this subject at some length, and after rejecting the possibilities of these being of (1) chromatic, (2) nucleolar, (3) chromidial, or (4) mitochondrial origin, concludes that they are transitory structures and "dass die Ectosomen als Abscheidungen Endprodukte des Kern-Zelle-Stoffwechsels aufzufassen sind," (p. 557). In this way the Keimbahn-determinants in copepods are satisfactorily explained, and a similar explanation may be applied to Sagitta, although with less certainty.

c. From a differentiated part of the cytoplasm. A review of the literature on the Keimbahn-determinants and the investigation of these substances in the eggs of insects force me to conclude that the fundamental organization of the egg is responsible for the segregation of the primordial germ cells, whereas the visible substances simply furnished evidence of this underlying organization. As I have stated elsewhere (Hegner, '08, p. 21), regarding the Keimbahn-determinants in beetles' eggs, "the granules of the pole-disc are therefore either the germ cell determinants or the visible sign of the germ cell determinants." The writer's experiments have thus far failed to determine the exact function of these granules. When the posterior end of a freshly laid beetle's egg is pricked with a needle, not only the pole-disc granules flow out, but also the cytoplasm in which they are imbedded (Hegner, '08). If a small region at the posterior end be killed with a hot needle the pole-disc is prevented from taking part in the development of the egg, but so also is the surrounding cytoplasm. Eggs thus treated continue to develop and produce embryos without germ cells, but, as a rule, a part of

the posterior end of the abdomen is also absent (Hegner, '11a). The pole-disc granules and the cytoplasm containing them are moved by centrifugal force toward the heavy end of the egg and the latter is proved to be quite rigid, but eggs thus treated do not develop sufficiently normally to enable one to decide whether the pole-disc produces germ cells in its new environment or not.

That the germ cells of *Chironomus* arise from a prelocalized substance was stated by Balbiani ('85) in these words, "les glandes genitales des deux sexes ont une origine absolument identifique, naissant de la même substance et au même point de l'oeuf." Later Ritter ('90) expressed the opinion that the 'Keimwulst' of *Chironomus* consists "aus feinkörnigem Protoplasma," an opinion concurred in by Hasper ('11) who terms it 'Keimbahnplasma.' The similar material in *Miastor metraloas*—the 'polares Plasma'—is considered a special sort of protoplasm by Kahle ('08) and I can confirm this for *Miastor americana*. Further evidence of the protoplasmic nature of the substances which become segregated in the primordial germ cells is furnished by Boveri's experiments on *Ascaris*. In 1904 this investigator concluded from a study of dispermic eggs that the diminution process is controlled by the cytoplasm and not by an intrinsic property of the chromosomes, and that the chromosomes of nuclei lying in the vegetative cytoplasm remain intact, whereas those of nuclei imbedded in the animal cytoplasm undergo diminution. This conclusion has been strengthened by more recent experimental evidence (Boveri, '10) both from observation on the development of dispermic eggs and from a study of centrifuged eggs (fig. 19, p. 378). Boveri's results furnish a remarkable confirmation of the conclusions reached by the writer from a morphological study of the germ cells of Chrysomelid beetles and expressed in the following words: "All the cleavage nuclei in the eggs of the above named beetles are potentially alike until in their migration toward the periphery they reach the 'Keimhautblastem.' Then those which chance to encounter the granules of the pole-disc are differentiated by their environment, i.e., the granules, into germ-cells; all the other cleavage products become somatic cells."

Here, however, the pole-disc granules were considered the essential substance.

The appearance of the Keimbahn-determinants at a certain time and in a certain place, and their determinate segregation point unmistakably to an underlying regulating mechanism. These phenomena have some definite relation to the fundamental organization of the egg and require an investigation of our present knowledge of this subject.

The isotropism of the egg, as postulated by Pflüger, and the 'cell interaction' idea, especially developed by O. Hertwig and Driesch, have given way before the beautiful researches tending to uphold the hypothesis of 'germinal localization' proposed by His and championed by so many investigators within the past two decades. The starting point for embryological studies has shifted from the germ layers to the cleavage cells and from these to the undivided egg. Organization, which Whitman ('93) maintains precedes cell-formation and regulates it, is now traced back to very early stages in the germ cell cycle and is held responsible for the cytoplasmic localization in the egg.

One of the fundamental characteristics of the egg is its polarity. It has been known for about thirty years that the eggs of insects are definitely oriented within the ovaries of the adults. Hallez in 1886, finding this to be true of the ova of *Hydrophilus* and *Locusta*, expressed the fact in his "Loi de l'orientation de l'embryon chez les insectes" as follows: "La cellule-oeuf possede la meme orientation que l'organisme maternal qui l'a produit: elle a un pole cephalique et un pole caudal, un cote droit et un cote gauche, une face dorsale et une face ventrale; et ces differentes faces de la cellule-oeuf coincident aux faces correspondentes de l'embryon." Moreover, gravity and the action of centrifugal force have no effect upon polarity of insect eggs (Hegner, '09b). Giardina ('01) has found that during the divisions of the oogonia in *Dytiscus*, a rosette of sixteen cells is produced, one of which is the oocyte and the other fifteen nurse cells. The rosette thus formed possesses a definite polarity coincident with the axis of the oocyte which is identical with that which was present in the last generation of oogonia. Similarly in *Miastor* (fig. 27) the

polarity of the oocyte is recognizable as soon as the mesodermal cells, which serve in this species as nurse cells, become associated with it.

The germ cells of other animals also possess a precocious polarity, as evidenced by their implantation in the germinal epithelium (e.g., Wilson, '03; Zeleny, '04, in *Cerebratulus*), the position of the nucleus, the formation of the micropyle (Jenkinson, '11), etc. This is true not only for the invertebrates, but, as Bartelmez ('12) claims, "the polar axis persists unmodified from generation to generation in the vertebrates and is one of the fundamental features of the organization of the protoplasm" (p. 310). Furthermore, experiments with centrifuged force seem to prove that the chief axis of the egg is not altered when substances are shifted about, but is fixed at all stages (Lillie, '09; Morgan, '09; Conklin, '10). Bilaterality also is demonstrable in the early stages of the germ cells of many animals, and like polarity, seems to be a fundamental characteristic of the protoplasm.

It is somewhat difficult to harmonize the various results obtained, especially by experimental methods, from the study of egg organization. As the oocytes grow, the apparently homogeneous contents become visibly different in some animals, and when the mature eggs develop normally these 'organ-forming substances' are segregated in definite cleavage cells and finally become associated with definite organs of the larva.

Conklin ('05) has shown "that at least five of the substances which are present in the egg [of *Cynthia*] at the close of the first cleavage, viz., ectoplasm, endoplasm, myoplasm, chymoplasm, and chordaneuroplasm, are organ-forming substances." Under experimental conditions

. . . . they develop, if they develop at all, into the organs which they would normally produce; and conversely, embryos which lack these substances, lack also the organs which would form from them.

. . . . Three of these substances are clearly distinguishable in the ovarian egg and I do not doubt that even at this stage they are differentiated for particular ends (p. 220). . . . The development of ascidians is a mosaic work because there are definitely localized organ-forming substances in the egg; in fact the mosaic is one of organ-form-

ing substances rather than of cleavage cells. The study of ctenophores, nemertines, annelids, mollusks, ascidians and amphibians (the frog) shows that the same is probably true of all these forms and it suggests that the mosaic principle may apply to all animals (p. 221).

The same writer has also proved from his study on *Phallusia* ('11) that these various substances exist even when they are not visible in the living egg. It is interesting also to note that Duesberg ('13) finds the 'myoplasm' of *Cynthia* to be crowded with plasmosomes, differing in this respect from other egg regions.

Experiments, especially those of Lillie ('06), Morgan and Spooner ('09), Morgan ('10) and Conklin ('10) have shown that in many eggs the shifting of the supposed organ-forming substances has no influence upon development, and leads to the conclusion that these visible substances play no fundamental rôle in differentiation, but that the invisible ground substance is responsible for determinate development. The eggs of different animals, however, differ both in time and degree of organization, and the conflicting results may be accounted for by the fact that specification is more precocious in some than in others.

The most plausible conclusions from a consideration of these observations and experiments are that every one of the eggs in which Keimbahn-determinants have been described, consists essentially of a fundamental ground substance which determines the orientation; that the time of appearance of Keimbahn-determinants depends upon the precociousness of the egg; that the Keimbahn-determinants are the visible evidences of differentiation in the cytoplasm; and that these differentiated portions of the cytoplasm are definitely localized by cytoplasmic movements, especially at about the time of maturation.

B. The localization of the Keimbahn-determinants

One of the characteristics of the Keimbahn-determinants is their regular appearance at a certain stage in the germ cell cycle, according to the species in which they occur, and their constant localization in a definite part of the egg, or in one or more definite cleavage cells. Keimbahn-determinants are recognizable in

many insect eggs before fertilization is accomplished, and even before the oocyte has reached its maximum size. We know that in *Chironomus* the 'Keimwulst' (Ritter, '90) or 'Keimbahnplasma' (Hasper, '11) is present when the egg is laid, at which time the pronuclei as a rule have not yet fused. This is true also of the 'Dotterplatte' in *Calliphora* (Noack, '01). There can be little doubt, however, that these substances are present as such in the eggs before fertilization, judging from our knowledge of the history of similar materials in the eggs of other insects. The 'pole-dise' in the eggs of Chrysomelid beetles (Hegner, '08; Wieman, '10a) and the 'polares Plasma' in *Miastor* (Kahle, '08; Hegner, '12) are recognizable some time before fertilization and cannot therefore arise because of any influence exerted by the spermatozoon. Moreover, in *Miastor* the eggs thus far examined have all been parthenogenetic. In parasitic Hymenoptera the 'nucleolo' leaves the germinal vesicle in both fertilized and parthenogenetic eggs before the egg is laid. In only one animal, not an insect, has a similar occurrence been noted, namely, in *Polyphemus*, where, according to Kühn ('11, '13) the Keimbahn-determinants consist of the remains of one or more nurse cells (fig. 18). In the Daphnidae (Weismann and Ischikawa, '89) the 'Paracopulationszelle' arises from material cast out by the germinal vesicle; in *Aequorea* (Haecker, '92) the 'Metanucleolus' is likewise derived from the germinal vesicle; in *Ascaris* (Boveri, '92) chromatin diminution occurs during the two to four-cell stage; in *Cyclops* (Haecker, '97, '03) and other copepods (Amma, '11) the 'Aussenkörnchen' or 'Ectosomen' become visible soon after fertilization (*Diaptomus*), but usually not until the pronuclei fuse (other species); in *Sagitta* the 'besonderer Körper' (Elpatiewsky, '09, '10) or 'Keimbahnchromidien' (Buchner, '10b) appear to arise de novo after fertilization, although, if Buchner's contention that they are the remains of the accessory fertilization cells be correct, they should be classed with the 'Nahrzellenkern' described by Kühn ('11, '13) in *Polyphemus*.

It is thus evident that the Keimbahn-determinants become visible, wherever they have been described, either just before

or just after the eggs are fertilized, or, in parthenogenetic forms, shortly before maturation and cleavage are inaugurated.

The localization of the Keimbahn-determinants at the time of their appearance seems to be predetermined. In insects the posterior end of the egg is invariably the place where these bodies occur. In species whose eggs undergo total cleavage they are, as a rule, under normal conditions, segregated in one definite blastomere from the two-cell stage up to the thirty-two cell stage, and are then distributed among the descendants of the single primordial germ cell. In *Ascaris* it is normally the cell at the posterior (vegetative) pole that fails to undergo the diminution process. It seems therefore that there must be some mechanism in the egg which definitely localizes the Keimbahn-determinants.

The segregation of these substances in one blastomere at the first cleavage division is a result of their previous localization, but in later cleavage stages events are more difficult to interpret. Both Haecker ('97) and Amma ('11) have attempted to explain the distribution of the 'Ectosomen' in copepods by postulating a dissimilar influence of the centrosomes resulting in the segregation of these granules at one end of the mitotic spindle in the dividing stem-cell. According to Zeigler's hypothesis, the centrosomes during unequal cell division are heterodynamic, and Schönfeld ('01) believes that the synizesis is due to the attraction of the chromosomes by the centrosomes. It is well known that in many cases where unequal cell division occurs, one aster is larger than the other, and this may be the true interpretation of the phenomena, but to the writer it seems more probable that the entire cell contents undergo rearrangement after each cell division, possibly under the influence of the material elaborated within the nucleus and set free during mitosis. Elpatiewsky ('09) also believes in the unequal attractive force of the centrosomes in *Sagitta*, as indicated in the following quotation:

Nach der vierten Teilung kommt der besondere Körper in dem Wirkungskreis eines Zentrosomas, nämlich desjenigen, welcher näher der Polarfurche liegt. Fast die ganze 'Energie' dieses Zentrosomas wird für die Ueberwindung der *Vis inertiae* des besonderen Körpers

verbraucht; dieser wird dem Zentrosoma genähert und umschliesst es wie mit einer Kappe, so dass er im optischen Durchschnitt stets Hufeisen oder Sichelform aufweist. Infolge davon wird die Wirkung dieses Zentrosomas auf das Zellplasma nur sehr schwach, dieses Zentrosoma kann nur einen kleinen Plasmateil beherrschen, und die resultierende Zelle wird viel kleiner, als die Schwesterzelle. Diese kleine Zelle, die den besonderen Körper bekommen hat, liegt näher zum vegetativen Poles, als die grössere Schwesterzelle, und stellt die erste Urgeschlechtszelle G (d^{111}), die grössere Schwesterzelle die erste Urentodermzelle E (d^{112}). vor (p. 231).

In *Ascaris*, certain copepods, *Sagitta*, *Polyphemus* and certain *Daphnidae*, the Keimbahn-determinants are not segregated in one cleavage cell after about the thirty-two cell stage, but their substance is distributed at the next division between the daughter cells. In the insects, such as *Chironomus*, *Miastor* and *Chrysomelid* beetles, where, on account of the superficial cleavage, the Keimbahn-determinants are not segregated in blastomeres, the primordial germ cells, from the beginning, consist almost entirely of the Keimbahn material or this material plus the matrix in which it is imbedded. Hence in these cases the Keimbahn-determinants are localized at a determined point during each cleavage stage, instead of being carried about by the movements of the egg contents or of the blastomeres, but, as in the eggs which undergo total cleavage, the determinants are distributed between the daughter cells as soon as the primordial germ cells are established. The reason for this appears to be that localizations occur in holoblastic eggs at each cleavage and that not until the thirty-two cell stage or thereabouts does the Keimbahn material become entirely separated from other organ-forming substances and segregated in a single cell. When this point is finally reached this Keimbahn material must necessarily become divided between the daughter cells.

In practically all known cases the daughter cells of the primordial germ cells are equal in size and each receives an equal portion of the Keimbahn-determinants. This is certainly to be expected from their constitution and future history. *Sagitta*, however, differs in this respect for the remains of the 'besonderer Körper' appear to be unequally distributed between the two

daughter cells of the primordial germ cells (fig. 22) and both Elpatiewsky ('09, '10) and Stevens ('10), therefore, consider this as probably a differential division whereby in this hermaphroditic animal the substance of the male primordial germ cell is separated from that of the female. More work is necessary to make certain of this point.

Conclusion. Keimbahn-determinants are definitely localized in the egg and in definite cleavage cells. This localization is first observable just before or just after the eggs are fertilized, or, in parthenogenetic forms, shortly before maturation and cleavage are inaugurated. Some mechanism in the egg must be responsible for this localization. Heterodynamic centrosomes may have some influence so far as the segregation of the Keimbahn-determinants in cleavage cells is concerned, but the movement of the egg contents seems to be a more probable cause of localization.

C. The fate of the Keimbahn-determinants

It is unfortunately impossible to trace the Keimbahn-determinants throughout the entire germ cell cycle. The question of their fate, however, is an important one. As we have seen, they become apparent shortly before or just after the inauguration of the maturation divisions, and remain intact for a brief period during the early cleavage stages. They persist in insects as definitely recognizable granules (fig. 8, *F*) for some time after the primordial germ cells are segregated; then they gradually break up into finer particles, leaving no trace of their existence behind except in so far as they give the cytoplasm of the germ cells a greater affinity for certain dyes. In *Chironomus* they may still form distinct masses after the definitive germ glands have been formed (fig. 1). The ectosomes in the copepods are temporary bodies which appear to rise *de novo* during the formation of each mitotic figure in the early cleavage stages; then break down and disappear. Practically all of the other Keimbahn-determinants persist during early cleavage and then disappear as distinct visible bodies as soon as the primordial germ cells

are definitely segregated. What becomes of them during the comparatively long period between their disappearance in the primordial germ cells and their reappearance in the oocytes or mature eggs can only be conjectured. They seem to disintegrate into very fine particles which become thoroughly scattered within the cell body and mixed with the cytoplasm. It has been suggested (p. 397) that they may retain their physiological characteristics and become concentrated again in the growing oocytes into morphologically similar bodies, increasing in the meantime, by multiplication or in some other way, until they equal in mass those of the preceding generation of germ cells. On the other hand, they may all, like the ectosomes of copepods, be temporary structures, produced at a certain time and place under similar metabolic conditions, and becoming associated with particular parts of the cell contents, may thus be constant in their distribution.

Several ideas have been advanced regarding the fate of the eliminated chromatin in *Ascaris*. The ends of the chromosomes which are cast out into the cytoplasm are not equally distributed among the daughter cells nor does there appear to be any mechanism for their definite unequal division. These facts argue against the theory that these cast out chromatin bodies serve as determinants and also make improbable the hypothesis that they enable the somatic cells to differentiate, whereas the germ cells which do not undergo the diminution process remain in an indifferent condition since their cytoplasm lacks this material (Montgomery, '11, p. 792). However, the fact that during the early cleavage divisions in some animals (p. 465) large amounts of chromatin escape from the nucleus and are differentially distributed to the daughter cells, is evidence that nuclear material may play some important rôle in the progressive changes of cleavage cells.

It has been shown that in many animals the germ cells do not multiply for a considerable period during the early developmental stages. This period also coincides with that during which the Keimbahn-determinants, as a rule, disappear. For example, the germ cells of Chrysomelid beetles multiply until there are about sixty-four present, at which time they constitute a group

at the posterior end of the egg and the embryo has just started to form; no further increase in number occurs until the larval stage is reached and the definitive germ glands are established. As soon, however, as the embryo has reached a certain developmental stage, the germ cells migrate into it, and it looks very much as though they remain quiescent until the somatic cells are "able to protect, nourish, and transport" them.

The number of primordial germ cells during the 'period of rest' is perhaps most definitely known in *Miastor*, where, as one group of eight and later as two groups of four each, they are present throughout a large part of embryonic development.

In vertebrates also, a long period exists, during which division of the primordial germ cells does not take place (fig. 24) and, at least in several species, certain cell contents (the mitochondria) remain in an indifferent condition (Rubaschkin, '10; Tschaschkin '10; fig. 23, *B*). These facts all indicate that these cells remain in a primitive condition and do not undergo the histological differentiations characteristic of somatic cells, a view which, however, has been objected to (Eigemann, '96). The disappearance of the Keimbahn-determinants and the yolk globules of vertebrates during this period have suggested that these substances are nutritive in function, furnishing energy to the migrating germ cells.

The fact of this long rest period, followed by rapid multiplication of the oogonia and spermatogonia, during which no important specializations occur, and later succeeded by the remarkable changes which occur in both the oocytes and spermatocytes has led to the suggestion (Montgomery, '11, pp. 790-792) that in the germ cell cycle there is a series of changes parallel with that of the somatic cycle. In the development of both cycles preformation and epigenesis proceed at the same time. The chromosomes seem to be preformed elements of the germ cells, since they are apparently the most stable constituents. The cytoplasm, on the other hand, undergoes a series of epigenetic changes such as the formation of an idiozome, the development of mitochondria, the appearance of a sphere and the metamorphosis of the spermatozoon.

Finally, we must inquire into the fate of the Keimbahn-determinants in the male germ cells. Does the Keimbahn material in these cells increase in amount, as has been suggested for the oocytes, and is it localized in the spermatogonia, spermatocytes, or spermatozoa as a definite, visible substance? We know from the investigations of Meves ('11) that the plastosomes in the spermatozoon are carried into the egg, in the case of *Ascaris*, and there fuse with the plastosomes of the ovum. Whether Keimbahn-determinants act in a similar manner is unknown. There are, however, certain cytoplasmic inclusions in the male germ cells which have been compared with similar structures in the oocytes; for example, the chromatic body described by Buchner ('09) in the spermatogenesis of *Gryllus* (see p. 415), and the plasmosome which is cast out of the nucleus of the second spermatogonia in *Periplaneta* and disintegrates in the cytoplasm (Morse, '09). That Keimbahn-determinants from the spermatozoon are not necessary for the normal production of germ cells is of course evident, since some of the species with which we are best acquainted (for example, *Miastor*) are parthenogenetic.

7. CONCLUSIONS AND SUMMARY

1. The most interesting period in the germ-cell cycle is that extending from the formation of the ultimate oogonia and spermatogonia to the complete segregation of the germ cells in the developing egg. A little known and important part of this period is that during which, in some animals, visible substances (Keimbahn-determinants) peculiar to the germ cells, appear, become localized in a definite part of the egg or in certain blastomeres, and are equally distributed among the primordial germ cells (pp. 376-379).

2. The Keimbahn in animals was first traced in dipterous insects. Keimbahn-determinants appear in the eggs of all Diptera that have been carefully studied. The most detailed reports have been upon *Miastor*, *Chironomus*, and *Calliphora* (figs. 1-2, pp. 380-387).

3. In *Miastor* there are a definite number of cell divisions during the multiplication of the oogonia, namely, six. The somatic cells lose part of their chromatin by diminution processes, whereas the germ cells possess a complete amount of chromatin. The nurse cells are of mesodermal origin. A peculiar mass of cytoplasm becomes situated at the posterior end of the oocyte; within this one of the first eight cleavage nuclei (with a complete amount of chromatin) becomes imbedded; it is then cut off from the rest of the egg as the primordial germ cell. The origin of this peculiar mass of cytoplasm could not be determined, but several hypotheses are offered to account for its genesis (figs. 27-40, 3-5, pp. 387-398).

4. The eggs of the ovoviviparous dipteron, *Compsilura* and of the willow-cone gall fly, *Cecidomyia*, contain Keimbahn-determinants which have a history like that of similar bodies in other insects (figs. 41-43, 6, pp. 398-399).

5. An early segregation of germ cells has been reported for certain Chrysomelid beetles and Keimbahn-determinants have been found in the eggs of those carefully examined. A résumé of the writer's previously published results is given (figs. 7-8, pp. 400-408).

6. An examination of all stages in the early cleavage of Chrysomelid eggs failed to reveal a chromatin-diminution process such as occurs in *Ascaris* and *Miastor*. The conclusion is reached that the cleavage nuclei are all potentially alike and that the cytoplasm controls their differentiation into the nuclei of blastoderm cells, primordial germ cells, and vitellophags. What appears to be amitotic nuclear division among the vitellophags is described (figs. 44-57, 7-8, pp. 408-413).

7. No nuclear changes were observed in the germ-cell cycle of *Leptinotarsa* resembling those recorded by Giardina ('01) and others in *Dytiscus* resulting in the formation of nurse cells and ultimate oogonia (pp. 413-417).

8. The pole-disc granules in Chrysomelid eggs form a recognizable mass, just before the oocyte reaches its full size. This genesis could not be definitely determined, but several methods of origin are suggested. The growth of the oocyte is described and figured (figs. 58-66, 9, pp. 417-420).

9. In the testis of *Leptinotarsa* the germ cells in each cyst arise from a single spermatogonium. Spindle remains connect the daughter spermatogonia up to the time when sixty-four cells are present in each cyst. This process is homologous to the differential divisions in *Dytiscus* and other beetles, and certain Hymenoptera, during which an ultimate oogonium and a definite number of nurse cells arise from a single oogonium (figs. 68-74, 10-12, pp. 420-424).

10. What appears to be amitotic nuclear division was found among the nurse cells of *Leptinotarsa*, but no nuclear phenomena were observed among the oogonia or spermatogonia which could be interpreted as amitosis and which could not be regarded as phases of mitosis (fig. 67, p. 427.)

11. A brief statement is given of certain phenomena that have been recorded during the segregation of the germ cells in the Hymenoptera (figs. 13-14, pp. 428-432).

12. The Keimbahn in the Crustacea is best known in certain Cladocera and Copepoda. In some species the Keimbahn-determinants seem to be temporary bodies which represent the "Endprodukte des Kern-Zelle-Stoffweschels." In others they appear to originate from nurse cells which enter the oocyte (figs. 15-18, pp. 432-441).

13. In several species of *Ascaris* a determinate segregation of germ cells has been recorded and a chromatin-diminution process discovered. The most recent work indicates that this diminution process is controlled by the cytoplasm and is not initiated by the nuclei (figs. 19-21, pp. 442-446).

14. The origin of the 'besondere Körper,' which serves as a Keimbahn-determinant in *Sagitta*, has not been determined. It apparently is unequally distributed when the primordial germ cell divides, and the daughter cell which receives the larger portion is considered the primordial spermatogonium; the other is the primordial oogonium (figs. 22, pp. 446-449).

15. The vertebrates do not furnish as favorable material for germ cell studies as do the invertebrates. No definite Keimbahn-determinants have been discovered in them but bodies have been described in the cell body of some of them which give

us hopes of tracing the germ-cell cycle back to cleavage stages. A peculiar rod has been found in the spermatogonia of man which is regarded as a Sertoli-cell-determinant (figs. 23-25, pp. 449-456).

16. Metanucleoli and granules of various kinds have been described in the mature eggs and early cleavage stages of a number of animals, which may, upon closer study, be found to play some rôle in the segregation of the germ cells (fig. 26, pp. 456-460).

17. A table is given (p. 461) of the principal described cases of the occurrence of the Keimbahn-determinants in animals. These bodies in certain species arise from nuclear substances, such as metanucleoli, chromidia, or the 'achromatishen Kernkörper' (pp. 462-469).

18. Yolk globules are characteristic of the primordial germ cells and may play some rôle in their genesis; nurse cells are known to become Keimbahn-determinants in some Crustacea; and in certain other Crustacea the Keimbahn-determinants are considered metabolic products; the mitochondria of germ cells differ from those of the surrounding somatic cells (pp. 469-474).

19. Observations and experiments indicate that the egg is definitely organized and that this organization is continuous throughout the germ cell cycle. The Keimbahn-determinants are bodies which enable us to determine the position of that part of the egg substance which controls the production of the primordial germ cells, and to identify the stem cell and ultimately the primordial germ cells when they are definitely established (pp. 474-478).

20. Keimbahn-determinants become visible at a definite time and place, usually just before or just after maturation takes place and cleavage begins. The localization of these bodies is determined by the organization of the cytoplasm and takes place during cleavage, either under the influence of the centrosomes, or, more probably, by rearrangements of the egg contents (pp. 478-482).

21. The Keimbahn-determinants become distributed apparently equally (except in *Sagitta*) between the daughter cells of the primordial germ cell. As a rule they then gradually disintegrate and disappear, and hence cannot be traced throughout

the entire germ cell cycle. It seems probable, however, that the particular kind of cytoplasm (germ-plasm) marked by the presence of these bodies is continuous throughout the germ cell cycle. In some animals (e.g., *Miastor*, fig. 32) it constitutes the entire substance (with the exception of the nucleus) of the primordial germ cell; this is also true in other animals, but, as shown in figure 17, a succession of cleavage divisions occurs before the germ-plasm is entirely separated from the somatic-plasm. The nature of the Keimbahn-determinants is uncertain, but their origin from metanucleoli, nurse cells, and possibly the nutritive stream suggests that they may play a rôle in the nutrition of the germ cells during the period extending from their segregation until the formation of the definitive germ glands (pp. 482-485).

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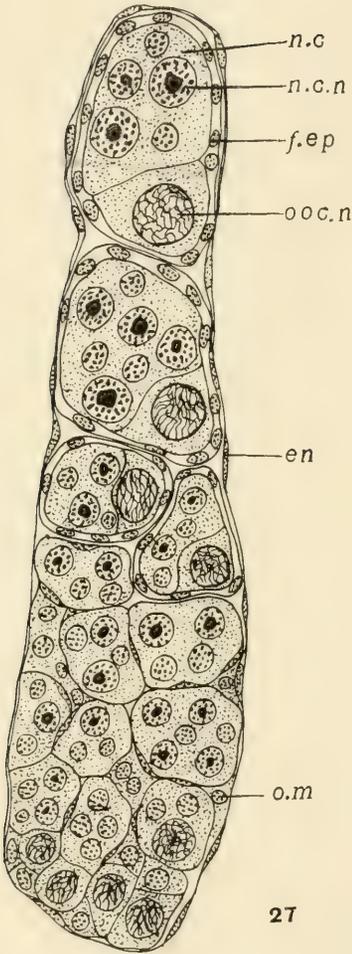
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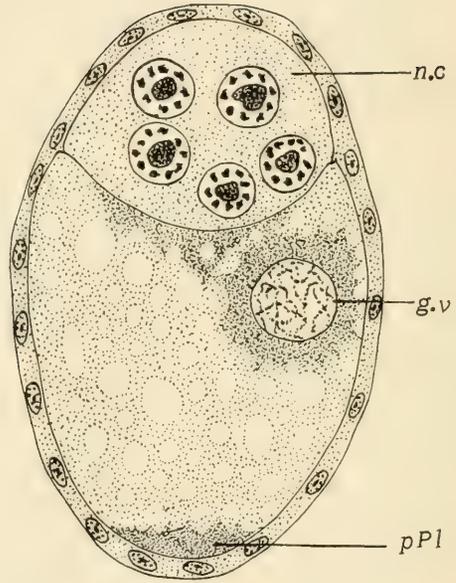
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ABBREVIATIONS

<i>b.c.</i> , blastoderm cell	<i>n.c.</i> , nurse chamber
<i>c.</i> , cytoplasm	<i>n.c.n.</i> , nurse cell nucleus
<i>ch.</i> , chorion	<i>o.m.</i> , ovarian mesoderm
<i>cMp.</i> , 'Chromosomenmittleplatte'	<i>ooc.</i> , oocyte
<i>c.n.</i> , cleavage nucleus	<i>ooc.n.</i> , oocyte nucleus
<i>cR.</i> , 'Chromatinreste'	<i>oog.</i> , oogonium
<i>en.</i> , ovarian envelope	<i>p.b.</i> , polar body
<i>ep.</i> , epithelial cell	<i>pd.</i> , pole-disc
<i>f.ep.</i> , follicular epithelium	<i>p.g.c.</i> , primordial germ cell
<i>f.n.</i> , female nucleus	<i>p.Pl.</i> , 'polares Plasma'
<i>g.v.</i> , germinal vesicle	<i>s.</i> , spermatozoon
<i>m.</i> , mesoderm	<i>spg.</i> , spermatogonium
<i>m.s.</i> , maturation spindle	<i>t.c.</i> , terminal cap



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EXPLANATION OF FIGURES

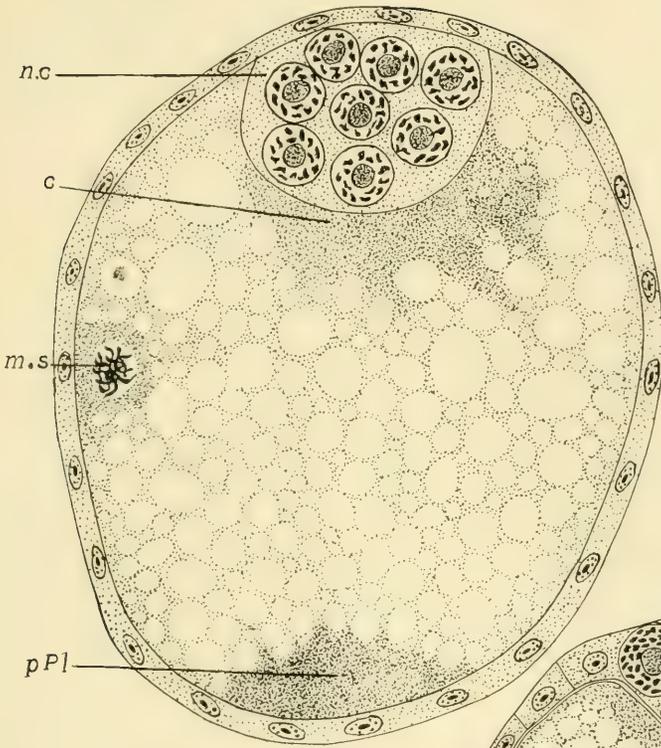
Miastor americana Felt

27 A section through the ovary of *Miastor* showing the accumulation of mesoderm cells to form the nurse chamber (*n.c.*), and the follicular epithelium (*f.ep.*).

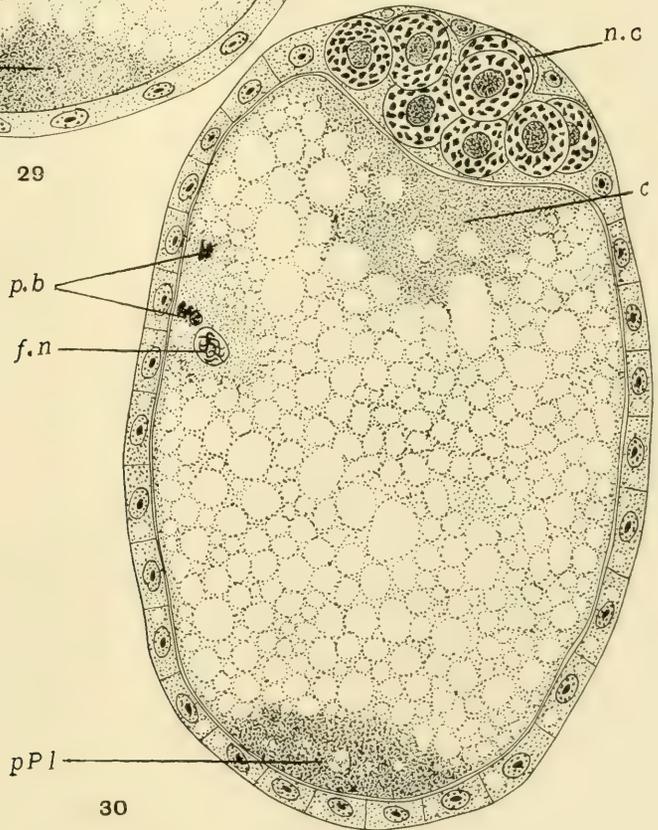
28 Longitudinal section through a nearly grown oocyte.

29 Longitudinal section through an oocyte at the time of polar body formation.

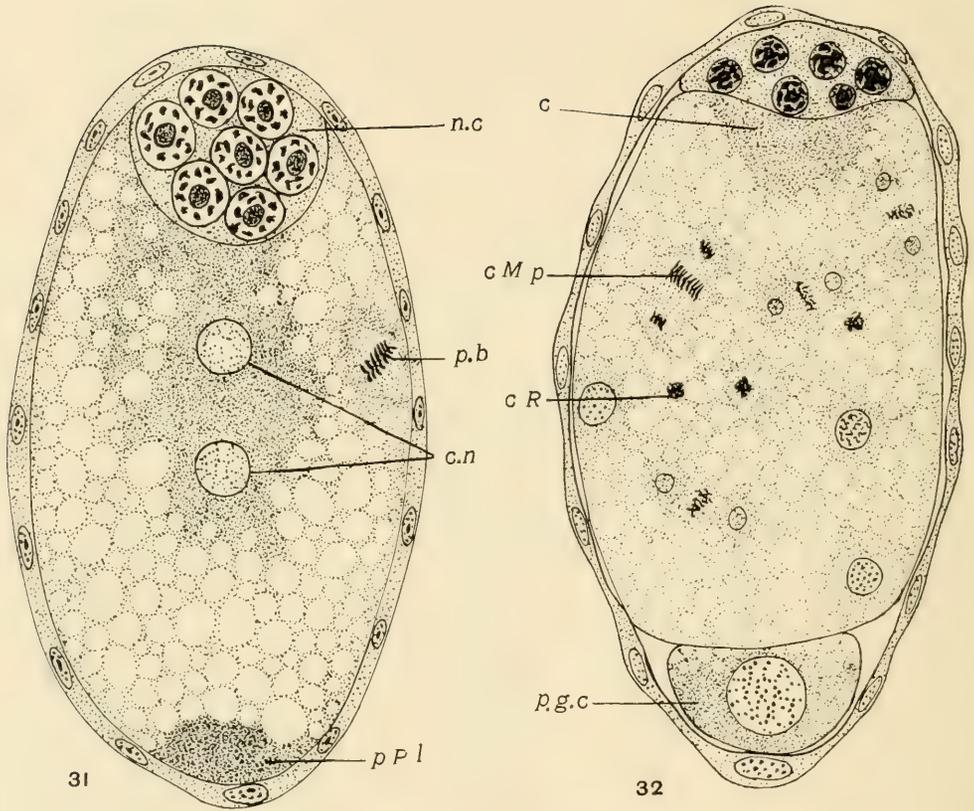
30 Longitudinal section through a mature egg showing the female nucleus (*f.n.*), and the polar body (*p.b.*) already divided.



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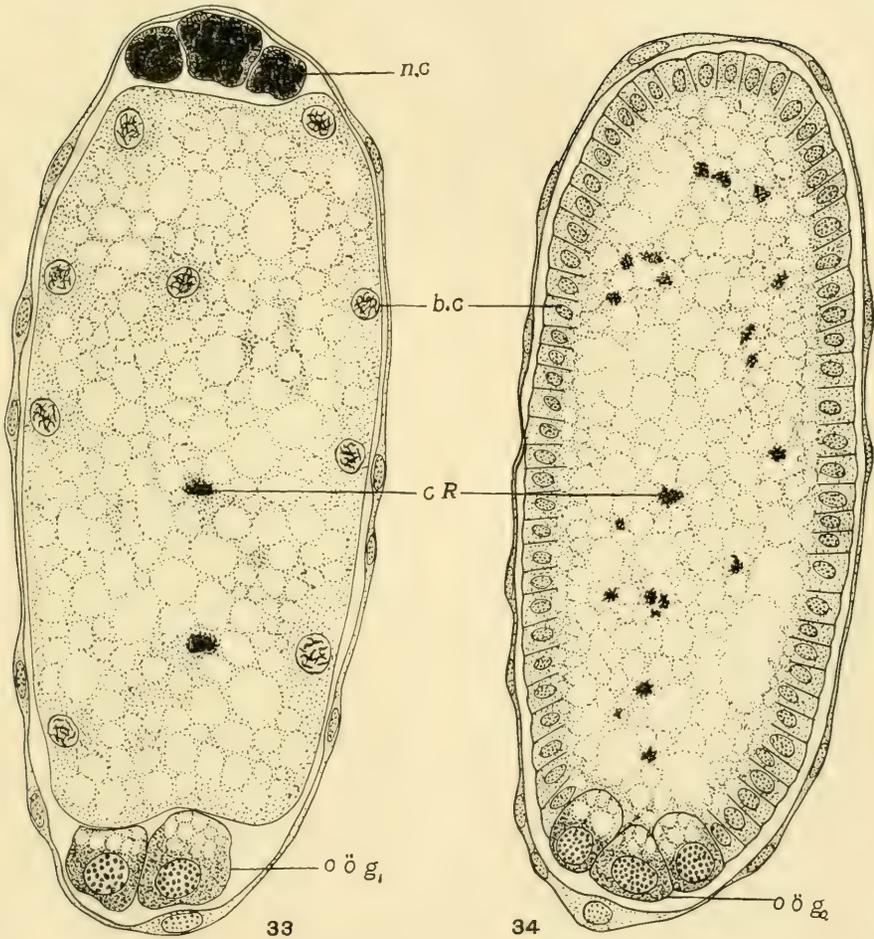


EXPLANATION OF FIGURES

Miastor americana Felt

31 Longitudinal section through an egg containing two cleavage nuclei (*c.n*) and a dividing polar body (*p.b*).

32 Longitudinal section through an egg with a single primordial germ cell (*p.g.c*), several nuclei undergoing the chromatin-diminution process, and several clumps of cast-out chromatin (*c.R*).

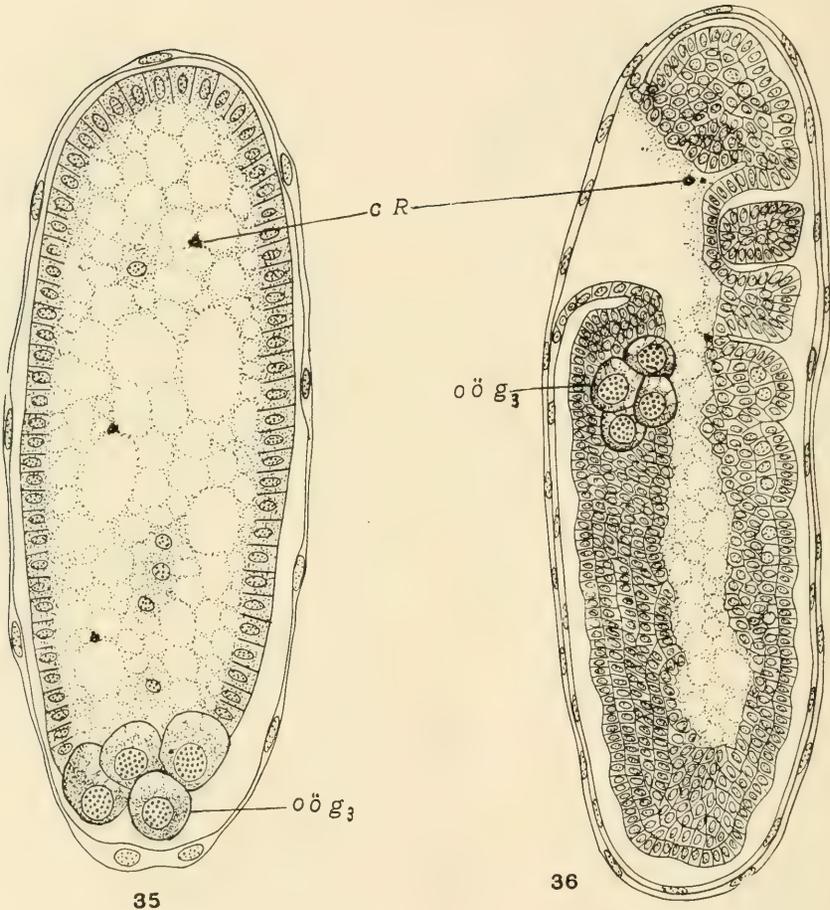


EXPLANATION OF FIGURES

Miastor americana Fell

33 Longitudinal section through an egg with two primordial germ cells (*oog*₁). The somatic cells (*b.c*) are preparing to form the blastoderm.

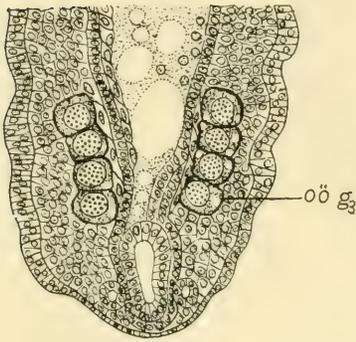
34 Longitudinal section through an egg with four germ cells (*oog*₂).



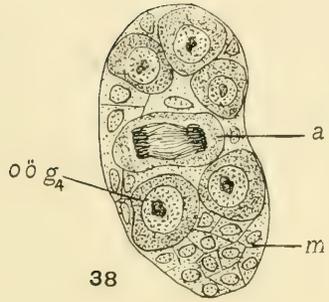
EXPLANATION OF FIGURES

Miastor americana Fell

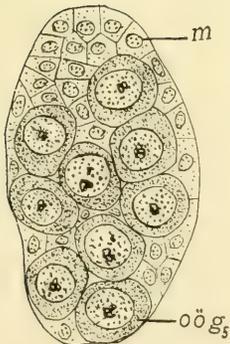
- 35 Longitudinal section through an egg with eight germ cells ($oo g_3$).
36 Sagittal section through an embryo with eight germ cells ($oo g_3$) near the end of the tail-fold.



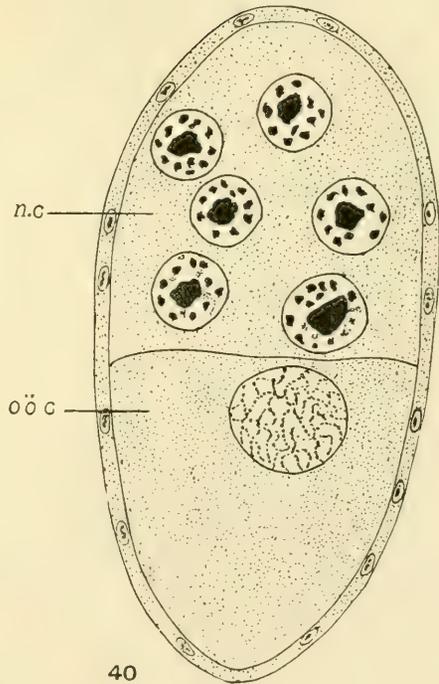
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EXPLANATION OF FIGURES

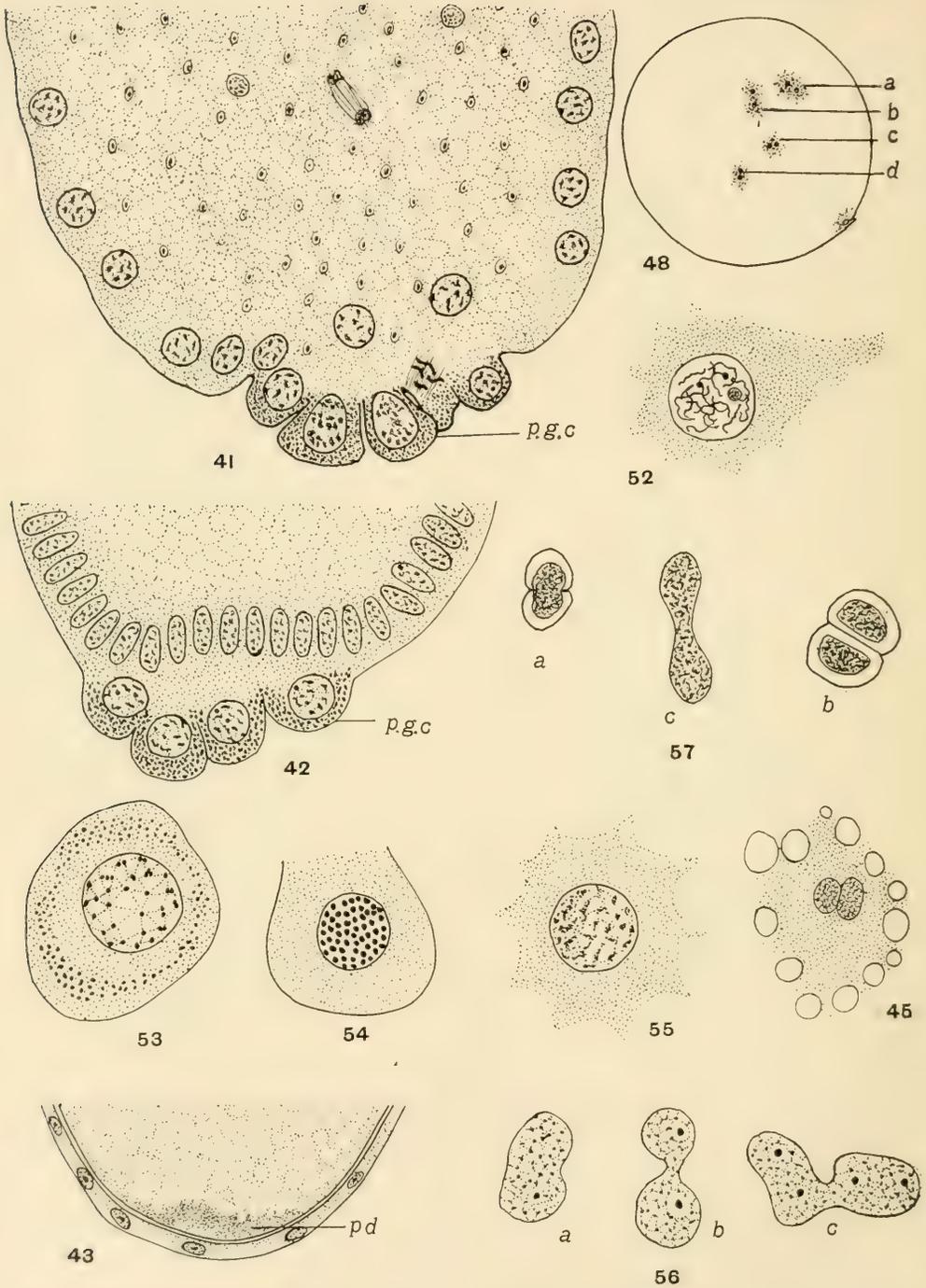
Miastor americana Felt

37 Frontal section through the posterior end of an older embryo showing two rows of four germ cells each (oog_3).

38 A section through an ovary containing eight oogonia (oog_4), one of which is dividing by mitosis (a).

39 A section through an ovary containing sixteen oogonia (oog_5).

40 A section through a young oocyte (ooc) and the accompanying nurse cells ($n.c.$).



41 *Compsilura concinnata*; section through posterior end of egg showing formation of primordial germ cells (*p.g.c.*).

42 A slightly later stage than in figure 41.

43 *Cecidomyia strobiloides*; section through posterior end of egg just before deposition.

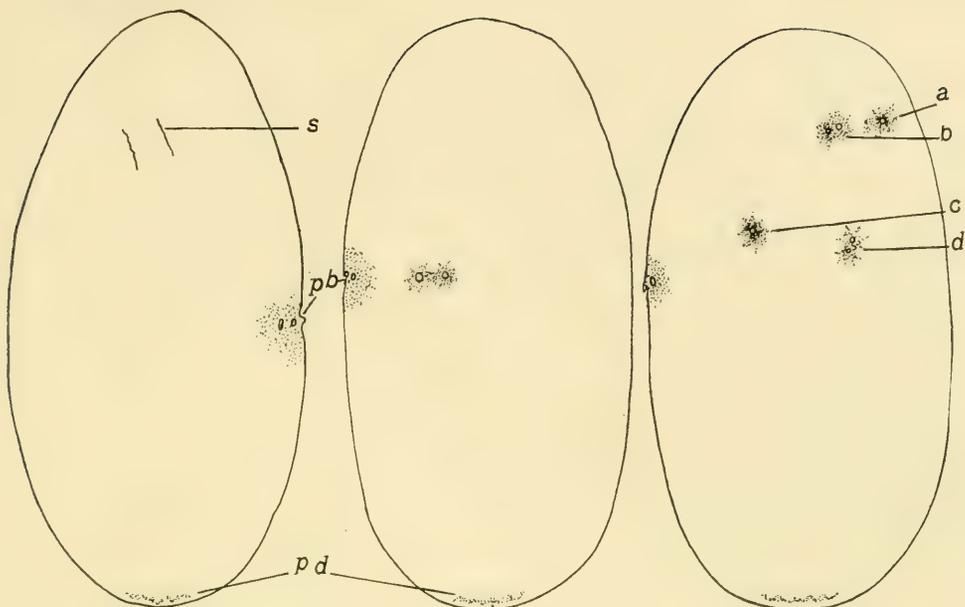
44-55 *Calligrapha*.

44 Section through an egg containing one polar body (*p.b.*), a female nucleus, and two spermatozoa (*s.*).

45 The fusion of male and female nuclei.

46 Egg containing two polar bodies and two cleavage nuclei.

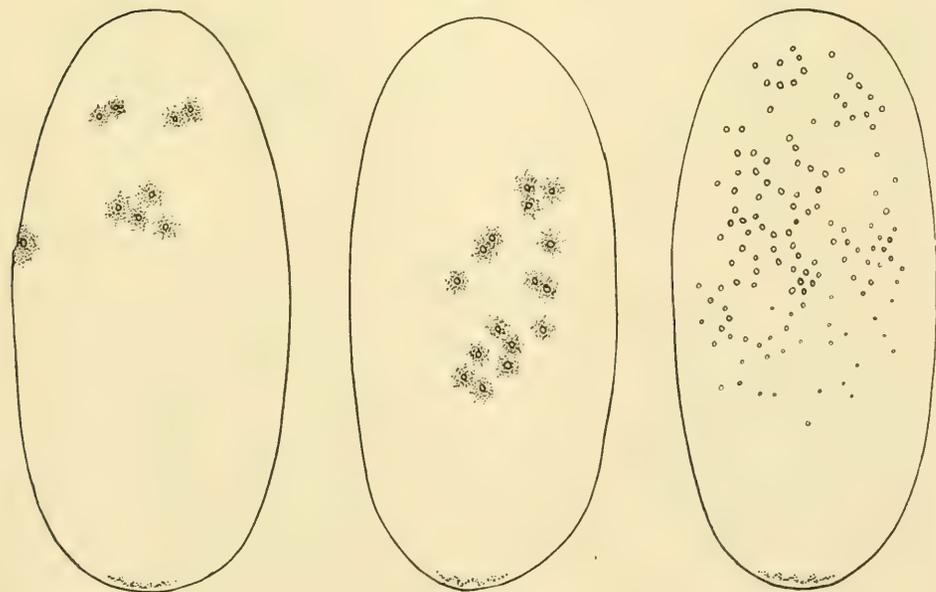
47 Egg containing four cleavage nuclei.



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48 Transverse section of egg containing four cleavage nuclei.

49 Egg containing eight cleavage nuclei.

50 Egg containing sixteen cleavage nuclei.

51 Egg containing 133 cleavage nuclei.

52 One cleavage nucleus from egg shown in figure 50, enlarged.

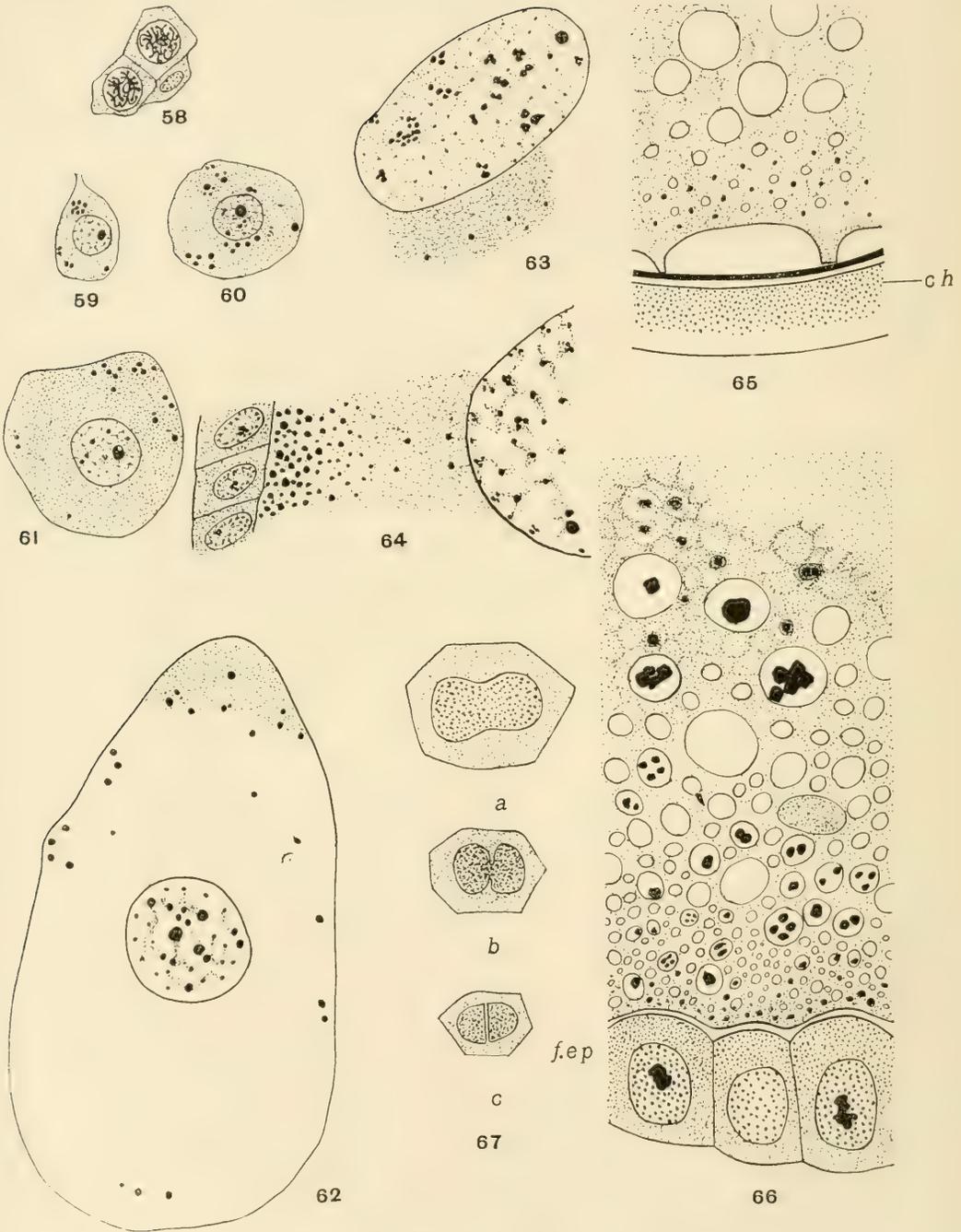
53 Primordial germ cell just after separation from the egg.

54 Blastoderm cell.

55 Vitellophag.

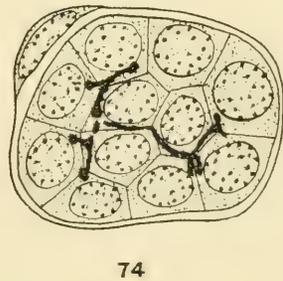
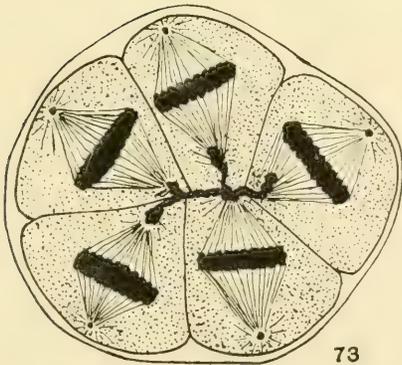
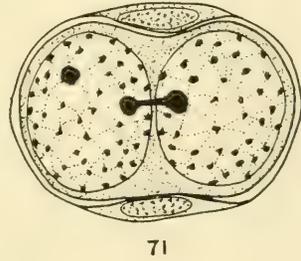
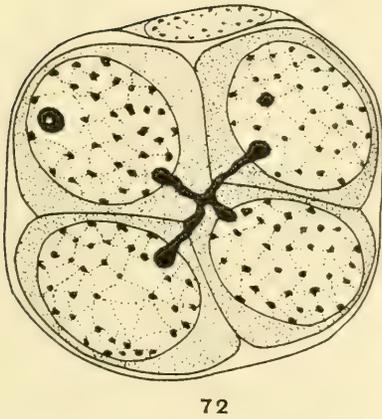
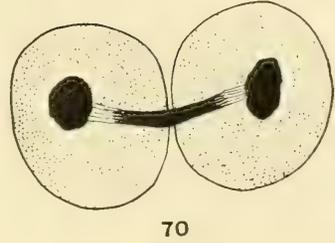
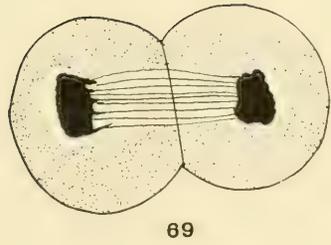
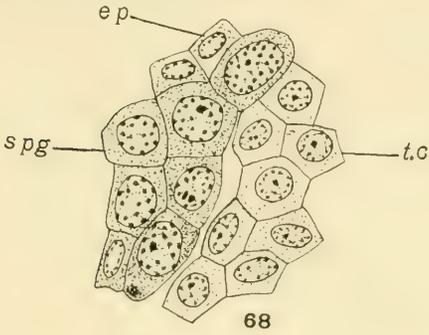
56 *Leptinotarsa decemlineata*; nuclear division of vitellophags.

57 Nuclear division of vitellophags in a centrifuged egg.



Leptinotarsa decemlineata

- 58 Two oocytes and one epithelial cell from position 58 in figure 9.
- 59 Oocyte from position 59 in figure 9.
- 60 Oocyte from position 60 in figure 9.
- 61 Oocyte from position 61 in figure 9.
- 62 Oocyte from position 62 in figure 9.
- 63 Part of oocyte from position 63 in figure 9.
- 64 Part of oocyte from position 64 in figure 9.



65 Part of posterior end of oocyte from position 65 in figure 9.

66 Part of posterior end of egg shortly before ready to lay.

67 Nuclear division among nurse cells.

68 Spermatogonia (*spg*), cells from terminal cap (*t.c*) and epithelial cells (*ep*).

69 Mitotic division of spermatogonium.

70 Later stage of same process.

71 Binucleated spermatogonial cell within epithelial envelope.

72 Four spermatogonia connected by spindle remains.

73 Spermatogonia from a cyst containing eight cells.

74 Section through a cyst containing 32 spermatogonia.

SOME FEATURES IN THE DEVELOPMENT OF THE
CENTRAL NERVOUS SYSTEM OF
DESMOGNATHUS FUSCA¹

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FIFTY-EIGHT FIGURES (EIGHT PLATES)

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INTRODUCTION

Desmognathus fusca is one of the most widely distributed of the eastern urodeles. It was chosen for this study, among other reasons, because: it was easily available; the eggs during earlier stages are not pigmented; and the persistence of embryological characters (Fish '95) and the retention of teleostean charac-

¹ The writer wishes to express his appreciation of the help and encouragement received from Prof. B. F. Kingsbury and Dr. William A. Hilton.

ters (Wilder '99, '04, and Hilton '04, '09) have been noted. This species also develops under conditions which are rather unusual for the tailed amphibia, namely, the space in which each larva develops is very limited; this, together with an unusually large and dense yolk mass, causes a twisting of the embryo and a marked reduction in the size of the cavities. These latter conditions are evidently largely contributory to the various modifications which will be noted.

MATERIAL AND METHODS

Various features in the development of the nervous system have been traced from the first appearance of the medullary plate (about 60 hours) to the adult. For convenience the developing eggs and larvae up to the time of hatching have been divided into twelve stages. As a large number of eggs in each stage have been secured and as it was impossible for the specimens placed in any stage to be at exactly the same degree of development, a practically continuous series from the first appearance of the medullary plate up to the time of hatching has been secured. This is especially true of Stages VI to XI.

Several fixers have been used, namely: Gilson's, Zenker's, Flemming's (stronger sol.), Hermann's, Perenyi's, HgCl_2 plus glacial acetic acid, platinic chloride (0.25 per cent), and formalin (10 per cent). Formalin was found to be the most satisfactory fixer for both section and surface study. It differed from the other fluids used in that good fixation could be secured although the egg membranes were present.

Celloidin imbedding was used throughout for the stages up to hatching. Due to the density of the yolk satisfactory results with paraffin could not be secured and double imbedding with paraffin and celloidin was not as successful as the celloidin alone.

Most of the series were stained with hematoxylin (chloral and Delafield's) and eosin. As the work was largely morphological, a series of models have been made and from these the relations of the various sections can be secured.

Stages

It has not been possible to raise this form in the laboratory, so that the ages of the larvae have not been ascertained and consequently have not been available for the classification into stages. The body length, also, has been of but little direct value. More use could not be made of it because the larvae flex in two planes as well as undergoing an axial rotation. Thus it is only after hatching that lengths can be given. In the description under the various headings the different characters used to determine the stage will be given. This can be supplemented by consulting figures 15 to 26. The specimens portrayed in these drawings were selected from a large amount of material as being the most normal.

CLOSURE OF THE NEURAL TUBE

The dense yolk mass appears to affect strikingly the closure of the neural tube. Its effect upon some of the earlier processes before the formation of the nervous system is noted by Hilton ('09). In his conclusions is the following: "The blastopore formation is much like other Amphibia from the surface but seems to be entirely different from other forms described in the way the cells go to form the archenteron by growing down into a nearly solid mass of yolk." In contrast to most Amphibia in which the cavities are well marked, this species, due to the influence of this dense yolk mass, has the various cavities, including the neurocoele, greatly reduced. In the formation of the neural tube the medullary folds first meet in the hind-brain region. From this point closure takes place both caudad and cephalad and in a manner typical of some of the lower forms, eg., *Bdellostoma*. That is, from the hind-brain caudad only a morphological not an actual neurocoele is present. The actual cavity forms intrasegmentally, first appearing in the center of each of the segments, and these gradually enlarge and coalesce, this process proceeding from the head caudally.

At the time of the closure of the neural folds and as late as Stage VII it is not possible to distinguish the three germ layers in the tail region, all being fused in a caudal mass which appears to be

a growth center. No neurenteric canal could be distinguished nor would we expect to find one under these conditions. In the head region the dorso-ventral diameter of the neural cavity up to Stage VII is very narrow, the dorsal and ventral walls nearly touching each other. By Stage VIII a well marked cavity is present.

Although in the closure of the medullary folds, *Desmognathus* is apparently similar to some of the lower forms, this is not due, I believe, to any phylogenetic relation but rather to the mechanical influence of the large and compact mass of yolk and the small space in which the processes of growth must take place. It is an interesting example of the effect of mechanics which might lead to a wrong phylogenetic interpretation.

THE PINEAL REGION

This term is used in the sense introduced by Minot ('01). It includes the paraphysis, the posterior commissure and the structures in the roof of the fore-brain lying between them. This region has received attention from numerous investigators so that the literature about this rather peculiar portion of the brain is quite extensive. That these investigators concur for the most part in their results, although working on widely diversified forms, attests the relative phylogenetic simplicity of this region. This has enabled the structures to be homologized throughout the vertebrate series.

To contribute one more form to the series described, I have traced the development of this region in *Desmognathus*. Several stages have been modelled to show the topographical relations and the intervening stages have been studied from cross and sagittal sections.

Description

The foldings in the fore-brain and their relation to the neuromeres have been taken up under a discussion of "Segmentation."

At Stage IX (figs. 1, 37) a point in the development has been reached when the parts of this region are unmistakable. Figure 1 is a medial section through the pineal region. For relations

consult figure 37 which was drawn from the same specimen. Three infoldings are prominent dorsally, the cephalic fold (the velum transversum) the most so. Cephalad to this is the paraphysial arch which as yet is indistinct and shows no evidence of a paraphysial evagination. The cells of this arch, as well as those of the velum, are arranged in a single or irregular double layer. Posterior to the velum there is an arch limited caudally by a prominent infolding in which the posterior commissure will develop. At about the mid-point in this arch, there is a slight projection inward, which represents the constriction which was prominent in figure 29 and separated the roof of the diencephalon into two segments. The first of these segments is the post-velar arch and as yet it has not begun to lengthen more rapidly than have the other parts of the roof. Only at this early stage (fig. 29) is the roof of the diencephalon distinctly divided into two segments and by the next stage this dividing point will have disappeared. Caudad to the diencephalic arch lies the first mid-brain neuromere. The cell arrangement of the constriction which divides the mid-brain from the diencephalon is different from that of the velum transversum. In the former the invagination apparently is due to a proliferation of cells, while in the latter it is a simple infolding, there being no thickening (if anything, a flattening) of the cells. This might lend some support to the contention of Johnston ('09) that the formation of the velum is primarily due to the withdrawal of material for the formation of the optic vesicles.

Just cephalad to the di-mesencephalic infolding is a dorso-caudal outgrowth. This is the rudiment of the posterior portion of the epiphysis and in this evagination a cavity will later appear.

By Stage X (figs. 2, 38) the paraphysis has become a well marked evagination composed of simple cuboidal cells. Cephalad to the paraphysial out-pocketing is a second out-growth which reaches its maximum development at this stage. In specimens between figures 1 and 2 at this region two evaginations of about equal size can be seen. The caudal one grows the more rapidly and forms the paraphysis, the cephalic one becomes less distinct and probably contributes to the true paraphysis. The second

evagination was present in all the series of this age which I examined and shows but little variation, apparently having the same fate in all cases. In Reptilia three paraphysial evaginations have been noted (Warren '09).

Warren ('05) in discussing the growth of the paraphysis and epiphysis says:

The roof of the fore-brain has now descended to such a degree that the opening of the paraphysis is on a level with the tip of the velum. The velum itself has lost its cephalic layer, and consists of one layer only, which however, is much longer than the velum in fig. 5. If figs. 4, 5 and 7 are compared it will be seen that the distal end of the paraphysis is practically at the same distance from the ectoderm in each case. As the paraphysis has developed during those stages into a long tube, its growth must have occurred by a downward extension of the neighboring parts into the cavity of the fore-brain.

I would not agree with the author that this is the process taking place. Rather than being a downward extension of the neighboring parts it is a dorsal extension of the parts bordering these 'neighboring parts.' That is, it is a growth out of the brain in which the ostium of the paraphysis and the tip of the velum remains practically as a fixed point. This viewpoint would affect the interpretation that the cephalic layer of the velum becomes the caudal layer which I do not see can be the case. The telencephalon must have a dorso-caudal wall and it seems to me that the simplest interpretation is that the cephalic wall of the velum remains as such and that the post-velar arch is lengthened by its own intrinsic growth. The post-velar arch is a region of rapid growth, this growth continuing and forming the diencephalic plexus. The anterior layer of the velum, likewise, is a region of rapid growth, thus forming the telencephalic plexus. Although the growth forming the paraphysis is at first localized, later, a general growth takes place, forming the telencephalic plexuses. This latter process takes place later than the formation of the diencephalic plexus.

At this stage the constriction separating the first and second diencephalic neuromeres has disappeared. The epiphysis, distinctly formed, is located at the position shown in the earlier stage (fig. 37) and a short distance caudad to it lies the developing

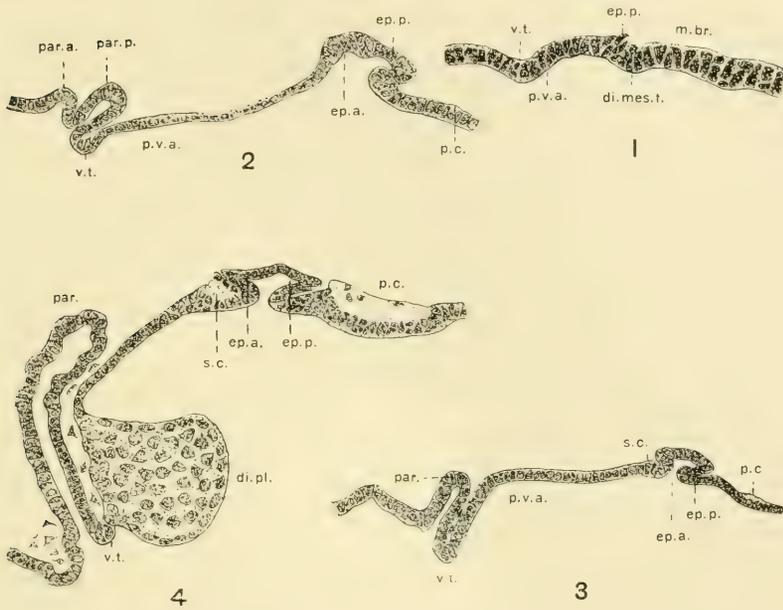


Fig. 1 A medial sagittal section of an embryo of Stage IX. For relations see figure 37. $\times 125$.

Fig. 2 A medial sagittal section of an embryo of Stage X. For relations see figure 38. $\times 125$.

Fig. 3 A medial sagittal section of an embryo of Stage XI. $\times 125$.

Fig. 4 A medial sagittal section of an embryo of Stage XII. $\times 125$.

REFERENCES

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| <i>ep. a.</i> , anterior evagination of epiphysis | <i>par. a.</i> , anterior paraphysial evagination |
| <i>ep. p.</i> , posterior evagination of epiphysis | <i>par. p.</i> , posterior paraphysial evagination |
| <i>di. mes. f.</i> , di-mesencephalic fold | <i>p. c.</i> , posterior commissure |
| <i>di. pl.</i> , diencephalic plexus | <i>p. v. a.</i> , post-velar arch |
| <i>m. br.</i> , mid-brain | <i>s. c.</i> , superior commissure |
| <i>par.</i> , paraphysis | <i>v. l.</i> , velum transversum |

posterior commissure, indicated by an area of ectoglea. Figure 2 gives the typical appearance of the epiphysial formation of this age. It is composed of a well marked epiphysial arch, in the caudal portion of which is the out-pocketing, mentioned in the previous stage, which now contains a cavity. In the cephalic part of the arch is a much less pronounced solid diverticulum and in this a

cavity will later appear, forming the anterior part of the adult epiphysis. This indicates, I believe, that the epiphysis is double. Both diverticula, morphologically, have distinct openings, but, due to their proximity and to the drawing out of the epiphysial arch, they finally come to have a common ostium leading into the third ventricle. In this way what is apparently a single organ is fundamentally double. That the posterior outgrowth arises before the anterior one, strengthens this conclusion. As mentioned by most other workers the epiphysis touches directly the body ectoderm, there being no intervening mesenchyme. The habenular commissure is not yet evident.

By Stage XI (figs. 3, 33, 39,) the paraphysis has formed an extensive tube but, as yet, with no secondary diverticula. The anterior paraphysial diverticulum has nearly disappeared, an indication, only, of it being seen immediately cephalad to the ostium of the paraphysis. The habenular commissure has become very slightly evident, while caudad the posterior commissure is prominent and is farther removed from the epiphysis than in the earlier stages. This does not necessarily indicate, however, that the growth of the commissure itself has been caudad. If this were the case the commissure would belong to the roof of the mid-brain. It indicates rather, I think, a lengthening of the region immediately caudad to the epiphysis. In later stages (figs. 4,5) the posterior commissure and the epiphysis again approach each other because of the increase in size of both of these structures. The epiphysis has been placed in the first diencephalic segment by several writers, and since the posterior commissure later lies in contact with it, this relation has been taken as evidence that the roof of the second diencephalic segment (synencephalon) does not lengthen. With this interpretation I can hardly agree. The first appearance of the epiphysis is next to the di-mesencephalic constriction. Then the portion of the roof between it and the posterior commissure lengthens as does the rest of the roof of the diencephalon, thus causing the separation of these two structures. It would be peculiar if a small part of the diencephalon, such as is represented by the synencephalon of Kupffer, remained alone free from growth while all other parts of the brain were undergoing such rapid extension.

The large epiphysial arch has disappeared as such and the epiphysis now appears as a definite single evagination. Quite a small cavity is present in the cephalic portion, however, while the posterior portion has a well marked lumen.

By Stage XII (figs. 4, 35) the habenular commissure is well marked so that all the features enumerated by Minot ('01) as

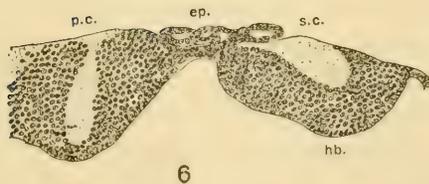
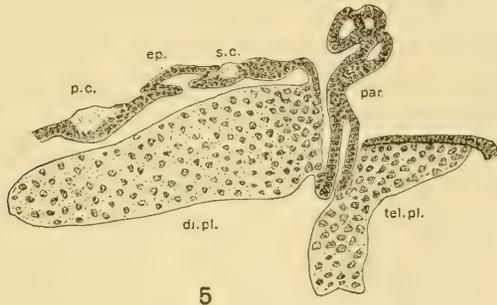


Fig. 5 A medial sagittal section of a 13 mm. larva. $\times 75$.

Fig. 6 A medial sagittal section of an 8 cm. adult (full grown). $\times 75$.

REFERENCES

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| <i>di. pl.</i> , diencephalic plexus | <i>p. c.</i> , posterior commissure |
| <i>ep.</i> , epiphysis | <i>s. c.</i> , superior commissure |
| <i>hb.</i> , habenula | <i>tel. pl.</i> , telencephalic plexus |
| <i>par.</i> , paraphysis | |

characteristic for this region have been described. The paraphysis has lengthened and secondary diverticula are appearing in it. The post-velar arch has further increased in extent, its anterior part entering into the formation of the diencephalic plexus, the remainder being, as yet, an unpleated membrane. Both the cephalic and posterior portions of the epiphysis have

well formed lumina opening into a common cavity, this communicating with the third ventricle by a wide ostium.

In a larva of 13 mm. (figs. 5, 41) the wall of the velum, anterior to the paraphysis, has become plicated, forming a plexus which intrudes into the lateral ventricles (fig. 41). In addition to this, there is an unpaired plexus which goes towards the floor of the fore-brain, into the aula. These relations are the same as described so clearly by Warren ('05) for *Necturus*. The paraphysis is much convoluted and has a rich blood supply. The relative distance from the tip of the velum to the epiphysis is much less than in the previous stage. This is due apparently to the forward growth of the mid-brain and the caudal growth of the telencephalon. This shortening of the 'twixt-brain roof is also increased by the straightening out of the brain which in Stage XII has a strong diencephalic flexure.

In a larva of 17 mm. the structures described in the previous stages are present, as is true for the remaining stages. The plexuses have become much more extensive, and the habenulae are broadly connected across the mid-line so that the roof of the third ventricle is quite thick for some distance in front of the superior commissure. The epiphysis, because of its increase in size, has come to overlap both the superior and posterior commissures. As yet no secondary diverticula have appeared in it nor could a communication between it and the third ventricle be detected in this or the succeeding stages. Specimens of 19, 23, 26 and 30 mm., have been examined but need no description.

In a 38 mm. specimen (fig. 40) the epiphysis has become divided into secondary diverticula, the cells forming its roof having become more flattened than those of the floor. In this, in a 52 mm., and in an 8 cm. specimen the blood supply of the epiphysis and paraphysis is abundant, the supply of the latter being the richest. In this organ a greater amount of space is taken up by the blood vessels than by the gland itself. In an 8 cm. specimen (full-grown adult) the epiphysis is relatively less extensive than in the 38 mm. specimen. The superior commissure and habenulae have undergone considerable growth.

Discussion

The literature on the embryology of the pineal region treats the subject, in general, from two points of view: (1) the development of the structures, considering them intrinsically, and (2) their significance in determining the segmentation of this region. For a more extensive summary of the literature than is here given, consult Gaupp ('97) and Warren ('05, '11).

In the forms below amphibia two epiphysal evaginations have been usually described. Hill ('91), in *Coregonus* and ('94) in *Salmo*, *Catostomus*, *Stizotiedion*, and *Lipomis*, describes two evaginations, the anterior one becoming constricted off and lying between the thalamencephalon and mesencephalon and never reaching an eye-like structure. He, however, homologizes the anterior vesicle to the lacertilian epiphysis. In *Petromyzon*, Studnicka ('95) describes two outgrowths from the roof of the thalamencephalon and homologizes the anterior one (parapineal organ) with the anterior vesicle of Hill. In *Amia*, Kingsbury ('97) states that the anterior vesicle persists and has a fiber connection with the left habena. Eycleshymer and Davis ('97) also worked on this form and my figures correspond closely to theirs. My figure 1 gives much the same appearance as their figure 1, with the exception that in my figure an active cell migration dorso-caudally is suggested in the posterior vesicle. In their figure of a 5 to 6 day embryo (fig. 3) the posterior vesicle has a lumen and the anterior epiphysis is a mass of cells with no lumen as yet. Compare my figure 2 with this. My figure 3 corresponds to their figure 8. Their figures, thus, up to this stage, correspond closely to mine; from this stage on, however, as shown by their drawings, the anterior vesicle becomes constricted off, loses its cavity and shows marks of degeneration. In *Desmognathus* both vesicles remain, and up to about a 17 mm. stage have a common opening into the III ventricle. Terry ('10) in *Opsanus* describes two outgrowths, the anterior one being solid. Locy ('93) in *Acanthias* finds a double epiphysis and traces its origin to a pair of accessory optic vesicles in the medullary plate stage. If this observation prove to be correct it explains phylogenetically the

double epiphysis, but does not indicate why one should arise earlier than the other as has been demonstrated to be the case by Eycleshymer and Davis ('97) and others.

In amphibia the epiphysis is small. With one exception (Cameron '03) the latter organ has been described as a single diverticulum: De Graaf ('86) and Blanc ('00) in Triton; Leydig (53), Burekhardt ('91), Galeotti ('97) in *Salamandra maculata* and *S. perspicillata*; Orr ('89) in *Amblystoma mexicanum*; Eycleshymer ('92, '95) in *A. punctatum*; Mrs. Gage ('93) in *Diemyctylus*; Kinsgbury ('95) and Warren ('05) in *Necturus*. Cameron ('03) in a brief paper, with no description or figures, stated that in certain amphibia (*Rana*, *Bufo*, Triton) the epiphysis arises as two primary outgrowths, these being placed one on each side of the mesial plane.

Warren ('05) suggests that there is some sort of a proportion in the relative development of the paraphysis, epiphysis, and parietal eye, for in urodela, where there is no parietal eye, and a small epiphysis, the paraphysis reaches its maximum development. Certainly in amphibia the paraphysis is much larger than in the higher or lower forms. That the epiphysis is correspondingly small might suggest a phylogenetic vicarious action between the two structures.

Reptilia have been a favorite field for work on the epiphysis and pineal eye. In most reptilia the epiphysis is at first double, the anterior vesicle then becomes constricted, migrates forward, lodges in a foramen in the skull, and is known as the parietal eye. In certain forms, however, the epiphysis has been described as lacking: Sorensen ('93, '94) and Reese ('10) in the alligator, and Voeltzkow ('03) in the crocodile and caiman.

In *Desmognathus* the paraphysial arch and paraphysis have nothing distinctive. The two paraphysial evaginations were found in all the series examined. The posterior evagination continues to grow, absorbing the anterior one and becoming the paraphysis of the adult.

The epiphysis forms in the caudal portion of the second diencephalic neuromere, just cephalad to where the posterior commissure appears. In *Desmognathus* it appears earlier than the

paraphysis, but the difference in time of their origin is very slight.

In forms on either side of the urodela we have the epiphysis usually double. It has never been considered double in urodela, to the writer's knowledge, except by Cameron ('03). I believe that in my description I have shown that it is fundamentally double in this form. The two epiphysial outpocketings are closely associated, both in position and time of origin. The posterior vesicle, however, antedates the anterior one slightly in two respects: the solid outgrowth of the former appears earlier than in the latter; the cavity of the posterior outgrowth appears earlier than that of the anterior one. That they later have a common opening and that they never become separated is nothing fundamentally against their being two morphological structures. In amphibia the epiphysis is more rudimentary than in pisces or reptilia. This might account for the two vesicles remaining together. If the epiphysis of this form had reached a greater development, I think that we logically could have expected the anterior vesicle in the later embryonic stages to have become constricted from the posterior vesicle.

The posterior commissure first forms near the epiphysis in the constriction which separates the diencephalon from the mesencephalon. By the growth of that portion of the roof lying between the posterior commissure and the epiphysis, the two structures become separated. They again approach each other due to their extension and because the roof of the diencephalon assumes a different direction in its growth, thickening instead of lengthening.

Summary

The epiphysis of *Desmognathus fusca* is morphologically double.

The posterior outgrowth forms first; it is followed by an anterior evagination which forms independently.

As growth proceeds they come to have a common ostium into the third ventricle.

The epiphysis is formed on the second diencephalic segment.

THE DEVELOPMENT OF THE HYPOPHYSIS

The literature shows a considerable diversity of opinion in regard to the origin of the hypophysis. The three views held in regard to its formation are: that it is of ectodermal origin, that it is of entodermal origin, and that both ectoderm and entoderm enter into its formation. The literature has been frequently reviewed. Minot ('92), Kingsley and Thyng ('04) and Tilney ('11) each have good reviews.

The view that the hypophysis is ectodermal in origin has the largest number of supporters, among whom some of the best known are: Goette ('75) in *Bombinator*; Mihalkovics ('75) in the goose, rabbit and dog; Kölliker ('79) in mammals; Kraushaar ('84) chiefly on *Mus musculus*; Hoffman ('86) in the bony fishes; Scott ('87) in *Petromyzon*; Gaupp ('93) in *Lacerta* and *Anguis*; Salzer ('87) in the pig and guinea-pig; Haller ('98) in a series of forms; Minot ('98), Corning ('99) in *Rana*; Zeleny ('01) in chelonia (*Aspidonectes*, *Chelydra*, *Chrysemys*); Kingsley and Thyng ('04) in *Amblystoma*.

Giving the hypophysis as ento-ectodermal in origin are Hoffman ('86) in *Reptilia*; Gregory ('03) in *Salmo alsaticus*, *S. salar*, and *Esox*; Kupffer ('94) in *Rana*; Valenti ('95), Nusbaum ('95) in the dog.

Prather ('00) in *Amia* finds the hypophysis to be entirely entodermal. Kingsley and Thyng ('04) think that not early enough stages were selected to show its real origin in this form. Tilney ('11) gives a good review of the literature but adds nothing to the embryology, the paper being chiefly on the comparative histology of the gland. There seems to be a tendency among text-books to consider the question as settled, and that the hypophysis is entirely of ectodermal origin, but much of the work will have to be confirmed before such a generalization will be warranted.

In *Desmognathus*, I have found it difficult to determine with certainty whether the entoderm contributed to the organ or not. There is a very close association between the ectodermal hypophysial invagination and the pre-oral entoderm up to my Stage X, when the separation becomes very definite. At times the relation

of the entoderm to the ectoderm was so close that there appeared to be a fusion of the two. This form, also, is not the most favorable for the study of this problem because cavities and separations which would be present in many other forms are not apparent here.

Figure 7 shows a median sagittal section of an embryo in an early Stage VIII. By the cephalic growth of the embryo the space (*s*) is formed, beneath which is the ectoderm covering the yolk mass. Above this space is the outer layer of the ectoderm and then the stratum basale (nervous layer of ectoderm). No indication of a thickening of the stratum basale can be seen to indicate the formation of the hypophysis. Figure 8 is a sagittal section of an embryo in a later Stage VIII. The rudiment of the hypophysis is seen as a thickening of the stratum basale. The ectoderm blends so with the entoderm that it is difficult to determine the boundaries of each.

Figure 9 is a cross-section through the hypophysial anlage of an embryo in a Stage VIII. This is the earliest series in which I could recognize the anlage of the hypophysis in cross-section. It appears as a medial thickening and shows no indication of a paired origin as described by Gaupp ('93), Pollard ('94), Lundborg ('94), Gregory ('93), and Kingsley and Thyng ('04). Gaupp's models showing this condition are particularly instructive.

Figures 27 and 28 show that laterad to the hypophysis there is a thickening and a folding of the ectoderm. This lateral thickening, however, is entirely distinct from the true hypophysial ingrowth and does not enter into the hypophysis.

Figure 37 shows a median sagittal section of a larva in Stage IX. The hypophysis has increased in size and is still very intimately associated with the entoderm. It is broadly connected with the oral ectoderm. Figure 10 shows a transsection through the posterior portion of the hypophysis in an embryo of the same age as figure 39.

By Stage X (fig. 38) the distance between the oral ectoderm and the hypophysis has increased greatly, due to the cephalic flexure and the growth of the embryo. The hypophysis, however, is still connected by a well marked double strand of cells with the

oral ectoderm. The hypophysis can now be easily distinguished from the entoderm. Just dorsal to the hypophysis is a well marked infundibular recess, which is more prominent at this stage than at any other. The walls of the infundibulum have become much thinned.

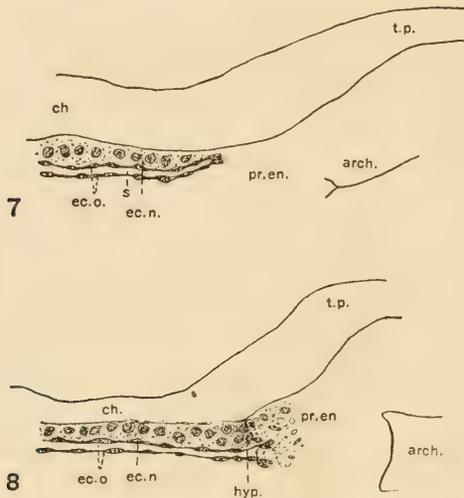


Fig. 7 A medial sagittal section through the infundibular region of a larva of Stage VIII. $\times 125$.

Fig. 8 A medial sagittal section of same region. Larva of a later Stage VIII. $\times 125$.

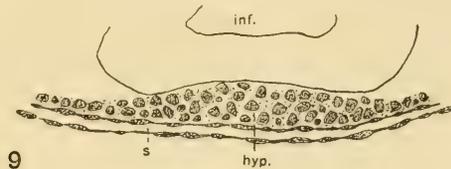
REFERENCES

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|---|---|
| <i>arch.</i> , archenteron | <i>hyp.</i> , hypophysis |
| <i>ch.</i> , optic chiasma | <i>pr. en.</i> , pre-oral entoderm |
| <i>ec. n.</i> , nervous layer ectoderm (stratum basale) | <i>s.</i> , space formed by the cephalic growth of embryo |
| <i>ec. o.</i> , outer layer of ectoderm | <i>t. p.</i> , tuberculum posterius |

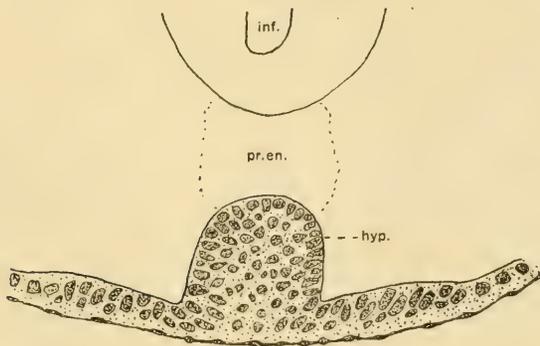
Stage XI (fig. 39) shows but little advance in the growth of the hypophysis. There has been an ingrowth of mesoderm between the anterior commissure and the optic chiasma.

In later stages the strand connecting the hypophysis and oral ectoderm is lost. It is difficult to determine the fate of the cells composing this strand. I was not able to distinguish them from the surrounding mesenchymal cells. I believe the hypophysis in

this form to be entirely ectodermal, the only question being at the very early stages where it was impossible to distinguish absolutely between the ectoderm and entoderm. Figure 40, of a 38 mm. specimen, gives practically the adult conditions.



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Fig. 9 A transsection through the hypophysis of a larva of Stage VIII. $\times 125$.

Fig. 10 A transsection through the hypophysis of a larva of Stage IX. $\times 125$.

REFERENCES

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|------------------------------------|---|
| <i>hyp.</i> , hypophysis | <i>s.</i> , space formed by the cephalic growth of embryo |
| <i>inf.</i> , infundibulum | |
| <i>pr. en.</i> , pre-oral entoderm | |

SEGMENTATION

A study of segmentation may well be divided into two parts, namely, that bearing on (1) the medullary plate, and (2) the neural tube. Neuromeres in each of the above stages have been described, but the problem of tracing the one into the other has not been satisfactorily completed. In the closed neural tube, investigators have reached fairly definite and uniform results concerning the definition, number, and relations of the neuromeres of the hind-brain. There are less uniform conclusions on the fore-brain.

Among the earlier workers who noticed foldings in the hind-brain region and attached segmental significance to them, may be mentioned: von Baer ('28), Remak ('50), Dursey ('69), Dohrn ('75), Foster and Balfour ('76), Béraneck ('84), Kupffer ('85), Orr ('87), Hoffman ('88), and Miss Platt ('89).

In the fore-brain, McClure ('89) working on the chick, *Anolis* and *Amblystoma* first described a segmentation and stated that the neuromeres conformed to those of the hind-brain. Waters ('92) confirmed McClure's observations and tried to explain why the neuromeres appeared at such a late stage. Herrick ('92) believed that the neuromeres described for the fore-brain were only dorsal diverticula and not homologous to the ventral diverticula of the mid- and hind-brain. Johnston ('05, '09) described a segmentation of the fore-brain, as did Warren ('11). Most of the above workers have described five to nine segments for the hind-brain, two in the mid-brain, two in the diencephalon, and one in the telencephalon.

Turning now to the problem of the segmentation of the medullary plate, we find considerable diversity of opinion. Zimmerman ('91) found eight 'primäre Abschnitte.' These divided later and formed thirteen. Kupffer ('85, reprinted in '93) found eight neuromeres. Froiep ('91, '92, '93) found in Triton and Salamandra five and four neuromeres respectively. In his second paper he was skeptical of the segmental value of the neuromeres. Loey ('94) in a preliminary paper, described in *Acanthias* and *Amblystoma* a distinct segmentation and in a fuller account ('95) he adds to the number of forms described. Eycleshymer ('95) working on *Amblystoma* found certain irregularities on the medullary plate and folds but believed that they were artifacts. Neal ('96, '98) was a severe critic of Loey's work. Hill ('00) worked on the chick and teleosts and traced the neuromeres from the medullary plate into the neural tube. Kupffer ('03) was skeptical of Hill's work. Griggs ('10) described for *Amblystoma* a segmentation of the neural plate but not of the folds. Loey stated that in this form the neural plate was smooth but that the folds were segmented. Smith ('12) found in *Cryptobranchus* that three transverse grooves appeared in order on the medullary plate and could be

traced into the region of the medulla oblongata of the adult. Cephalad to these were six more or less irregular and transitory grooves. His figures and description indicated considerable regularity in the segmentation of the medulla oblongata and caudad to it, while cephalad to it there is a greater irregularity.

Description

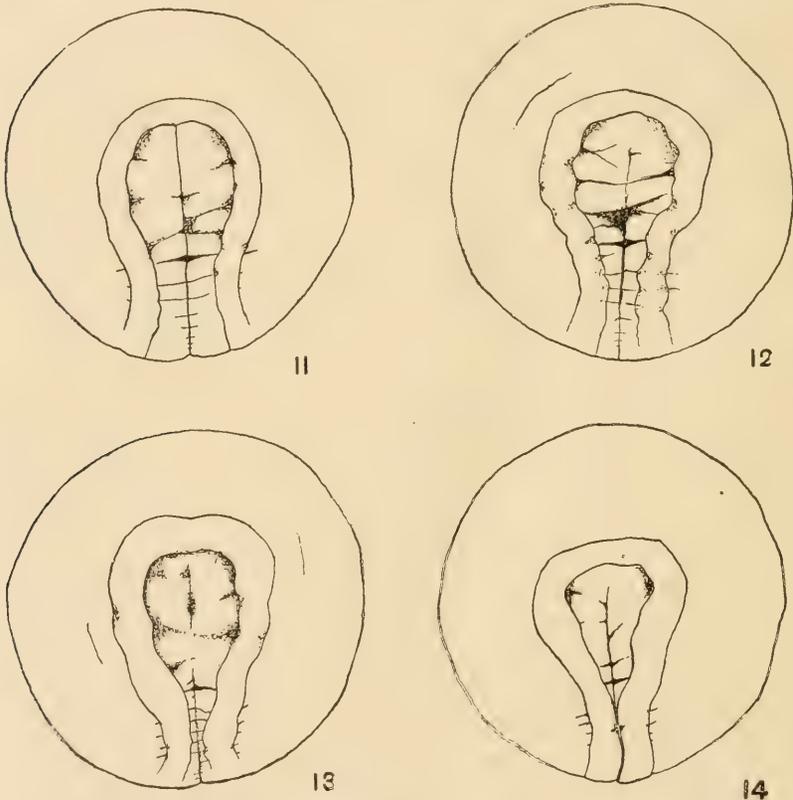
The neural plate up to closure. Several hundred eggs were collected for this study. In the summer of 1911 the eggs then collected were fixed in formalin. Later these were examined and camera sketches made of a number. Again in 1912 a considerable number were collected, these being studied and sketched before and after fixation. They were found to correspond very closely. In several cases eggs were examined at periods in their growth and the irregularities, described farther on, were found to persist.

Figures 11 to 19 show typical specimens. I have portrayed the actual conditions as accurately as possible and present these, selected from a number of drawings, as being from representative specimens. A slight beading is indicated. Its irregularity in size, number, and position makes its segmental significance very questionable.

In most of the specimens a depression extends across the caudal portion of the procephalic plate. This I take to be the transecephalic groove of Griggs. Its position and prominence vary considerably. In specimens younger than those figured, and less often in the later stages before closure of the medullary plate, I have been able to see transverse markings in the procephalic plate which were very transient, varied in position in different individuals, and which I was not able to trace through from one stage to another in living specimens. Caudad to the transecephalic groove the markings in the plate were more uniform and persisted longer and were subject to but slight variation. In some specimens they corresponded closely to the outpocketings in the medullary folds but they failed to do this in other specimens.

Another noticeable feature in embryos of this stage is the plications in the neural folds themselves. These are observed with

less difficulty than the other two features described and so are more accurately portrayed. They appear in the medullary folds at an early stage, and in the consecutive observations which I have made on developing eggs I have been able to trace them through from the wide-open medullary plate to a stage between figures 18



Figs. 11 to 14 Camera sketches of the open medullary plate. $\times 15$.

and 19, when I could trace them no longer because of their fusions and absorption into the expanding prosencephalon. From the drawings, it will be noticed that in the younger stages this irregularity is partially due to a thickening of the neural folds. They have the appearance of being caused by the medullary folds increasing in length faster than does the base which supports

them. In the later stages (fig. 18) the lateral irregularities do not correspond to the medial ones. It would easily be possible to select from the material a series of specimens which would show a uniform development and fate of these foldings, but after the examination of a large number of specimens, I have not been able to satisfy myself that they have a definite fate. However, these foldings can be traced through from the earliest appearance of the medullary folds to near the time of the fusion and formation of the neural tube and they appear to be due to a rapidly lengthening line of tissue upon a less rapidly lengthening base. At the beginning of my study upon this problem I felt that each of the three characteristics that I have described had a segmental significance and studied them with the expectation of showing this as well as their uniformity but have been forced to change my views because of the evidence presented.

The neural tube. Better to study segmentation in the neural tube, several models have been made. Figures 27 and 28 are from a model of an embryo in an early Stage VIII. The eye-vesicles have not yet invaginated to form the optic cup and the otic vesicles are still in broad communication with the body ectoderm.

In the medial view a very slight dorsal and lateral constriction separates the fore-brain from the mid-brain. This infolding is not well marked but can be definitely distinguished by the cell arrangement. In the floor the folds marking where the anterior commissure and optic chiasma will develop are prominent. It is evident that the optic vesicles communicate with each other by both the pre- and post-optic grooves. The mid-brain is separated into two neuromeres by a very slight ridge. Posterior to the mid-brain a neuromere is marked off by a pronounced constriction. This neuromere later forms the cerebellum. The neuromeres of the medulla are very prominent.

Figures 29 and 30 are from a model of an embryo in a late Stage VIII. A slight constriction representing the velum transversum has appeared. Between this and the infolding in which the posterior commissure develops is a constriction which divides the roof of the diencephalon into two segments. This infolding is

more prominent at this stage than in any subsequent one. In this specimen the fold in which the posterior commissure develops is farther caudad than normal, thus lengthening the second diencephalic segment and shortening the first mid-brain segment. The optic vesicles communicate more distinctly by the preoptic recess than in the previous stage.

Figures 31 and 32 are from a model of an embryo in a rather late Stage IX. The velum transversum has become prominent, as has also the infolding in which the posterior commissure will develop. In the formation of these two constrictions, the one separating the diencephalon into two parts has become less prominent and is seen in the broad arch which forms the roof of the diencephalon. The cells, however, show a typical neuromeric arrangement. The mid-brain neuromeres have become more prominent and a thinning in the roof of the medulla caudad to the cerebellar neuromere is evident. Laterally the ridge separating the diencephalon from the mesencephalon is much better shown than in the previous stage. This appears to be due in its ventral portion to the lateral expansion of the hypothalamus. As in the previous models, the otic vesicle lies opposite the third and fourth medullary neuromeres. The landmarks of the brain are now so distinct that there can be no mistake about the identification of the various parts. In the next model (figs. 33 and 34) all indications of the neuromeres have disappeared.

Discussion

Upon the medullary plate and folds of the forms which have been examined, it is evident that more or less regular ridges exist. Loey, Hill, Smith, and Griggs have put forth evidence that they have segmental value. Neal questions the observations of Loey; Kupffer is surprised that the observations of Hill have been so generally accepted. The form in which Griggs found transverse markings upon the medullary plate Loey had previously regarded as free from such markings, while observing a segmentation of the neural folds.

Caudad to and including that portion of the cephalic plate entering into the medulla oblongata, investigators have found a

much greater regularity than in the anterior portions. In this latter situation the variation in the observations upon different species and upon individuals of the same species throws some doubt in my mind upon the segmental value of the structures described. It seems possible or even probable that these structures are not segmental but due to growth processes entirely distinct from segmentation. It has been suggested that where there is a rapid growth and migration there is also, usually, an irregularity of the surface. Thus the ridges observed by Smith ('12) lying external and parallel to the medullary folds are explained by him as due to the rapid shifting of material in this region, concomitant with the closing of the neural tube. In the medullary plate there is a rapid lengthening, consequently a rapid migration of material, this migration taking place more longitudinally than laterally. In this way the oblique and transverse ridges can well be explained. Further than this, I believe that phylogenetically we can consider this as more probable than that they are segmental structures. The view generally held that segmentation should appear first upon the open medullary plate and thus precede mesodermal segmentation, appears to me as incorrect. Rather a segmentation of the neural plate or tube would follow mesodermal segmentation and be due to the development of the centers necessary to control the actions of the various body segments. If this be true, we would not expect to find neuromeres in the cephalic portion of the medullary plate.

For the closed neural tube there has been commonly described three neuromeres in the primitive fore-brain, two in the mid-brain and five to seven in the hind-brain. To the hind-brain neuromeres segmental value has been accorded by most workers. This should be supplemented by demonstrating the continuity of these structures (or lack of it) with the neuromeres of the medullary plate stage. In the fore- and mid-brain a second complication is met, namely, whether the smaller divisions (neuromeres) appearing in these segments are primary, or the primitive fore- and mid-brain segments primary, and the 'neuromeres' secondary. Upon this point there is a division of opinion, Hill and Locy, for example, believing that the so-called 'neuromeres' ante-

date the primitive fore-brain and mid-brain vesicles, Neal and Warren that they do not. The problem, difficult because of minuteness of the foldings and the danger of artifacts, will have to be worked out also in terms of other correlated structures. I have made no attempt to do this because of the difficulty of the technique in this form and because of the obliteration of cavities which in other forms would be prominent. In *Desmognathus* the 'neuromeres' of the fore- and mid-brain appear at about the same time as the primitive brain vesicles. From the evidence I have seen, however, the primitive fore-brain vesicle antedates slightly the smaller segments (neuromeres). In the mid-brain both appear at about the same time, so far as I can determine.

Summary

No evidence is found in the cephalic portion of the medullary plate, of divisions to which a segmental value should be assigned.

There are irregularities and folds in this portion of the medullary plate which are normal but not constant.

The primary fore-brain vesicle becomes secondarily divided into three segments; the cephalic one is included in the telencephalon, the other two in the diencephalon.

The mid-brain vesicle is composed of two segments. These appear at the same time as the primary vesicle.

GENERAL MORPHOLOGY

Fish ('94) noted the preservation of embryological characters in the adult brain of *Desmognathus*. Herrick ('10) states that, judging from the figures of Fish, the fore-brain of *Desmognathus* is intermediate between that of a 17 mm. and a 35 mm. *Amblystoma* larva in regard to the less extensive development of the primordium hippocampi and nucleus medianus septi and the more extensive septum ependymale. The papers of Herrick and Johnston have been especially valuable and my indebtedness to them will be evident.

The fore-brain

As Herrick ('10) has pointed out, the cerebral hemispheres of amphibia are divided into five parts which he terms olfactory bulb, ventro-medial, ventro-lateral, dorso-lateral, and dorso-medial parts. These divisions correspond to Gaupp's lobus olfactorius, emenentia septalis, ganglion basale, formatio pallii lateralis and emenentia pallii medialis. The two lateral parts are separated by the sulcus limitans lateralis (Gaupp) and the two medial parts by the fissure limitans hippocampi (Herrick).

In *Desmognathus* the relations are very much complicated, up to and slightly after hatching, by the prominent flexure in the dien-cephalic region. Figures 35 and 36 show the medial and lateral aspects of the brain of a larva just before hatching, or Stage XII. Figure 42, the plane of which is shown by the dotted line *a-a* (fig. 35), is a section through a brain of this age. The walls, which later form the pars ventro-lateralis and dorso-lateralis, are very massive, the medial walls being relatively thin, i.e., two to three cells thick. The thickness of the lateral walls has reduced the lateral ventricle and also the ventriculus impar so that they are much smaller, relatively, than in the earlier stages. By examining the series of models (figs. 27 to 33) a relative decrease in the size of the ventricles of the fore-brain and an increase in the lateral walls up to this stage will be noted.

In a 13 mm. larva the cerebral flexure is much less pronounced and consequently the relations simplified. Figure 43 is through the caudal part of the olfactory bulb. There is no fusion of olfactory bulbs in this or the later stages as in the frog. Figure 44 is just cephalad to the interventricular foramina. The membranous septum is not nearly as extensive as it is at a later stage, when a great increase in the length of the brain takes place. The ventro-lateral part (striatum complex) is so massive that it nearly obliterates the ventral part of the lateral ventricle. The dorso-medial part, however, is less developed than in a later stage. The cells also are arranged less densely here than in the other parts and approach nearer to the surface. This will form the primordium hippocampi.

Figure 45 is through the posterior poles of the cerebral hemispheres, caudad to the foramina of Monroe. The hippocampal commissure is seen, and separated from it by a layer of cells is the anterior commissure. The ventral sulcus of the diencephalon, which can be traced to the interventricular foramina, is present and separates the hypothalamus from the pars ventralis thalami. The sulcus medialis is indistinctly seen separating the pars dorsalis thalami from the pars ventralis and, as pointed out by Herrick, is a continuation of the sulcus limitans of His. By tracing through the series at this region the lateral extension of the cavity, in the dorsal region, can be identified as the sulcus dorsalis. Between this and a stage just before hatching, the brain has rapidly lengthened, with a partial loss of the cerebral flexure.

In a 23 mm. larva this process of lengthening has continued so that the brain has assumed nearly its adult shape. Figures 48 to 51 are transsections through the fore-brain of a larva of this size. By tracing through the series the septum ependymale is seen to have a much greater dorso-caudal extension than in the previous stage, but as yet there has been but a slight migration of cells into it from the pars ventralis. The primordium hippocampi, however, has become much thicker. The sulci of the diencephalon are more distinct than in the previous stage.

In a 30 mm. adult the morphological features are about the same as described for the full-grown adult. The brain has increased in length over the previous stage and the sulci have become much more distinct.

The most striking feature of the fore-brain of this form is the great size of the lateral walls. The major part of the massive walls enter into the striatum complex or pars ventro-lateralis of the cerebral vesicles. When larvae of this form are compared with species such as *Amblystoma punctatum* or *Salamandra atra*, this massiveness is very striking. The relation of the plexuses, the velum transversum, and the paraphysis has been described under the "Development of the epiphysis." It has not been possible to identify the various tracts with the technique employed in this study.

The mid-brain

Like the fore-brain, the mid-brain at the time of hatching, is a massive structure, the cavity being reduced to a narrow slit. At this stage, a deep groove extends from the caudal recess of the mid-brain to the mesencephalic pit (fig. 35). In figure 33 is seen a depression—the first indication of this groove—in the dorso-caudal portion of the mid-brain. It becomes very prominent, but shortly after hatching, as shown by a 13 mm. larva (figs. 46 and 47) the cavity broadens out and this groove disappears. At this time also a distinct sulcus limitans becomes visible.

In a 13 mm. larva, and to a less extent in a 23 mm. specimen, the cinerea extends to the surface in the medial line. Not until after transformation is a zone of alba present between it and the surface. Figure 57 shows a division of the cinerea into an ectal and ental layer by a zone of alba.

The cerebellum

The separation of the cerebellum from the medulla, evident at an early stage, can be distinguished by a dorsal constriction between them and by a thinning of the roof from this groove caudad.

The cerebellum is a greatly differentiated segment of the brain. In Stage XII (fig. 35) it has assumed nearly its adult shape. It is composed of a commissural band of fibers surrounded by a small amount of gray matter. The gray matter in later stages shows a bilobed condition (consult figs. 47, 53 and 58).

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PLATE 1

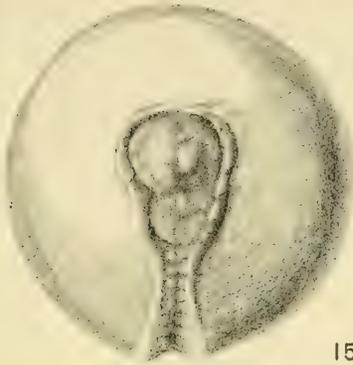
EXPLANATION OF FIGURES

- 15 to 19 Open medullary plate, Stages I to V. $\times 15$.
20 An embryo immediately after closure of the medullary folds, Stage VI.
 $\times 15$.

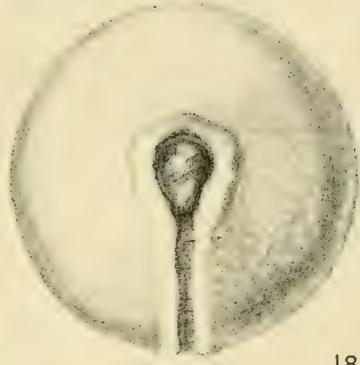
REFERENCES

m. br., mid-brain

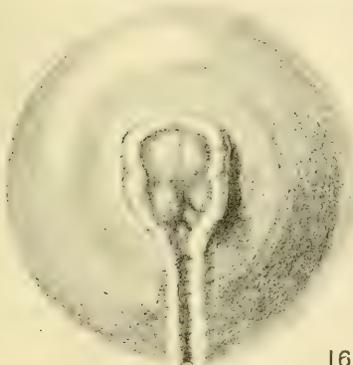
op. v., optic vesicle



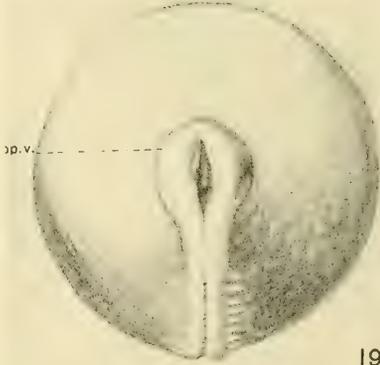
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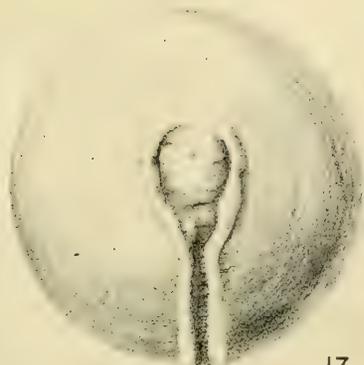
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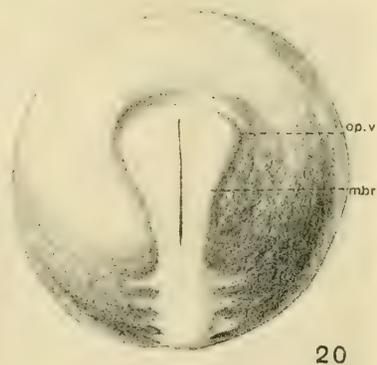
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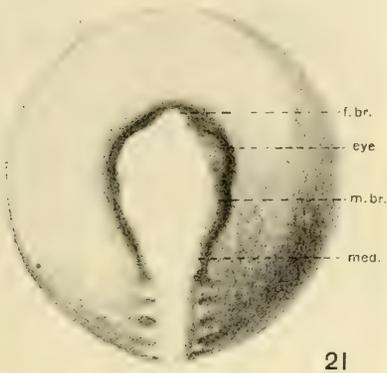
PLATE 2

EXPLANATION OF FIGURES

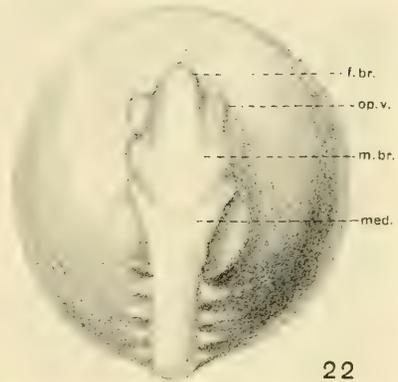
- 21 A larva before hatching, Stage VII. × 15
- 22 Same, Stage VIII. × 15.
- 23 Same, Stage IX. × 15.
- 24 Same, Stage X. × 15.
- 25 Same, Stage XI. × 15.
- 26 Same, Stage XII. × 15.

REFERENCES

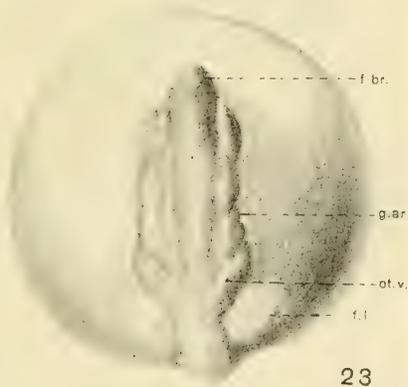
- | | |
|--------------------------------|--------------------------------|
| <i>f. br.</i> , fore-brain | <i>med.</i> , medulla |
| <i>f. l.</i> , fore-limb | <i>mx.</i> , maxillary process |
| <i>g. ar.</i> , gill arch | <i>olf. p.</i> , olfactory pit |
| <i>h. l.</i> , hind limb | <i>op. v.</i> , optic vesicle |
| <i>m. br.</i> , mid-brain | <i>ot. v.</i> , otic vesicle |
| <i>m. d.</i> , mandibular arch | |



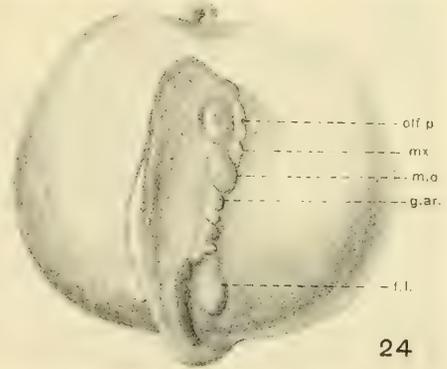
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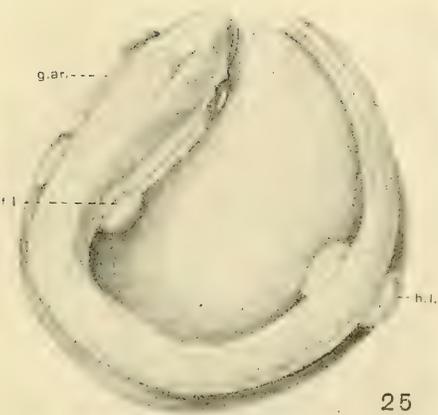
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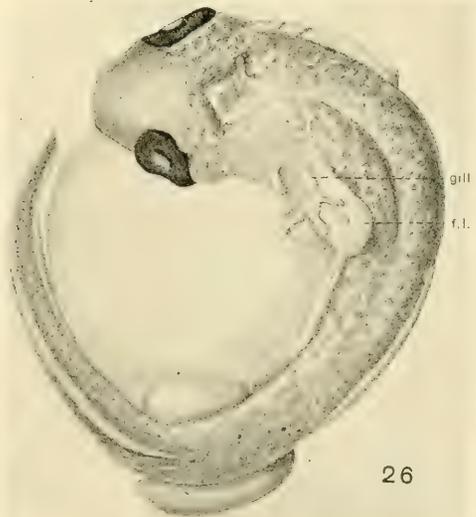
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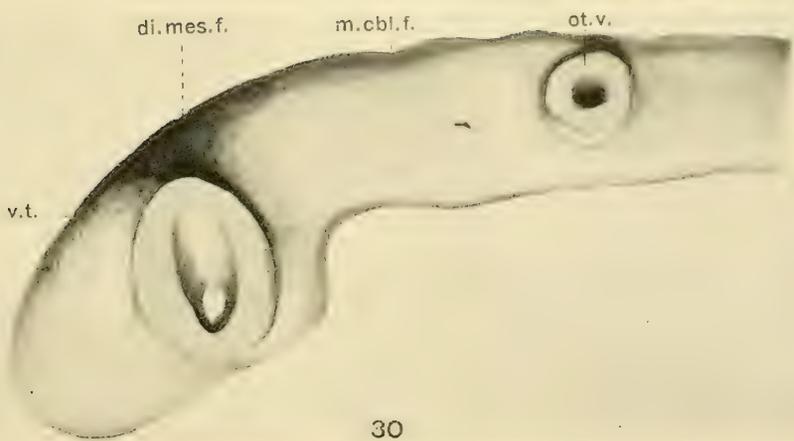
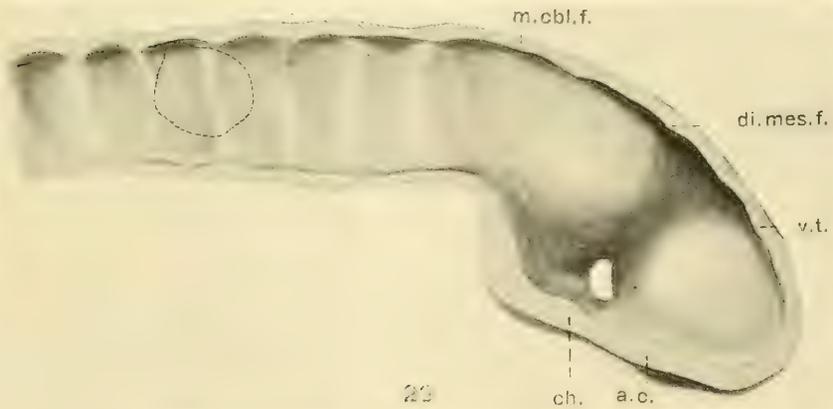
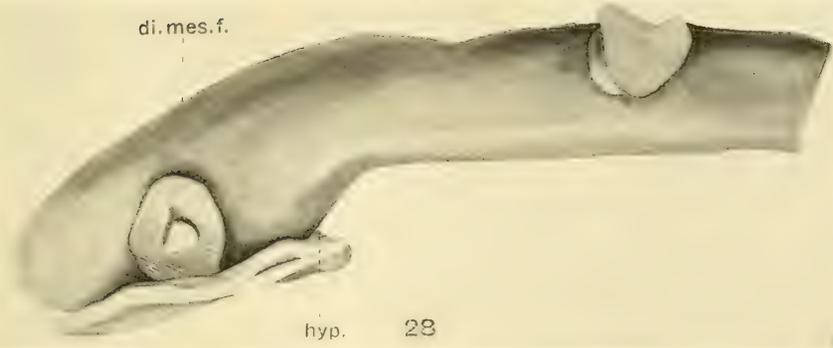
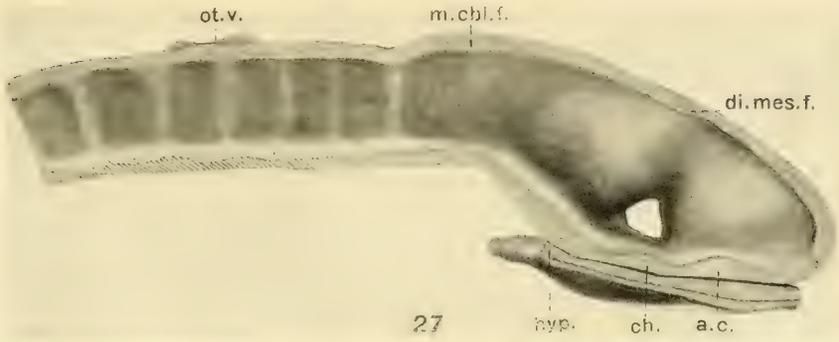
PLATE 3

EXPLANATION OF FIGURES

- 27 Medial section of a model, a young Stage VIII. × 75
28 Lateral view of same. × 75.
29 Medial view of a model, an older Stage VIII. × 75.
30 Lateral view of same.

REFERENCES

- | | |
|--|---|
| <i>a. c.</i> , anterior commissure | <i>m. cbl. f.</i> , mesencephalic-cerebellar fold |
| <i>ch.</i> , optic chiasma | <i>ot. v.</i> , otic vesicle |
| <i>di. mes. f.</i> , di-mesencephalic fold | <i>v. t.</i> , velum transversum |
| <i>hyp.</i> , hypophysis | |



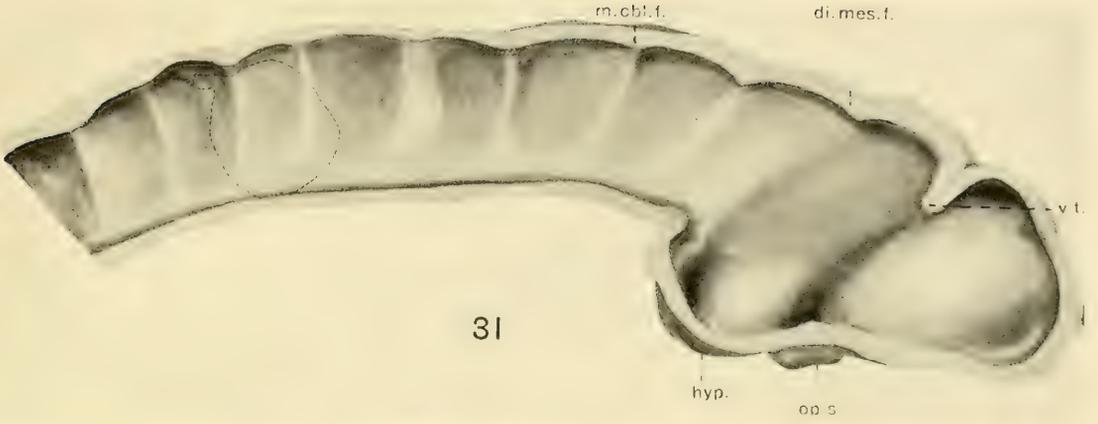
PLATES 4

EXPLANATION OF FIGURES

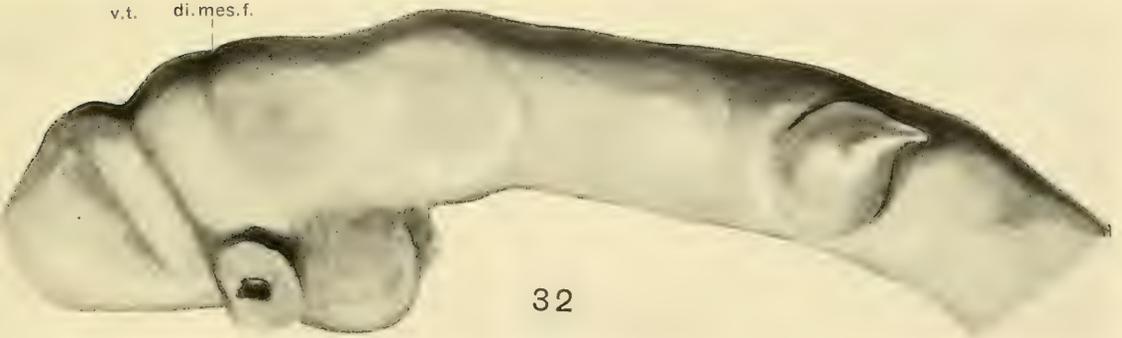
- 31 Medial view of a model, Stage IX. × 88.
- 32 Lateral view of same. × 88.
- 33 Medial view of a model, Stage XI. × 75.
- 34 Lateral view of same. × 75.

REFERENCES

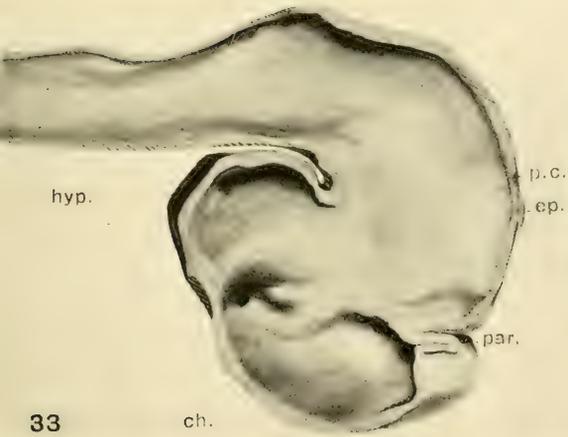
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| <i>ch.</i> , optic chiasma | <i>op. s.</i> , optic stalk |
| <i>ep.</i> , epiphysis | <i>par.</i> , paraphysis |
| <i>di. mes. f.</i> , di-mesencephalic fold | <i>p. c.</i> , posterior commissure |
| <i>hyp.</i> , hypophysis | <i>v. t.</i> , velum transversum |
| <i>m. cbl. f.</i> , mesencephalic-cerebellar fold | |



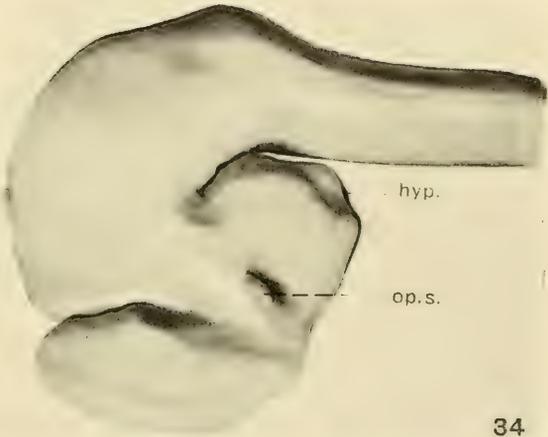
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PLATE 5

EXPLANATION OF FIGURES

- 35 Medial view of a model, Stage XII. $\times 75$.
36 Lateral view of same. $\times 75$.

REFERENCES

- | | |
|--|-------------------------------------|
| <i>a. c.</i> , anterior commissure | <i>ep.</i> , epiphysis |
| <i>cbl.</i> , cerebellum | <i>hyp.</i> , hypophysis |
| <i>cbl. c.</i> , cerebellar commissure | <i>op. s.</i> , optic stalk |
| <i>ch.</i> , optic chiasma | <i>p. c.</i> , posterior commissure |
| <i>di. pl.</i> , diencephalic plexus | <i>s. c.</i> , superior commissure |

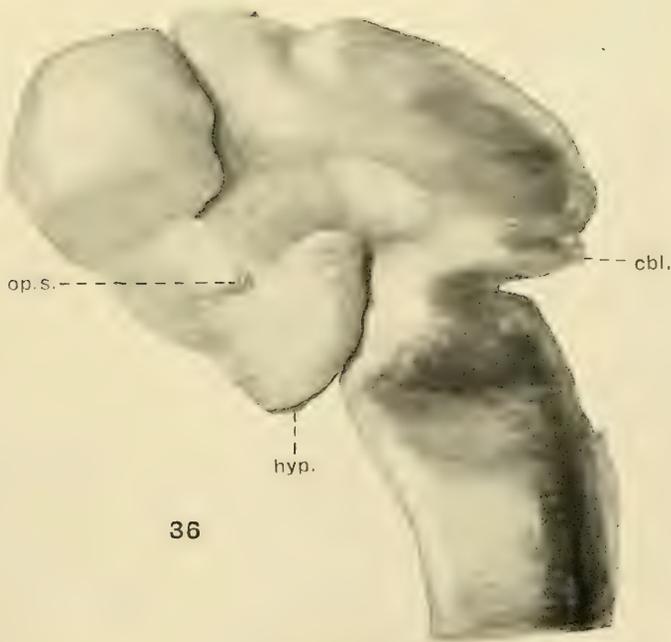
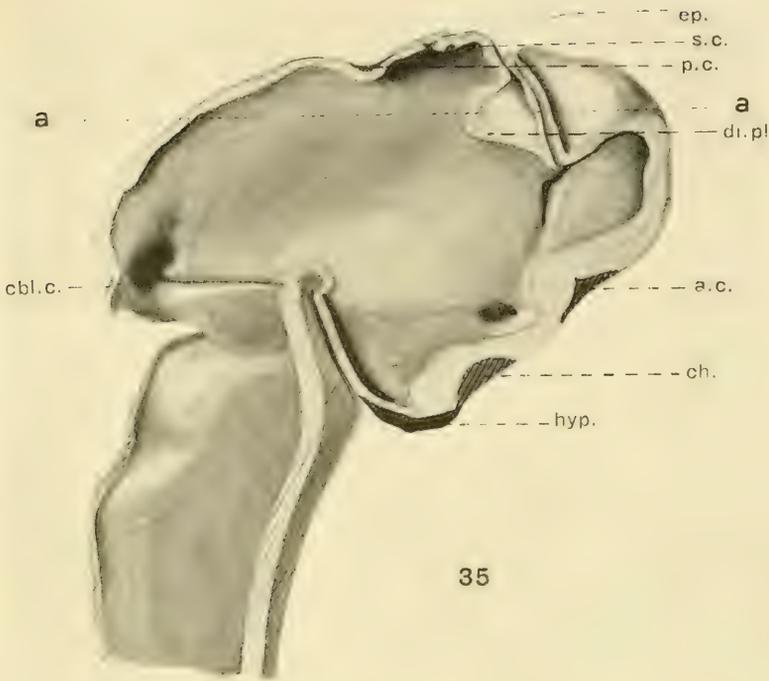


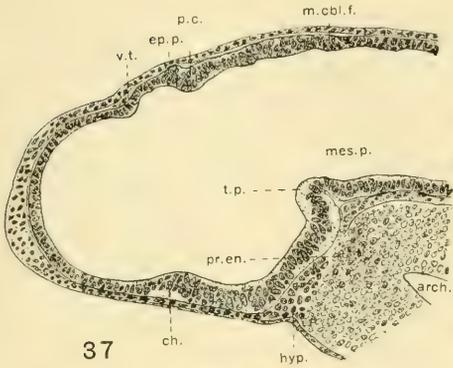
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EXPLANATION OF FIGURES

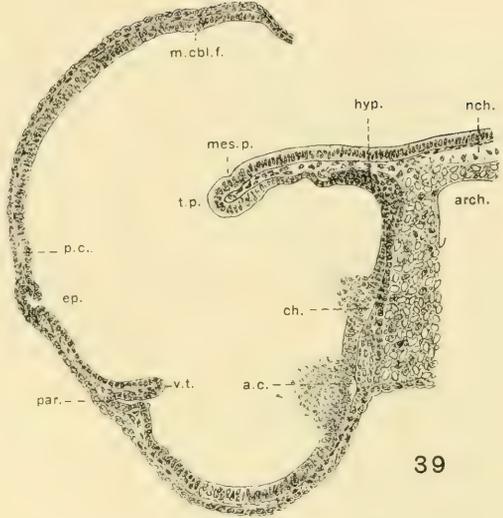
- 37 A medial sagittal section of a larva, Stage IX. $\times 75$.
38 Same, Stage X. $\times 75$.
39 Same, Stage XI. $\times 75$.
40 A medial sagittal section of a 38 mm. adult. $\times 47$.
41 Transsection through the habenulae and posterior part of the telencephalon, 13 mm. larva. $\times 75$.

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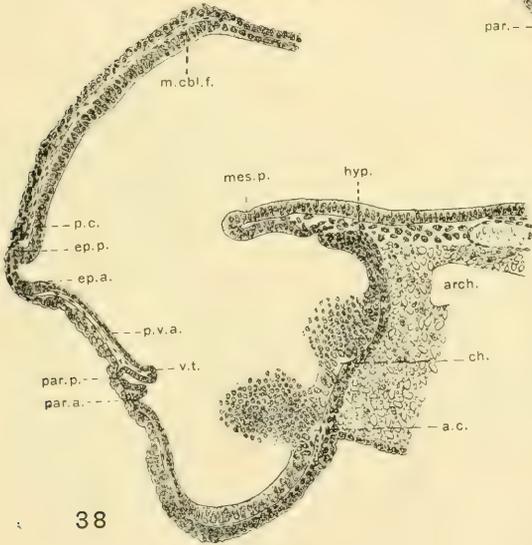
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| <i>a. c.</i> , anterior commissure | <i>nch.</i> , notochord |
| <i>arch.</i> , archenteron | <i>par.</i> , paraphysis |
| <i>b. v.</i> , blood vessel | <i>par a.</i> , anterior paraphysial evagination |
| <i>cbl. c.</i> , cerebellar commissure | <i>par. p.</i> , posterior paraphysial evagination |
| <i>ch.</i> , optic chiasma | <i>p. c.</i> , posterior commissure |
| <i>di. pl.</i> , diencephalic plexus | <i>p. hip.</i> , primordium hippocampi |
| <i>ep.</i> , epiphysis | <i>pr. en.</i> , pre-oral entoderm |
| <i>ep. a.</i> , anterior evagination of epiphysis | <i>p. v. a.</i> , post-velar arch |
| <i>ep. p.</i> , posterior evagination of epiphysis | <i>s. c.</i> , superior commissure |
| <i>hip. c.</i> , hippocampal commissure | <i>st.</i> , striatum |
| <i>hyp.</i> , hypophysis | <i>tel. pl.</i> , telencephalic plexus |
| <i>m. cbl. f.</i> , mesencephalic-cerebellar fold | <i>t. p.</i> , tuberculum posterius |
| <i>mes. p.</i> , mesencephalic pit | <i>v. t.</i> , velum transversum |
| <i>mpl.</i> , metaplexus | |



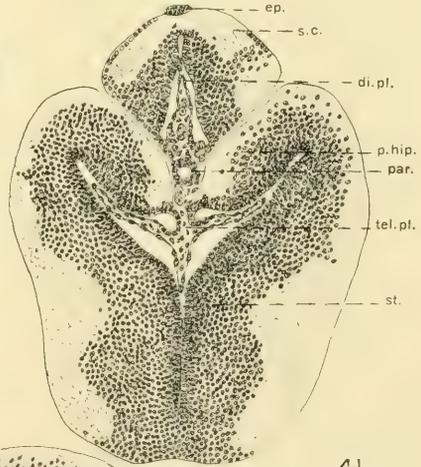
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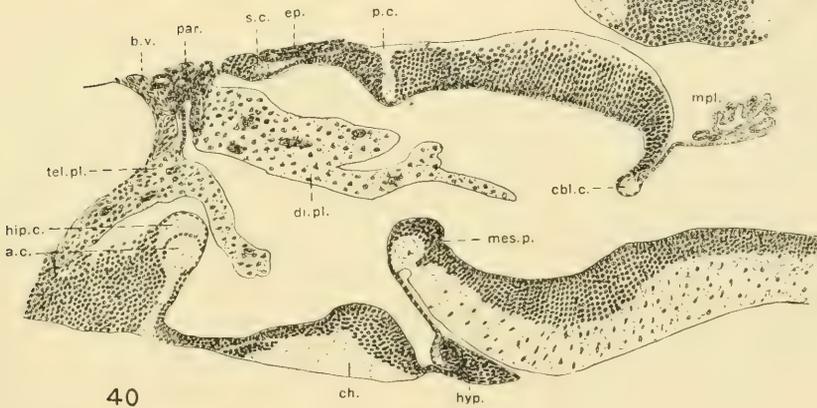
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PLATE 7

EXPLANATION OF FIGURES

- 42 Section of a larva, Stage XII. The plane is shown a dotted line a-a (fig. 36). × 50.
 43 Transsection through the posterior portion of the olfactory bulbs, 13 mm. larva. × 50.
 44 Transsection just cephalic to the interventricular foramina, 13 mm. larva. × 50.
 45 Transsection through the posterior poles of the cerebral hemispheres, 13 mm. larva. × 50.
 46 Transsection through the mesencephalon and hypothalamus, 13 mm. larva. × 50.
 47 Transsection through the cerebellum and posterior portion of the mid-brain, 13 mm. larva. × 50.
 48 Transsection through the olfactory bulb, 23 mm. larva. × 50.
 49 Transsection just cephalic to the interventricular foramina, 23 mm. larva. × 50.
 50 Transsection at the level of the anterior commissure, 23 mm. larva. × 50.
 51 Transsection through the habenulae and posterior poles of the cerebral hemispheres, 23 mm. larva. × 50.
 52 Transsection through the mid-region of the mesencephalon, 23 mm. larva. × 50.

REFERENCES

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|---|--|
| <i>a. c.</i> , anterior commissure | <i>p. hip.</i> , primordium hippocampi |
| <i>cbl.</i> , cerebellum | <i>p. po.</i> , polus posterior |
| <i>cbl. c.</i> , cerebellar commissure | <i>pr. r.</i> , preoptic recess |
| <i>fi. lat.</i> , fissura lateralis | <i>s. c.</i> , superior commissure |
| <i>hb.</i> , habenula | <i>s. d.</i> , sulcus diencephalicus dorsalis |
| <i>hip. c.</i> , hippocampal commissure | <i>s. ep.</i> , septum ependymale |
| <i>l. rec.</i> , lateral recess | <i>s. l.</i> , sulcus limitans |
| <i>l. v.</i> , lateral ventricle | <i>s. m.</i> , sulcus diencephalicus medius |
| <i>olf. n.</i> , olfactory nerve | <i>st.</i> , striatum |
| <i>op. s.</i> , optic stalk | <i>s. v.</i> , sulcus diencephalicus ventralis |
| <i>par.</i> , paraphysis | <i>tel. pl.</i> , telencephalic plexus |

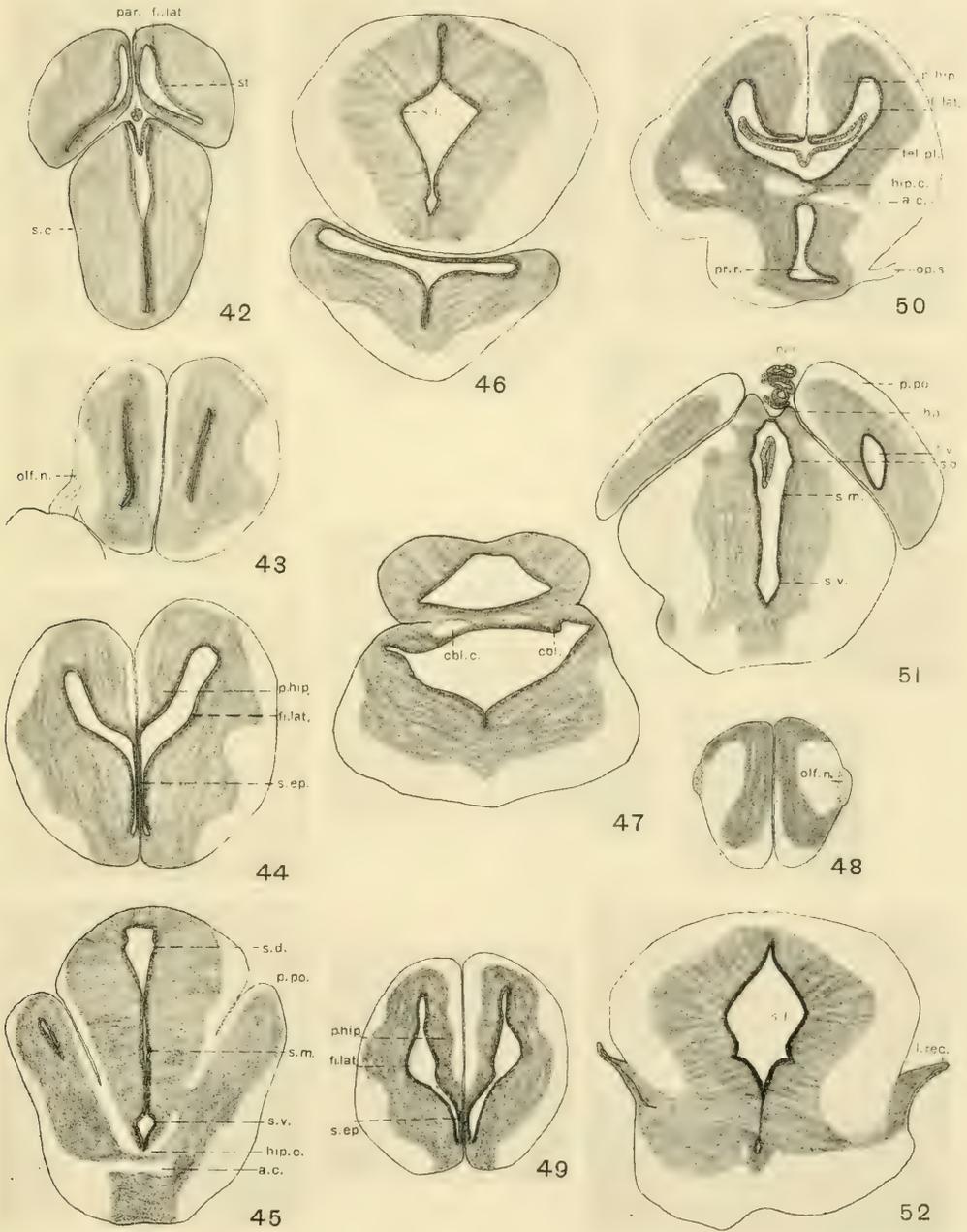


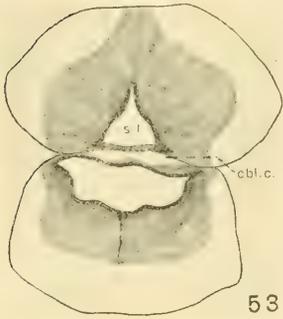
PLATE 8

EXPLANATION OF FIGURES

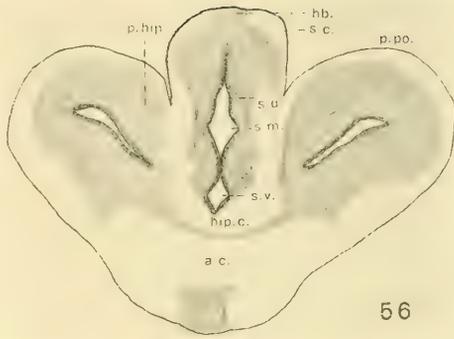
- 53 Transsection through the cerebellum and posterior portion of the mid-brain, 23 mm. larva. $\times 50$.
54 Transsection through the olfactory bulb, 30 mm. adult. $\times 50$.
55 Transsection just cephalic to the interventricular foramina, 30 mm. adult. $\times 50$.
56 Transsection through the posterior poles of the cerebral hemispheres, 30 mm. adult. $\times 50$.
57 Transsection through the mid-brain and hypothalamus, 30 mm. adult. $\times 50$.
58 Transsection through the cerebellum and posterior portion of the mid-brain, 30 mm. adult. $\times 50$.

REFERENCES

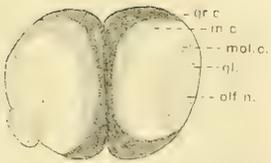
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|---|--|
| <i>a. c.</i> , anterior commissure | <i>olf. n.</i> , olfactory nerve |
| <i>cbl. c.</i> , cerebellar commissure | <i>p. hip.</i> , primordium hippocampi |
| <i>fi. lat.</i> , fissura lateralis | <i>p. po.</i> , polus posterior |
| <i>gl.</i> , olfactory glomeruli | <i>s. c.</i> , superior commissure |
| <i>gr. c.</i> , granule cell layer of olfactory bulb | <i>s. d.</i> , sulcus diencephalicus dorsalis |
| <i>hb.</i> , habenula | <i>s. ep.</i> , septum ependymale |
| <i>hip. c.</i> , hippocampal commissure | <i>s. l.</i> , sulcus limitans |
| <i>m. br.</i> , mid-brain | <i>s. m.</i> , sulcus diencephalicus medius |
| <i>m. c.</i> , mitral cell layer of olfactory bulb | <i>s. v.</i> , sulcus diencephalicus ventralis |
| <i>mol. c.</i> , molecular cell layer of olfactory bulb | |



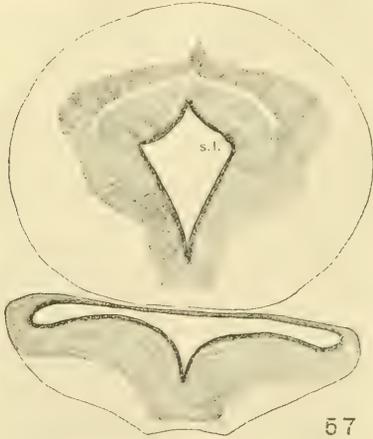
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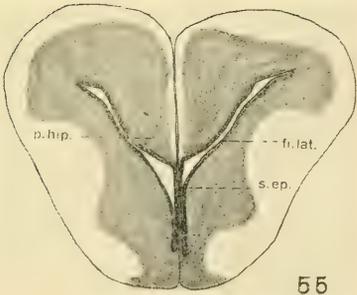
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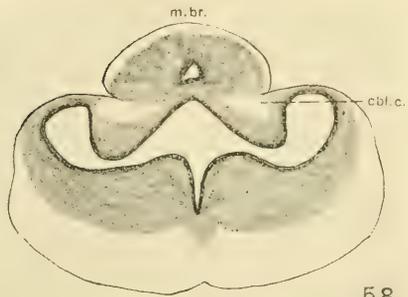
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CHROMOSOMAL VARIATIONS AND THE FORMATION
OF THE FIRST SPERMATOCYTE CHROMOSOMES
IN THE EUROPEAN EARWIG, FORFICULA SP.¹

FERNANDUS PAYNE

SIXTY-FOUR FIGURES

During the months of September and October, 1912, I collected a number of specimens of *Forficula* in the vicinity of Würzburg. The species remain in doubt, but probably most of my specimens are *auricularia*. From the chromosomal history, one other species may be present in my material. The gonads were fixed in Flemming and Bouin and the sections stained with Haidenhain's hemotoxylin. A superficial study of the slides indicated that irregularities in chromosome distribution were present. Nothing further was done, however, until my return to Bloomington. Upon looking up the literature, I found that a number of investigators had worked on *Forficula auricularia*, but their results were not in agreement.

As early as 1885 Carnoy described the number of chromosomes in the spermatocyte divisions as varying from 10 to 14. Two years later La Valette St. George ('87) described 12 in the first and 12 to 14 in the second spermatocytes. Sinety ('01) found no irregularities and gave the spermatogonial number as 24 and the spermatocyte 12. In two papers Zweiger ('06) works over the field in detail and finds the spermatogonial number 24 or 26 and the spermatocyte 12, 13 or 14. The observations of Stevens ('10) are more nearly in agreement with those of Sinety ('01). She finds the spermatogonial number 24, the first spermatocyte 12 and usually 12 in the second, although 11 and 13 chromosomes are occasionally counted in this division. She also describes an unequal pair of idiochromosomes. The explanations which these authors offer are no more in agreement than their observations.

¹ Contribution from the Zoological Laboratory of Indiana University, No. 134.

From these various descriptions and disagreements, it would seem that an excessively large number of errors have been made or that we really have irregularities in chromosome distribution. I believe the latter to be the case. In fact, my material shows this beyond a doubt and offers a plausible explanation for at least some of these irregularities. Stevens ('10) suggests that *Forficula auricularia* is a composite species, made up of several small species, which differ with respect to the number and behavior of the chromosomes. With this possibility in mind, I have studied the individuals singly and then compared the results. As we shall see, the differences within some individuals are as difficult of explanation as between different individuals.

DESCRIPTION

Specimen No. 29-2. In this specimen I found only one spermatogonial group which I was able to count. This showed clearly 24 chromosomes (fig. 1, *A*). The fact that the specimens were collected so late in the season accounts for the small number of spermatogonial divisions. A large percentage of the testes collected were full of mature spermatozoa. The first spermatocyte divisions show 12, 13 and 14 chromosomes (fig. 1, *L* and *M*; 14; *N*, 13 and *O*, 12). By counting only clear metaphase plates, I found 18 cells with 12, 13 with 13 and 3 with 14 chromosomes. These variations are found, not only within the testes of one individual, but, contrary to the observations of Zweiger ('06), they occur within a single cyst. Zweiger believed that a cyst arises from a single cell and hence all cells within the cyst contain the same number of chromosomes. He gives the spermatogonial number as 24 and 26. The half or reduced number would be 12 or 13. This, however, would not explain his 14 chromosome counts. According to his view such a group would have to arise from a spermatogonial cell with 28 chromosomes.

In my work a study of polar views failed to explain these variations, so I have resorted to a study of side views of metaphase plates, and believe I have found a true explanation. Why the chromosomes behave as they do thus causing these variations, I cannot say. Smear preparations would no doubt have helped

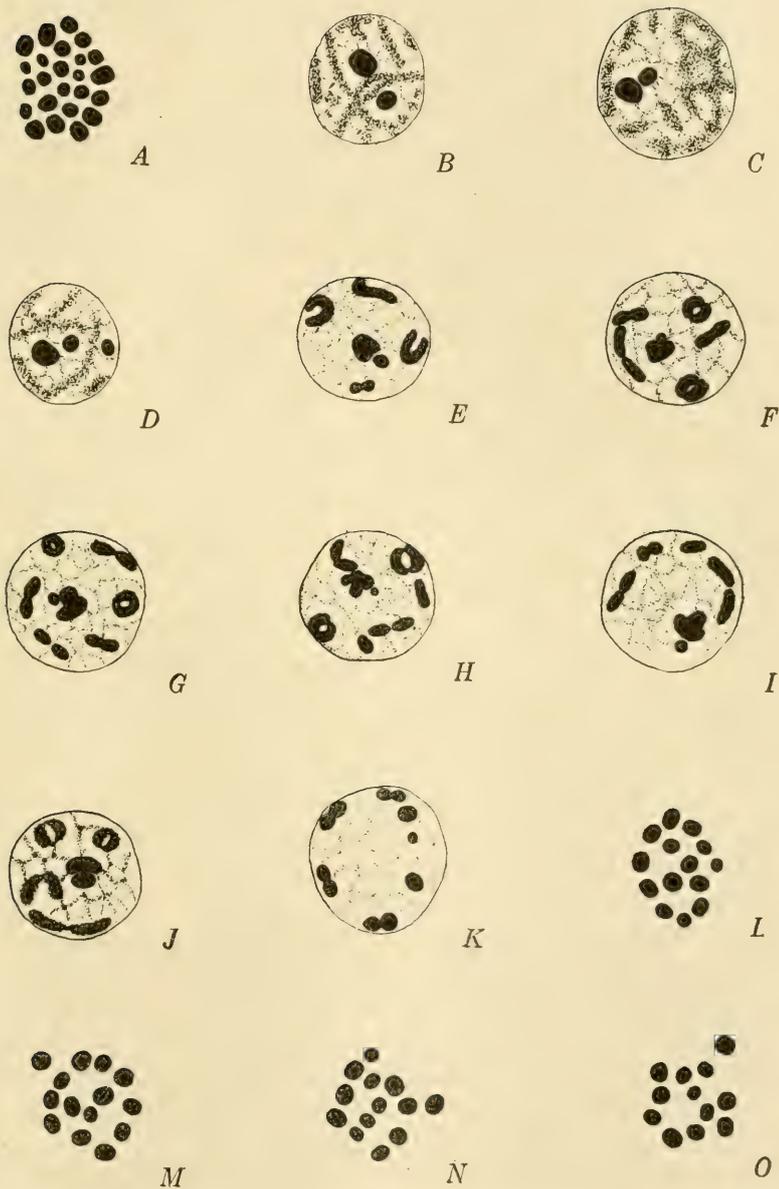


Fig. 1 Specimen 29-2. *A*, spermatogonial group with 24 chromosomes; *B*, *C*, *D*, early growth period, showing two or three black staining bodies; *E*, *F*, *G*, *H*, *I*, *J*, *K*, early prophases, demonstrating that the unequal pair arises from the dark staining nucleolus; *L*, *M*, first spermatocyte, polar view, showing 14 chromosomes; *N*, *O*, first spermatocytes with 13 and 12 chromosomes respectively.

in this study, but as no other material is available, my study is limited to sections. I realize that a study of serial sections of side views of a metaphase plate is beset with danger, as the knife may cut chromosomes so as to make them appear as two. However, this has not been such a serious difficulty as it might seem and in the following description I feel reasonably sure I have not made a mistake in this way. Further, these observations have been confirmed by a study of cells in which all the chromosomes are present in a single section. The greatest danger would be in cutting a double chromosome into its two components so as to make it appear as two single chromosomes. In my study of single chromosomes I have been very careful, when two or more are present in a single cell, to see that they do not lie opposite, that is, if the cell were reconstructed the two single ones would not fit together to make a bivalent.

Side views of metaphase plates with 12 chromosomes show all of them to be bivalent and dumb-bell-shaped (fig. 2, *L*, *M*, *N* and *O*). Stevens ('10) described in *Forficula auricularia* a pair of slightly unequal idiochromosomes. I find here also one of these 12 bivalents (fig. 2, *D*, *i*) is slightly unequal. Sometimes no size difference is visible in any of the bivalents and in no case do I find the difference as great as that described by Stevens. Since I have not been able to compare male and female groups, I cannot state definitely whether this unequal pair is related to sex. Even if I had the material for comparison, it is doubtful whether it would yield results, as the size difference is so small. I hesitate all the more in saying definitely that it is related to sex since the recent paper of Carothers ('13) describing among the autosomes an unequal pair.

Side views of metaphase plates with 13 chromosomes show 11 bivalent and two single ones (fig. 2, *D*, *E*, *F*, *G*, serial sections of one cell and *H*, *I*, *J*, *K*, of another). This indicates clearly, it seems to me, that two spermatogonial chromosomes have failed to conjugate at synapsis or have conjugated and then separated. A polar view of such a group looks very different from one with 12, yet the two are the same, the difference being that the components of one bivalent have remained separate in

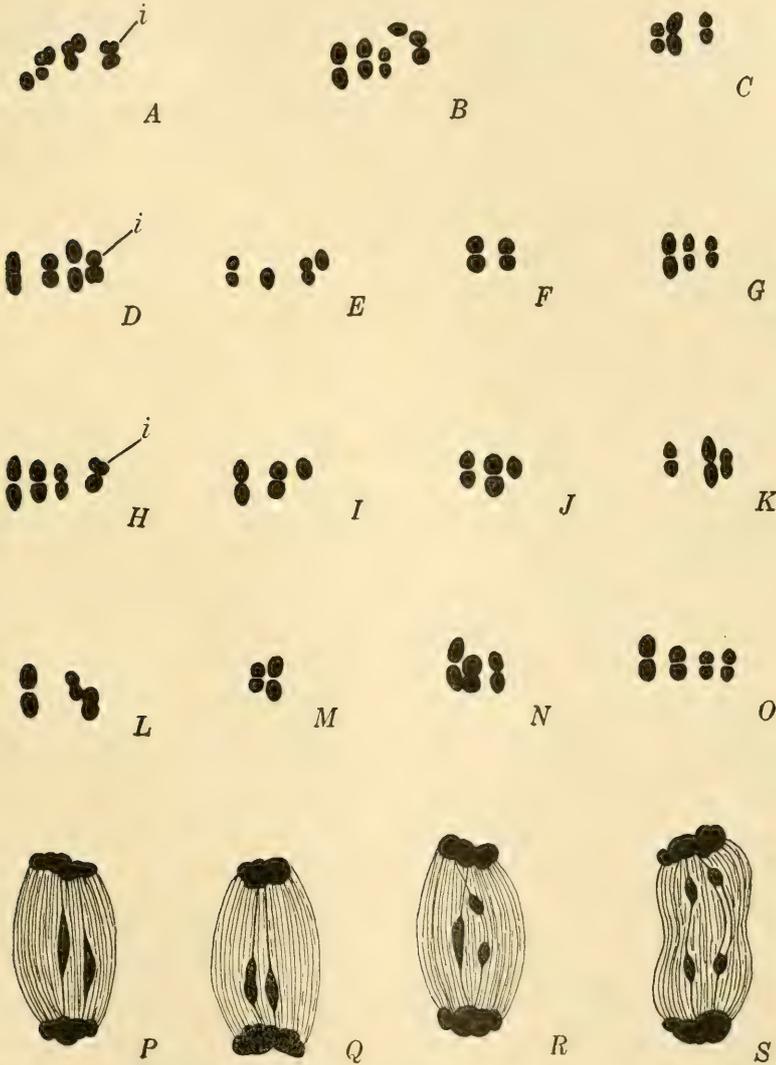


Fig. 2 Specimen 29-2. *A, B, C*, serial sections, side view, of a single metaphase plate, first division with 14 chromosomes, showing that four of these are univalent and 10 bivalent; *D, E, F, G*, serial sections of a metaphase plate with 13 chromosomes showing 11 bivalent and two univalent ones; *H, I, J, K*, same as *D, E, F, G*; *L, M, N, O*, serial sections of a metaphase plate with 12 chromosomes showing that all of them are bivalent; *P, Q, R, S*, anaphases of the first maturation division showing the behavior of the single chromosomes.

one case and have fused in the other. A few cells show 14 chromosomes. Side views of such cells show that ten of these are bivalent and four univalent. In this case the components of two bivalents have remained separate and the group as a whole is the same as the one where 12 and 13 chromosomes appear in polar views.

The interesting thing about these univalent chromosomes is not the fact that they are univalent, but their irregular behavior in the two divisions which follow. So far as I know, with the exception of the supernumerary chromosomes of *Metapodius* and *Diabrotica*, the behavior of any univalent chromosome in the two maturation divisions is regular, and such chromosomes divide in one or the other division. The rule is for bivalent chromosomes to divide twice and univalent chromosomes once. We would expect then these univalent chromosomes in *Forficula* to divide in one maturation division and not in the other. In the first maturation division I find that they may or may not divide and, whatever happens to them, they always lag behind the others. Figure 2, *S* shows both of them divided; *R* shows one divided, the other undivided. In *Q* it seems that both are passing undivided to one pole. In *P* both are elongated and it is impossible to say just what disposition will be made of them. Sometimes they become more drawn out, and in such cases as figure 4, *N* (a different individual), it seems very doubtful whether one lagging chromosome will ever reach either pole. These lagging chromosomes are the ones which Zweiger calls 'accessorische Chromosomen.' I have seen only one anaphase with four lagging chromosomes and it was not suitable for drawing. Hence I have not been able to make out the distribution of the univalents in the 14 chromosome groups. From this irregularity in the behavior of these single chromosomes, it will be seen that the number of chromosomes in the second maturation division will vary. This is the case, and by actual count of polar views, clear metaphase plates, I have found nine cells with 11 chromosomes; 65 with 12; 23 with 13 and four with 14. For these variations see figure 3, *B*, *C*, 14 chromosomes; *D*, *E*, *F*, 13 chromosomes; *G*, *H*, 12 chromosomes and *I*, *J*, *K*, 11 chromosomes.

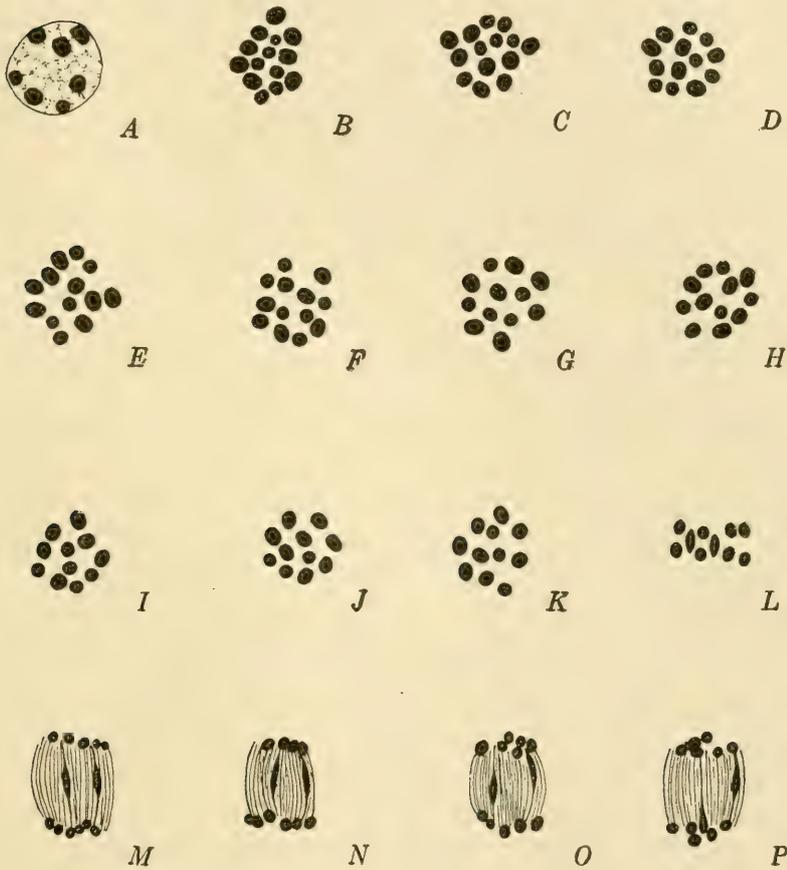


Fig. 3 Specimen 29-2. *A*, a resting nucleus between the first and second spermatocyte divisions; *B*, *C*, second spermatocyte divisions, polar views, 14 chromosomes; *D*, *E*, *F*, the same with 13 chromosomes; *G*, *H*, the same with 12 chromosomes; *I*, *J*, *K*, the same with 11 chromosomes; *L*, *M*, *N*, *O*, *P*, anaphases of the second division, side view, showing the behavior of the lagging chromosomes.

If there are 12 bivalents in the first division, all of them divide so that each secondary spermatocyte receives 12 chromosomes, and by actual count it is seen that more than two-thirds of them receive this number. When there are 13 chromosomes in the first division, two of these are univalent and divide irregularly. If both divide, each resulting cell receives 13 chromosomes;

if one divides and the other passes to one pole undivided, one cell will receive 12 and the other 13 chromosomes; if one passes to one pole and the other to the second pole undivided, each resulting cell will receive 12 chromosomes; lastly, if both single chromosomes pass to one pole undivided the result is one cell with 11 chromosomes and one with 13. Cells with 14 chromosomes are rather rare and, as shown above, are composed of ten bivalent and four univalent chromosomes. The irregular division of these four chromosomes might cause the variations, ranging from 10 to 14, the number which Carnoy ('85) actually described. Unfortunately my material does not show sufficient anaphases to demonstrate the distribution of them.

There is a short resting stage between the first and second maturation divisions (fig. 3, *A*). In the anaphases of the second, some of the cells show lagging chromosomes. While the evidence is not direct, these lagging chromosomes are no doubt the univalent ones which behave irregularly in the first maturation division. Judging from such figures as 3, *M*, *N*, *O*, and *P*, here again the behavior is irregular, and the spermatids undoubtedly receive numbers varying from 11 to 14 and perhaps from 10 to 14 chromosomes. All of these spermatids, I believe, develop into mature spermatozoa, as I have seen no sign of their degeneration. Whether all such spermatozoa function is impossible to say with the limited amount of material at my command. I hope to be able in the near future to obtain additional material for the study of this and other questions which remain unsolved.

A few words concerning the growth period may not be out of place before leaving this specimen, as it seems to be clearer here

Fig. 4 Specimen 29-3. *A, B*, spermatogonial cells with 24 chromosomes each; *C, D*, oogonial cells which have been included in this plate and which were the only ones which could be counted, 25 chromosomes (They were not very clear and I lay no weight on this evidence); *E, F, G*, serial sections, side view, of a metaphase plate of the first division with 13 chromosomes showing 11 bivalent and two univalent; *I, J, K*, serial sections of a metaphase plate with 12 chromosomes, all of which are bivalent; *H*, metaphase plate, pole view, first division, 12 chromosomes; *L*, metaphase plate, 13 chromosomes; *M, N, O*, anaphases of the first maturation division showing distribution of the univalents; *P, Q*, second spermatocyte divisions, metaphase plates, 13 chromosomes; *R, S*, the same with 12 chromosomes.

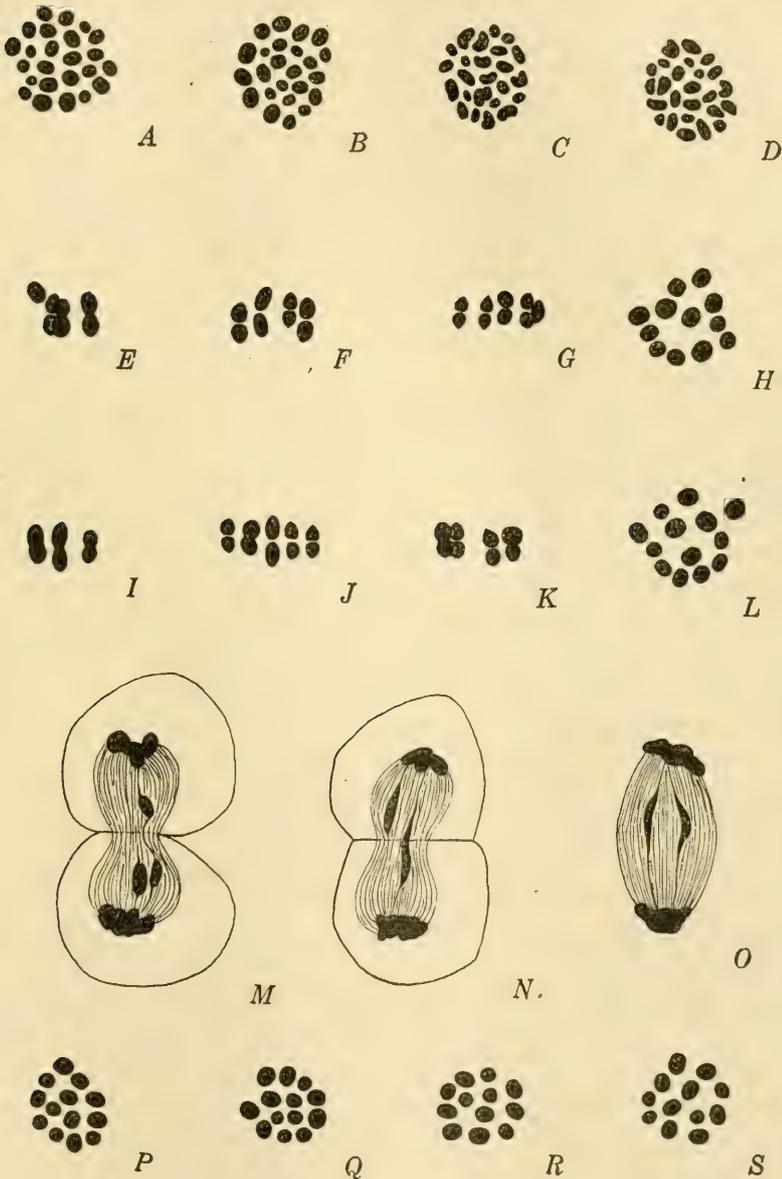


Figure 4

than in any of the others. Shortly after the last spermatogonial division and throughout the entire growth period, two and sometimes three chromatin nucleoli are present in the nucleus. In the prophases of the first spermatocyte division only one large nucleolus remains. Whether the second and third one disappear or whether all of them fuse, I cannot say. Stevens ('10) describes the second and third bodies as plasmosomes, but with hemotoxylin all of them stain alike. At any rate, from the single nucleolus there arises in late prophases the unequal pair of chromosomes. This is shown particularly well in figure 1, *J*. Most of the time a third small body is either in contact with them or lying very close (fig. 1, *F*, *G*, *H*, *I*). What this small body is I do not know, but believe it is not a chromosome as it is smaller than any of those which appear in the first spermatocytes. It persists until the late prophases (fig. 1, *K*) and then can be traced no further. It will be noticed in figure 1, *J*, that the size difference between the components of the unequal pair is greater during the early prophases than in metaphase.

Zweiger explains these irregularities in number by assuming that in cells with 12 chromosomes no accessory is present; in cells with 13, one accessory is present and in cells with 14, two accessory chromosomes are present. He further assumes that the accessory chromosomes divide in both maturation divisions, something which no accessory chromosome has ever been observed to do. I am using the word 'accessory' in the sense that it has been used by various authors in the recent work on chromosomes which are related to sex. It is a spermatogonial chromosome which has no mate with which to pair at synapsis and which appears in the spermatocyte divisions as a univalent chromosome and hence divides in only one of them. The spermatozoon which receives this chromosome is female producing and the other is male producing. It is readily seen that Zweiger's 'accessorisches Chromosom' is not of this type. He describes his 13 chromosome group as containing one accessory chromosome and assumes that such groups arise from spermatogonial cells with 26 chromosomes. Hence all 13 would be bivalent. My observations show clearly that when 13 chromosomes are present,

two of them are univalent and that their behavior in the maturation divisions is irregular.

As previously stated, Stevens finds 12 chromosomes in the first spermatocyte division and usually 12 in the second, although 11 and 13 are sometimes present. She accounts for 13 by assuming that the small idiochromosome may sometimes divide precociously. She sees no way of explaining the 11-group except by assuming that both idiochromosomes occasionally pass to one pole undivided in the first spermatocyte division. Later, I shall attempt to show that in some individuals the 13-group in the second division may be caused by the precocious division of one small chromosome.

Specimen 29-3. This is very similar to 29-2. Two spermatogonial counts show 24 chromosomes each (fig. 4, *A* and *B*). In the first division (fig. 4, *H* and *L*), I find 17 cells showing clearly 12 chromosomes and nine showing 13. Side views of metaphase plates again show 2 univalents in the 13-chromosome group (fig. 4, *E, F, G*). Anaphases also show the lagging chromosomes in some of the cells. Figure 4, *M* and *N* are late anaphases in which the cell plate has begun to form. In *M*, there is no doubt that one univalent has divided while the other is passing to one pole undivided. In *N*, one chromosome is passing to one pole undivided while the other is very much drawn out and it seems doubtful whether it will reach either pole. As in 29-2, the irregular behavior of these univalent chromosomes causes variations in the number of chromosomes in the second spermatocytes. The counts here gave 63 cells with 12 chromosomes and 15 with 13. It seems rather strange that no cells with 11 were present as these might be expected from what was found in 29-2. Four other specimens show the same irregularities as the two above. They did not contain as many dividing cells, however. Specimen 27-1, in the first division, showed one cell with 12 chromosomes, two with 13 and three with 14; in the second division two cells with 12 chromosomes and two with 13. Specimen 27-2, gave in the first division three cells with 12 chromosomes; in the second division 26 cells with 12 chromosomes and four with 13. In specimen 27-3, I found 15

cells with 12 chromosomes and two with 13 in the first division; in the second division, 20 counts showed 12 in all. From 12 counts of the first division in specimen 27-4, there were four with 12 chromosomes, three with 13 and one with 14. In the second division 22 counts were made. Fourteen of these showed 12 chromosomes, seven showed 13 and one showed 14.

Specimens 34-1. Stevens ('10) accounted for the occasional appearance of 13 chromosomes in the second division by supposing that the small idiochromosome sometimes divides precociously. In this specimen the evidence is in favor of a similar interpretation. Only four first division figures which could be clearly counted were present. All of them contained 12 chromosomes (fig. 5, *I* and *J*). In the second division, 125 counts gave 115 with 12 chromosomes and 10 with 13. If the 12-group is studied carefully, it can be seen that there are four chromosomes which are noticeably smaller than the others (fig. 5, *A*, *B*, *C*, *D*). A fifth one is present which sometimes appears about the size of these four, but more often it is just a little larger, intermediate between them and the larger chromosomes. In the 13-group five and sometimes six chromosomes are smaller than the others (fig. 5, *E*, *F*, *G*, *H*). Among these five or six, two are considerably smaller than the others, and it seems very probable that these two small ones have arisen by the precocious division of one of the smaller chromosomes which appears in the 12-group. Whether the chromosome which divides precociously is the small one of the unequal pair, is impossible to say. In fact, I am inclined to think that it is not, since the irregularities in the other specimens are not caused by this pair.

Specimens 31-1, 31-2, and 26. The latter two show no irregularities in either maturation division. Since 31-1 shows only one irregularity I have placed it in the same group. In this specimen, 39 counts of the first division showed 12 chromosomes. In the second division, 69 counts gave 12 chromosomes and one 13, the only irregularity observed. In 31-2, 34 counts of the first division gave 12 chromosomes and 70 counts of the second gave the same number. In 26, three cells of the first division gave 12 chromosomes and 22 counts in the second division also

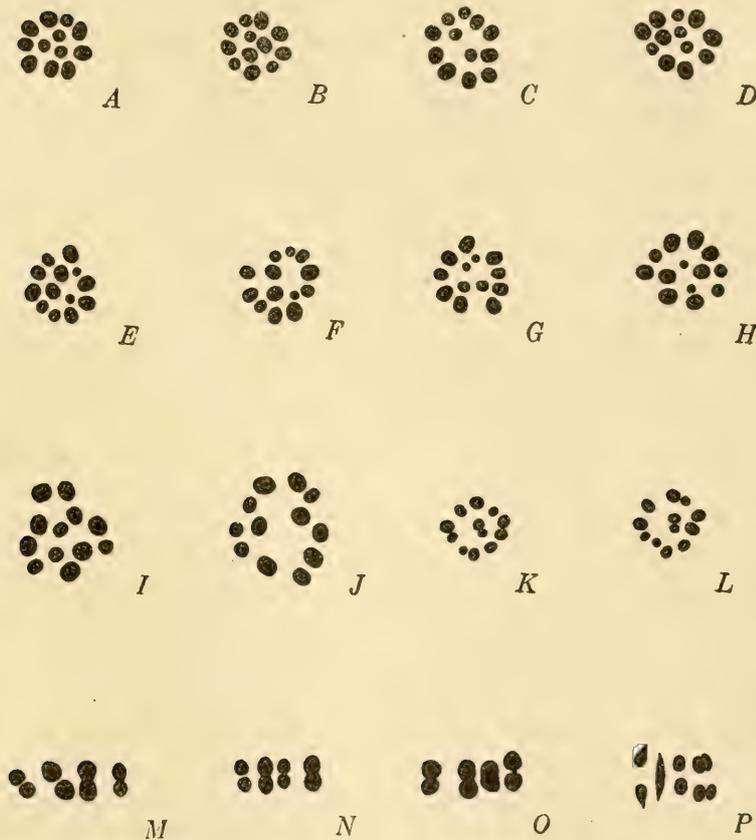


Fig. 5 Specimen 34-1. *A, B, C, D*, second spermatocyte divisions, pole views, showing 12 chromosomes; *E, F, G, H*, the same with 13 chromosomes; *I, J*, metaphase plates, first spermatocyte division, 12 chromosomes; *K, L*, second division anaphase, polar views, of both chromosome groups from the same spindle; *M, N, O*, serial sections, side views, of a metaphase plate of the first division showing that all chromosomes are bivalent; *P*, early anaphase of the second division showing one elongated chromosome.

gave 12 chromosomes. These specimens show clearly that by working on a limited amount of material, an observer might be led to believe that no irregularities were present. It is only by a comparative study of a large number of individuals that we may hope to reach a true solution of these difficulties.

Specimen 34-2 is the most puzzling individual of them all. The chromosome distribution is somewhat different from the others and this leads me to suspect that it may be a different species. The most striking variations are in the spermatogonial divisions. Two different cysts were found in division and variations were present in both. Among 33 counts of clear metaphase plates there were nine with 24 chromosomes, 16 with 25, seven with 26 and one with 27; (for these variations see fig. 6, *A*, 27; *B*, *C*, *D*, 26; *E*, *F*, *G*, *H*, 25 and *I*, *J*, 24 chromosomes). I am at a loss to explain these variations and certainly will not draw any conclusions from them. However, a few suggestions which the facts warrant may not be out of place. As there are a number of multipolar figures in the cyst, it is possible that these divisions may be pathological. Secondly it is possible that the chromosomes which behave irregularly in the spermatocyte divisions may also behave irregularly in the spermatogonial divisions. Thirdly, all counts (45) of the first spermatocyte division with the exception of one—and as we shall see this is really not an exception—show 13 chromosomes. Twelve of these 13 are bivalent and one single (fig. 6, *L*, *M*, *N*, three metaphase plates which have been smashed to cause the chromosomes to lie flat as in a smear preparation). Figure 7, *A*, *B*, *C*, are serial sections of a single cell, showing likewise 12 bivalent and one univalent chromosome. In case there are 14 present, 11 are bivalent and three univalent (fig. 7, *E* and *F*). So, as in the case of the other individuals, the two groups are in reality the same, although they appear different in polar views. The chromosomes then in the first division must have come from a spermatogonial cell with 25 chromosomes, 24 of them uniting two by two to form

Fig. 6 *Specimen 34-2*. *A*, spermatogonial group with 27 chromosomes; *B*, *C*, *D*, the same with 26 chromosomes; *E*, *F*, *G*, *H*, the same with 25 chromosomes; *I*, *J*, the same with 24 chromosomes; *K*, growth period showing nucleoli; *L*, *M*, *N*, first maturation divisions, metaphase, which have been smashed as in a smear preparation, showing 13 chromosomes, 12 of which are bivalent and one univalent; *O*, first division, polar view, 14 chromosomes; *P*, first division, 13 chromosomes (*O* and *P* are found in the same cyst); *Q*, *R*, anaphases, polar view, from the same spindle; *Q*, 13 chromosomes and *R*, 12; *S*, *T*, second spermatocyte divisions showing 13 and 12 chromosomes respectively.

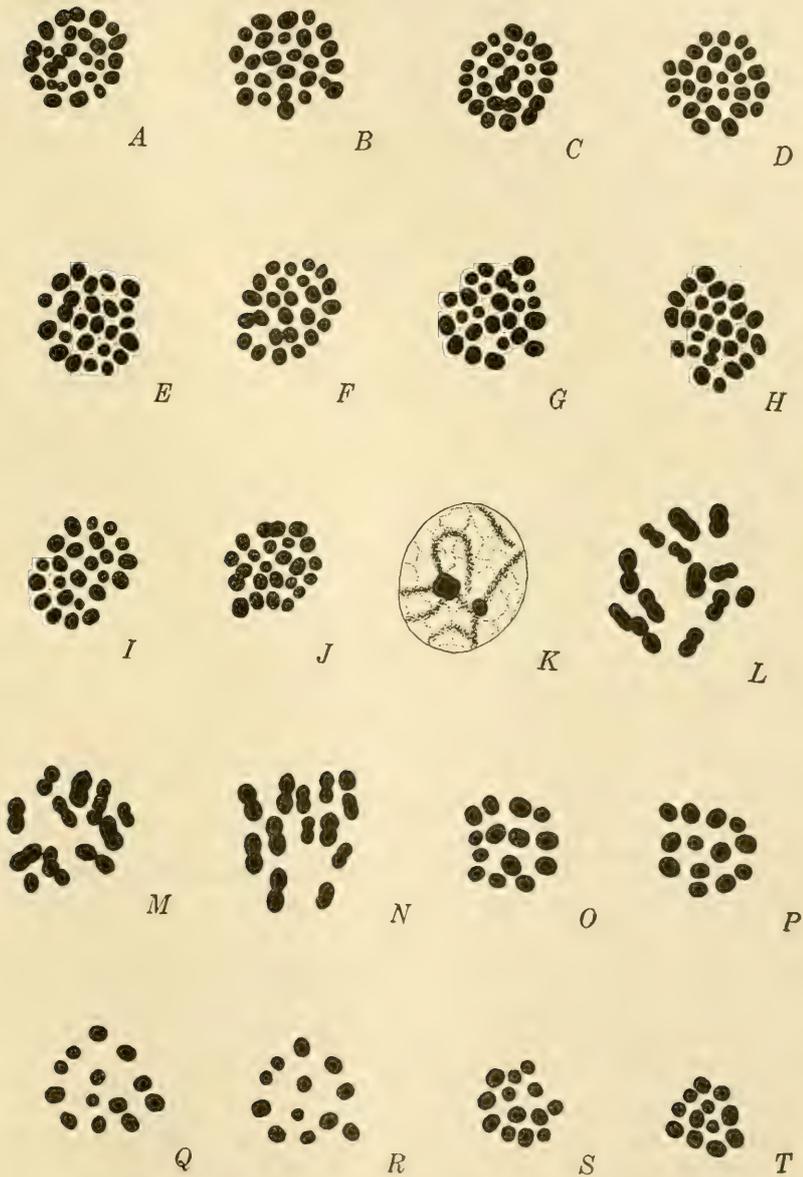


Figure 6

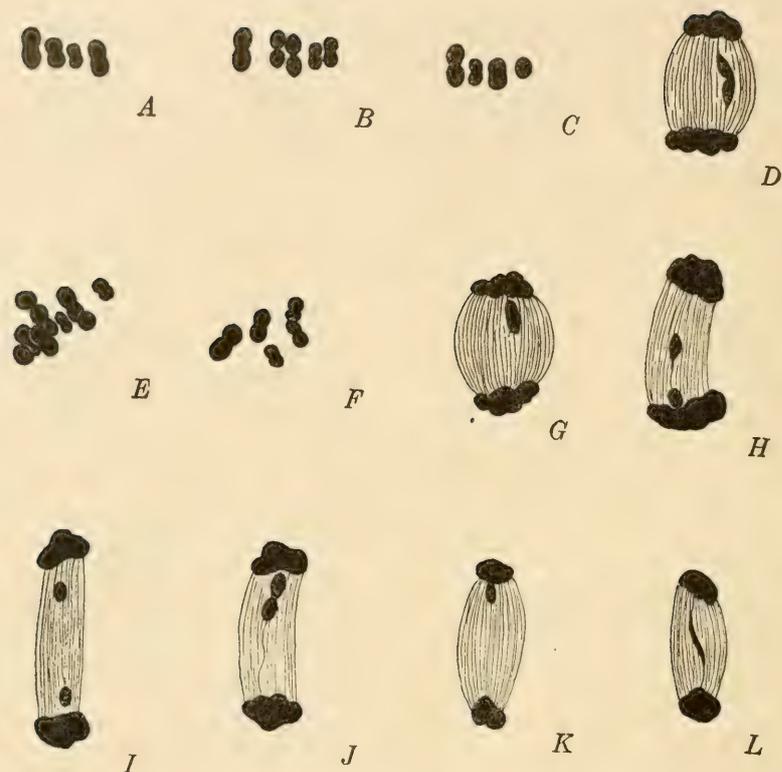


Fig. 7 Specimen 34-2. *A, B, C*, serial sections of a metaphase plate of the first division with 13 chromosomes, 12 of which are bivalent and one single; *E, F*, serial sections, first division, of a cell with 14 chromosomes, 11 of which are bivalent and three univalent; *D, G, H, I, J*, anaphases, first spermatocyte division, showing behavior of the single chromosome; *K, L*, anaphases of the second division showing a lagging chromosome here also.

the 12 bivalents and the other remaining single. From the spermatocyte chromosomes then, it would seem that 25 is the true spermatogonial number and the others (24, 26 and 27) are abnormal or at least not functional.

In this specimen I have not been able to make out an inequality in the two parts of any of the bivalents. Even though no size difference is recognizable, a pair corresponding to the unequal pair in the other individuals may be present. The nucleoli in the growth period look very much as they do in the other speci-

mens and there the unequal pair arises from the nucleolus in the prophases of the first spermatocyte division. A study of side views of metaphase plates of the first division indicates that we might have a case of a single unpaired idiochromosome. This seems very doubtful, however, since the behavior of the single chromosome in the maturation divisions is irregular. Its behavior is similar to that of the single chromosomes in the other individuals. Figure 7, *D*, *G*, *H*, *I* and *J* are anaphases of the first division and, I think, show clearly that this chromosome may pass to one pole undivided (*G*); that it may divide, one part passing to one pole and one to the other (*I*), or that it may constrict as if to divide and in this manner pass to one pole (*J*). Counts of the second division, metaphase plates, show 34 cells with 13 chromosomes and 34 with 12. This indicates an approximate equal distribution of the single chromosome. In anaphases of the second division (fig. 7, *K* and *L*) a single lagging chromosome is likewise present and no doubt it may or may not divide. My figures do not show it in division, but Zweiger ('06) and Stevens ('10) show a lagging chromosome in the second division which divides. Figure 7, *L*, is interesting in that it shows this chromosome very much elongated. In such cases it seems very doubtful whether it ever reaches either pole.

Formation of the first spermatocyte chromosomes

During the greater part of the growth period, and possibly all of it following synapsis, the chromatin threads appear double and made up of two rows of granules (plate 1, fig. 1). From these chromatin threads the dumb-bell-shaped spermatocyte chromosomes are formed. One of the intermediate stages in this transformation is a more or less typical ring, and it is the formation of this ring with which I am principally concerned. Two methods of ring formation have been described. Let us leave out for the present the question whether the longitudinally split thread is formed from chromosomes which have united end to end or side by side. One method is by the bending of the thread until the free ends come in contact. This method has been

described by Sutton ('02) in *Brachyostola* and Davis ('08) for several species of Orthoptera. Both these authors describe an end to end conjugation. The second method has been described by the Schreiners ('06) in *Tomopterus*, by Morse ('09) in *Periplaneta* and by others. In this case the ring is formed by the opening or moving apart of the two parallel parts of the split thread in the middle region, the ends of the two parts remaining in contact. These authors assume a side by side conjugation of maternal and paternal elements.

In *Forficula*, Zweiger describes ring formation as arising by the bending and coming together of the two ends of the chromatin thread, or what I may designate briefly as the bending process. This ring in later stages condenses into a dumb-bell-shaped chromosome. As he believed that the paternal and maternal chromosomes united end to end in synapsis, each part of the dumb-bell represented a single spermatogonial chromosome. Further, as the two parts separate, the first division, according to this scheme, would be reductional. Stevens ('10) describes no complete rings but finds only U's and V's. She believed the two ends of the U never come together, but straighten out again as condensation takes place. She also believed with Zweiger that telosynapsis takes place and that the first division is reductional.

My preparations of *Forficula* show clearly both methods of ring formation. Further, the rings are complete and not merely U's as described by Stevens. In fact, Zweiger's figures indicate that both methods are present. In his figure 60, which is supposed to show the various stages of ring formation and the condensation of this ring into a bivalent, it is a long jump from 'd' to 'e.' In fact, I think there is no connection between them. If we begin at 'd' and read in the opposite direction, 'd, c, b, a,' it will be seen that the split segment is opening up, as if in the initial stages of ring formation by splitting. I have seen no such irregular chromosomes as his figures 'f, g, h,' and hardly believe they occur in properly fixed material, although the two parts of the bivalent do bend around as he figures. While we have had both methods of ring formation described, *Forficula*, so far

as I know, gives the first example in which both methods are found in the same individual.

As previously stated, the doubly split chromatin threads remain throughout at least the greater part of the growth period (plate 1, fig. 1). I have not been able clearly to follow the pairing of the chromosomes in synapsis, and cannot say whether the two parts of the split thread are maternal and paternal elements which have conjugated side by side or whether the split is a division of single chromosomes which have united end to end. In such rings as figures 10 and 11, plate 1, there is no indication of the point where the two chromosomes join. Perhaps it appears later, but then the rings become lost among the others and it is impossible to trace them. In the early prophases, some of these threads open out, forming rings as shown in figures 2 to 11, plate 1. I have studied this point very carefully, being somewhat reluctant to believe that both methods could be present in the same species, but after going over the slides again and again, I have become convinced that such is the case. Plate 1, 12 to 17, shows various stages in the bending and twisting of these threads as they open out into rings.

Ring formation by bending is much clearer and here I have been able to trace the process in a number of different chromosomes. By careful study it is possible to recognize individually some of the chromosomes during this process of ring formation. This makes it possible to trace practically every step in the process and I have figured a complete series in the case of four chromosomes. Plate 1, figure 18, shows one of these chromosomes which can be distinguished by its shape and size. Figures 19 to 21 show clearly the formation of the ring by bending, while figures 22, 23 and 24 show the steps in the transformation into the chromosome of the first spermatocyte. By this series it is clearly seen that the composition of the bivalent chromosome (24) is the same as if the chromosome figured in 18, had condensed into a bivalent without passing through the ring stage. The split then in 18 is the line of the first maturation division. Whether this division is reductional, as believed by Zweiger and Stevens, I cannot say, as I do not know how the chromosomes

paired at synapsis. These authors believed that an end to end pairing took place, but so far as I can see they have no convincing evidence for such a conclusion. Much of our work on the question of the reductional and equational divisions is worse than worthless, because of the fact that writers have not considered the question of synapsis. It has been shown by Wilson and others that one pair of chromosomes may divide one way while another pair is dividing another way. How then, can we be sure of reductional and equational divisions without having traced the stages in synapsis and in the formation and division of the spermatocyte chromosomes? A second chromosome is shown in plate 1, figure 25, and the various stages of bending into a ring in figures 26 to 30. It is interesting that the rod and the ring in this case show no indication of the future division. A third chromosome in which I have given a more complete series of stages of bending is shown in plate 2, figure 1. The stages of transformation are given in figures 2 to 10. Later stages in the transformation of this ring could not be followed. A fourth chromosome which is rather remarkable in having an identification mark is shown in plate 2, figure 19. This is the large chromosome in this figure and its peculiar characteristic is the presence of two more or less oval bodies at the division point. In the prophases these bodies usually stain more intensely than the rest of the chromosome. Just what they mean, if anything, I do not know. In late stages of condensation they are lost to view. By means of these bodies, which are present in this chromosome and so far as I have been able to make out in no others, the chromosome can be easily followed in its transformation. Sometimes it is joined end to end with another chromosome (plate 2 figure 20). Another interesting fact in connection with this chromosome is that it may form a typical ring by bending (plate 2, figs. 21-25) or it may occasionally condense into a bivalent without passing through the ring stage (plate 2, figures 26 and 27). Three small chromosomes are recognizable in the late prophases. These are shown in plate 2, figure 11. They also may form a ring (12 and 13) or may condense into the bivalent without ring formation (14 and 15).

These facts are somewhat contrary to the view expressed by Baumgartner ('04) for *Gryllus*. In several species of this genus he describes each chromosome as having a definite shape and definite method of condensation into the spermatocyte chromosome. In *Forficula*, as shown above, some of the chromosomes may or may not pass through the ring stage. The rule however, seems to be the ring formation. Baumgartner brings his facts to the support of the hypothesis of the genetic continuity of the chromosomes, and I think rightly so. I do not consider the fact that a chromosome may or may not pass through the ring stage as evidence against such an hypothesis. The end result is the same in either case and I do not see how an occasional variation by the omission of a bend in the chromosome can be used as evidence against the hypothesis. No such rigid demands are made of any other hypothesis and certainly we have no laws of development or behavior without their occasional variations. If variations are found in what may be considered laws of development why is such rigidity demanded of a working hypothesis?

DISCUSSION

We have seen that the chromosomal variations in *Forficula* are different from any which have been previously described, and they can be explained without the assumption that *Forficula* is a composite species made up of several smaller ones. For the discussion, let us leave out of account the spermatogonial variations described in specimen 34-2, as they may be pathological. Restricting ourselves then to the spermatocyte divisions, we find not only variations in number, but also that some of the chromosomes behave irregularly in these divisions. The variations in number, as I have shown, are caused by the failure of some of the spermatogonial chromosomes to pair at synapsis, and hence such chromosomes appear in the spermatocyte divisions as single or univalent instead of bivalent chromosomes. From what we know of the behavior of chromosomes, we should expect these univalent ones to divide in one of the two maturation divisions and not in the other. In *Forficula* the behavior of these chromo-

somes is so irregular that I cannot say whether this rule is followed. Lagging chromosomes are seen in both divisions. In the first they may or may not divide. In the second they become very much elongated and while I have not seen them dividing, Zweiger ('06) and Stevens ('10) described lagging chromosomes in the second division which do divide. In some cases these lagging chromosomes become so drawn out that it seems doubtful whether they ever reach either pole of the spindle. Perhaps a failure to conjugate in synapsis is understandable, but the reason for the irregular behavior of the single chromosomes is not so clear. The only case approaching these irregularities is found in the supernumerary chromosomes of *Metapodius* and *Diabrotica*, but here such chromosomes are supposed to be functionless and on the road to degeneration.

I do not wish to draw any conclusions from the above observations, as the amount of material studied is too small. It might be well, however, to point out the principal questions which need further study. First, do the spermatids which receive 11, 12, 13 and 14 chromosomes become functional spermatozoa? Secondly, if so, do we find variations in the spermatogonial and oogonial number among different individuals of the species? Thirdly, if the spermatogonial and oogonial number is kept constant, what is the means of regulation? One other important problem which may be solvable in this material deserves mention. For some time it has been a disputed question whether the longitudinal split which appears in the chromatin thread following synapsis, is due to separation of maternal and paternal elements which have previously conjugated side by side, or whether it is a longitudinal splitting of chromosomes which have united end to end. In *Forficula* some of the chromosomes remain single and, so far as I have been able to make out, behave during the growth period as the bivalent autosomes. It seems to me that it may be possible to distinguish these single chromosomes from the double ones, at least in the early prophases of the first division, and if so, a comparison between the presence or absence of a longitudinal split in these single chromosomes with the same in the double chromosomes, would prove of interest and perhaps give us some light on the question of synapsis.

The fact that two methods of ring formation are present in *Forficula* might lead us to suspect that two methods of conjugation may be present or that we have some of the spermatocyte chromosomes dividing reductionally in one division and some in another. However, I cannot say definitely whether such is the case, as in ring formation by the opening up of the two parts of a longitudinally split thread, it is impossible to say exactly where the division occurs in the ring. Presumably it occurs where the ends of the double thread remain attached, but this is uncertain since all indications of this point of juncture are lost in later stages of condensation (plate 1, figures 8 to 11). Also it is impossible to say whether the cross division in such figures as shown in plate 1, figure 18, is reductional or equational, since it is not clear whether the chromosomes have conjugated end to end or side by side.

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PLATE I

EXPLANATION OF FIGURES

- 1 Nucleus in growth period.
- 2 to 11 Stages in the opening up of a longitudinally split thread to form a ring.
- 12 to 17 Shows twisting and bending in opening to form a ring.
- 18 to 24 Different stages of ring formation in the same chromosome by bending and the condensation of this ring into a bivalent chromosome.
- 25 to 30 Ring formation by bending in another chromosome.

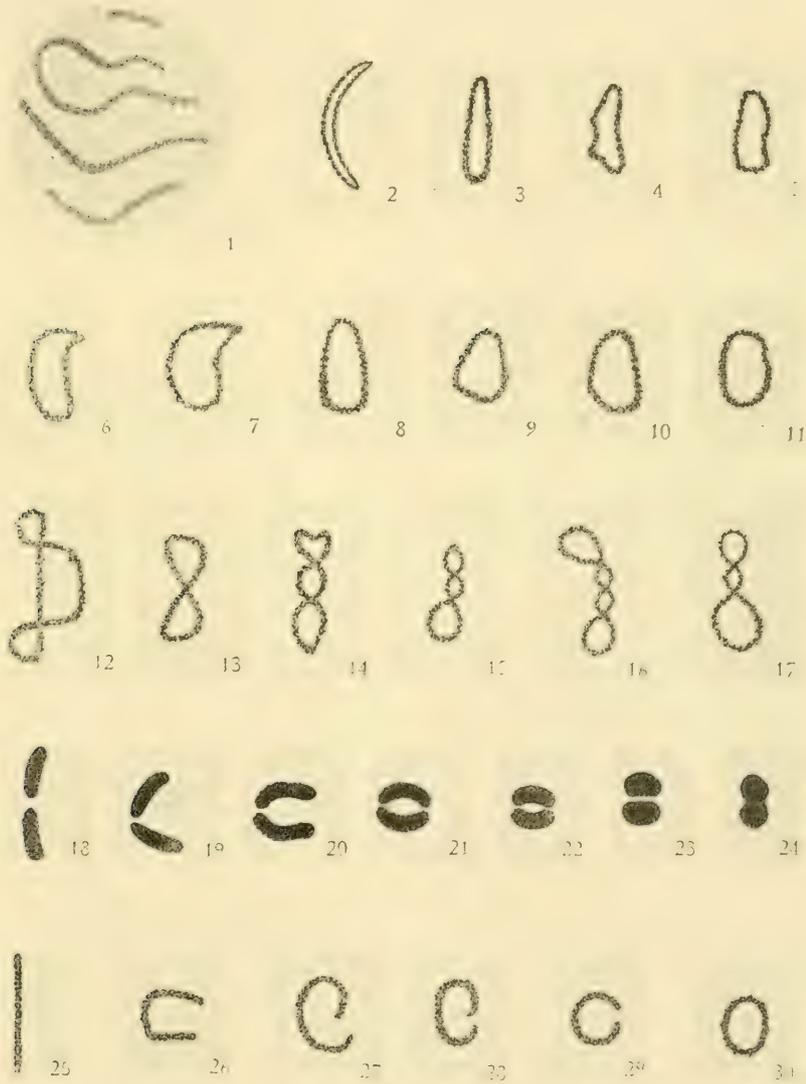
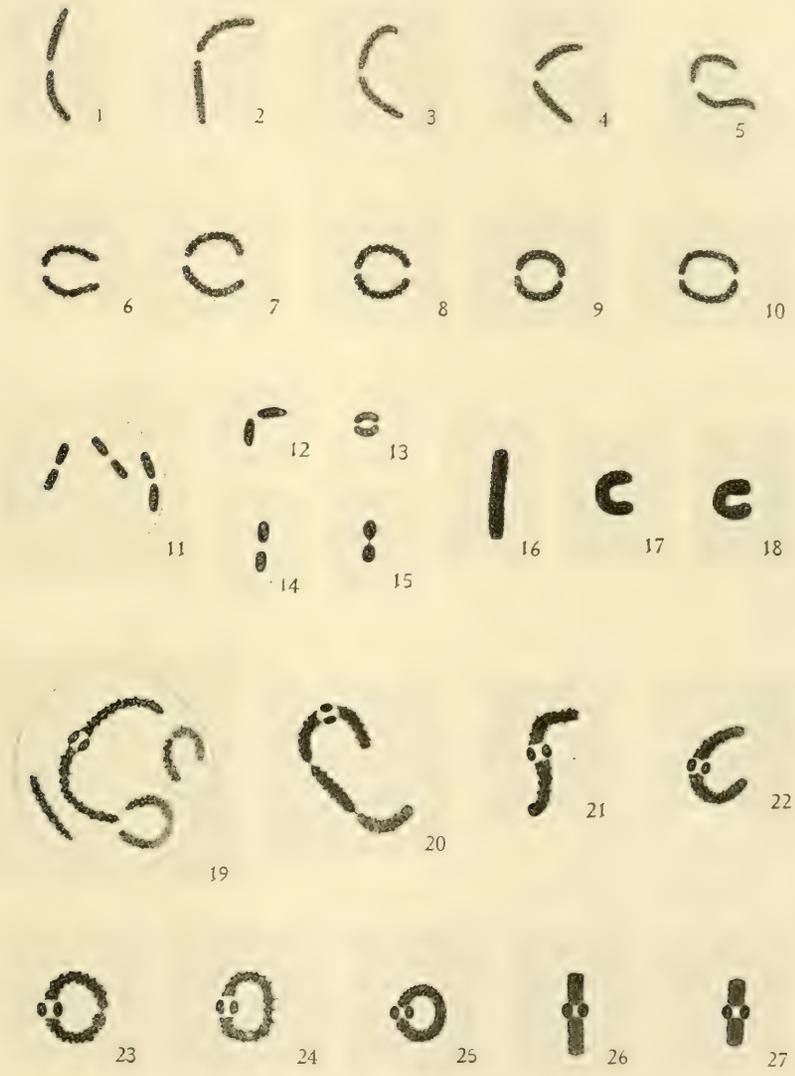


PLATE 2

EXPLANATION OF FIGURES

- 1 to 10 Successive stages of ring formation by bending in a third chromosome.
- 11 The three small chromosomes which can be recognized in later prophases of the first division.
- 12 to 13 Two stages of ring formation in the small chromosomes shown in 11.
- 14 to 15 Two stages in chromosome formation in these same small chromosomes without ring formation.
- 16 to 18 Stages of ring formation by bending in another chromosome.
- 19 Early prophase of the first spermatocyte division showing the presence of an elongated chromosome in which are two oval bodies. These serve as recognition marks.
- 20 to 24 Stages of ring formation in the chromosome shown in 19. In 20 it is joined end to end to another chromosome.
- 26 to 27 Two stages showing that the above chromosome (19) may condense into a bivalent without passing through the ring stage.



SPERMATOGENESIS OF THE AMERICAN CRAYFISH, CAMBARUS VIRILIS AND CAMBARUS IMMUNIS (?), WITH SPECIAL REFERENCE TO SYNAPSIS AND THE CHROMATOID BODIES

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ONE TEXT FIGURE AND TEN PLATES

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MATERIAL AND METHODS

The following studies were made upon the testes of two species of crayfish, *Cambarus virilis* and *Cambarus immunis* (?). *Cambarus virilis* was obtained in great abundance during the spring and summer of 1912 and 1913. This species is very common in the region about Madison, Wisconsin, and to it the writer devoted

most of his time. The other species, probably *Cambarus immunis*, was used for comparison, the slides having been prepared by Mr. Powers of Lincoln, Nebraska.

The testes of *Cambarus virilis* were fixed in various fluids, such as: (1) Bouin's fluid; (2) Carnoy's fluid; (3) Carnoy's and Lebrun's fluid; (4) Five per cent corrosive-acetic mixture; (5) Flemming's strong fluid; (6) Gilson's fluid; (7) Hermann's fluid; (8) Meves-Duesberg modified Flemming fixative; and (9) Zenker's fluid.

The best results were obtained with fluids (7) and (8). Zenker's fluid also gave fairly good results for fixing the gross structure of the cell, but it was not valuable for the study of details. The Hermann and Meves-Duesberg fluids were the most valuable for the structural study of the nucleus and the cytoplasm. The chromatoid bodies in the cytoplasm were best fixed by these reagents. Both these fluids, especially that of Meves-Duesberg, altered the cell configuration but slightly. The Hermann fixative was particularly valuable for the study of synapsis and for making chromosome counts.

Sections were cut from 4 to 7μ in thickness and stained in either of the following ways:

(1) Delafield's hematoxylin and eosin; (2) Heidenhain's iron-hematoxylin and acid fuchsin or Orange G; (3) Safranin with gentian violet, lichtgrün, or lichtblau; and (4) Sulfoalizarin-sauren natron and krystalviolet.

The Meves-Duesberg method gave the most beautiful results, and was perhaps the method of greatest importance for the study of the chromatoid bodies and the cytoplasmic granules (mitochondrial granules?). The essential steps in this method are as follows:

1. Material is fixed for twenty-four hours in a mixture containing, (a) 15 cc. of 1 per cent chromic acid, (b) 4 cc. of 2 per cent osmic acid, and (c) 3 drops of glacial acetic acid.

2. Wash for one hour in water and then for twenty-four hours in a mixture containing 100 cc. pyroligneous acid and 1 gram chromic acid.

3. Continue washing for twenty-four hours in 2 grams potassium bichromate dissolved in 100 cc. water.

*4. Then wash for twenty-four hours in water and run up through alcohols to xylol. Infiltrate with paraffin and imbed.

5. Cut sections 5μ thick, mount on slides and then run down to distilled water. Place into a 4 per cent iron-alum solution (at room temperature) for twenty-four hours.

6. Wash thoroughly in water until all trace of the iron-alum has been removed and then transfer to a solution of sulfoalizarinsäuren natron (made by taking one part of a saturated aqueous solution of the stain to from 80 to 100 parts of distilled water) for twenty-four hours.

7. Rinse in distilled water and place slide into a krystalviolet-anilin water solution [consisting of equal parts of a 3 per cent alcoholic solution of krystalviolet (3 grams to 100 cc. 95 per cent alcohol), and anilin water], and warm until the solution steams, keeping it here for about three minutes.

8. Wash in distilled water and transfer to 30 per cent acetic acid for one or two minutes. Then rinse in distilled water which removes the stain very rapidly. Continue washing in running water from five to ten minutes so as to remove every trace of acid. The alizarin stain appears reddish.

9. Dry the slide with filter paper and dip for a moment into absolute alcohol. Next place into bergamot oil until cleared, and thence into xylol. Then mount in Canada-balsam.

By this method the chromatic or nuclear elements are stained a beautiful purple with the krystalviolet, while the cytoplasm is stained a light reddish color with the sulfoalizarinsäuren natron.

Living testicular cells were also studied under the oil immersion. This was accomplished by teasing out the cells in Ringer's solution and preparing hanging drop cultures of the same. Methylin blue and lichtgrün were used as intravital stains for studying the details of these live cells.

Smear preparations were also made. They were fixed in either Bouin's, Carnoy's or Gilson's fluids and stained with Heidenhain's iron-hematoxylin and acid fuchsin. Bouin's fluid

gave the best result for smears, the cells undergoing almost no transformations in size and general appearance.

This problem was undertaken during the fall of 1911 under the direction of Prof. Michael F. Guyer, to whom the writer is much indebted for kindly help and criticism.

LITERATURE ON THE CYTOLOGY OF THE DECAPOD CRUSTACEA

Considerable has been written on the cytology of the Decapoda, especially on the genesis of the spermatozoa. This is due to the fact that the sperm of this group of Crustacea is so different in shape and form from those found in other animals, belonging to the type commonly known under the name of 'radial' spermatozoon.

Grobben ('78), was the first investigator to make a microscopical study of the sex organs of the decapod Crustacea. He worked on *Alpheus ruber*, *Palaemon rectirostris*, *Virbius viridis*, *Athanas nitescens*, *Astacus fluviatilis*, *Homarus vulgaris*, *Galathea squamifera*, *Eupagurus prideauxii*, *Eupagurus meticulosus*, *Pagurites maculatus*, *Palinurus vulgaris*, *Gebia littoralis*, *Calliaxis adriatica*, *Porcellana platycheles*, *Porcellana longicornis*, *Ethusa mascarone*, *Dromia vulgaris*, *Ilia nucleus*, *Stenorhynchus phalangium*, *Inachus thoracicus*, *Maja squinado*, *Lambrus angulifrons*, *Eurynome aspera*, *Pilumnus hirtellus*, *Portunus depurator*, *Carcinus maenas*, *Pinnotheres veterum* and *Pachygrapsus marmoratus*.

Grobben gives an excellent review of the earlier literature. From his review it is evident that earlier investigators had dealt only superficially with the sex organs of the decapods. He then describes in detail: (1) the structure of the testis, together with its position and form in the various species; (2) the origin of the spermatogonia, and (3) the development and structure of the spermatozoon. He dwells particularly on the origin of the spermatogonia. Upon examination of the tubules of the testis during the early stages of proliferation one finds two types of cells: (a) spermatogonia, containing large, round nuclei that are surrounded by a distinct mass of cytoplasm, and (b) irreg-

ular nuclei which are imbedded in a syncytial mass of protoplasm. These latter are the nurse cells, from which the spermatogonia are derived, the nurse cells increasing in size, becoming spherical in outline and then being surrounded by a distinct mass of cytoplasm. Grobben says:

Wir finden also dass zwischen Spermatoblast (English, spermatogonia) und Ersatzkeim (English, replacement cell) ebensowenig Unterschied besteht, wie zwischen Eizelle und Follikelzelle und die ausserordentliche Aenlichkeit beider Bildungen muss die schon so oft vorgebrachte Ansicht von der Homologie des Hodens und des Ovariums sehr unterstützen. Jeder Ersatzkeim ist potentia ein Spermatoblast, jede Follikelzelle potentia ein Ei. Es entstehen die Spermatoblasten durch Umwandlung der Ersatzkeime wie die Eier durch Umwandlung aus den Follikelzellen bilden.

In 1906, Grobben published a short article on the sperm of *Pandalus narwal*, *Pasiphaea sivado*, *Nephrops norvegicus*, *Xantho rivulosus*, *Homola spinifrons*, *Portunus corrugatus*, *Pagurus calidus*, *Pisa* and *Scyllarus arctus*. The spermatozoa of these species are very briefly described, and then their resemblances are traced with a view toward a systematic classification of the forms; (similar to Koltzoff's work).

Sabatier ('85, '93) studied the germ cells of many Crustacea including such forms as *Astacus fluviatilis*, *Pagurus striatus*, *Pagurus calidus*, *Pagurus angulatus*, *Paguristes maculatus*, *Eupagurus lucasi*, *Diogenes varians*, *Carcinus maenas*, *Maja verrucosa*, *Maja squinado*, *Palinurus vulgaris*, *Homarus vulgaris*, *Scyllarus arctus*, *Inachus scorpio*, *Stenorhynchus phalangium*, *Dromia vulgaris*, *Corystes dentatus*, *Palemon serratus*, *Palemon treillanus*, *Crangon*, etc. The species most studied were *Astacus fluviatilis* and *Pagurus striatus*.

Special attention was paid to the origin of the spermatogonia. The spermatogonia or the 'protospermatoblastes' as they are called by Sabatier, are, according to his account, derived from the replacement layer of cells found in the tubules of the testis, and these, in turn, are derived from the connective tissue layer that forms the skeleton of the tubule. During its early condition of development, the tubule presents the form of a hollow cylinder, whose walls are formed of a layer of connective tissue and some

special cells. Near the periphery of the tubule this connective tissue presents the appearance of a meshwork, with spaces of various dimensions.

The special cells are, on the one hand, *internal*, situated towards the center of the tubule below the connective tissue layer, and on the other hand, *intra-parietal*, situated in the spaces of the connective tissue layer toward the periphery. The former elements (internal) form a circular layer, a sort of epithelium within the tubule, while the latter (intra-parietal) elements remain scattered throughout the lacunae of the connective tissue. These cellular elements, he asserts, are but two forms of the same substance; both appear almost similar, multiply by amitosis, and give rise ultimately to replacement cells.

The replacement cells consist of large nuclei situated within a common mass of protoplasm. They grow rapidly, become round in outline and are soon surrounded by a distinct mass of protoplasm, thus forming the spermatogonia (protospermatoblasts). These then go to form the spermatocytes, or the so-called 'deutospermatoblastes,' which in turn given origin to the spermatozoa.

Gilson ('86) made a comparative study of the spermatogenesis of *Astacus fluviatilis*, *Homarus vulgaris*, *Pagurus calidus*, *Pagurus striatus*, *Eupagurus prideauxii*, *Calibanarius misanthropus*, *Paguristes maculatus*, *Galathea strigosa*, *Maja verrucosa*, *Xantha rivulosus*, *Carcinus maenas*, *Inachus scorpio*, *Stenorhynchus phalangium*, *Acanthonyx lunulatus*, *Dromia vulgaris*, *Dorippe lanata* and *Ethusa mascarone*.

In the main, Gilson confined himself to the development of the spermatogonia and to the transformations undergone by the spermatids in developing into the spermatozoa. His results are as follows:

1. *Spermatogonial development.* At certain periods, the tubules of the testicular cavity are found to be lined with a mass of undivided protoplasm, which is studded with numerous scattered nuclei containing clumps of fragmented chromatin. These nuclei undergo direct division throughout most of the year. But when proliferation commences, the homogeneous protoplas-

mic mass fragments, thereby becoming individualized around many of these scattered nuclei, thus forming 'mother cells' (métrocytes) from which all of the elements in the spermatogenesis originate during the following season of reproduction.

According to his account the nuclei of these primary mother cells (first métrocytes) sooner or later reconstruct the chromatin into a spireme. During this process they are often seen to divide amitotically. When the spireme is completely formed, division by mitosis makes its appearance. From now on active proliferation takes place as is shown by the migration of numerous 'mother cells' (métrocytes) from the primitive protoplasmic syncytium lining the walls of the tubules, into the interior of these cavities.

The mother cells (métrocytes) multiply solely by direct division (la segmentation binaire), and give rise successively to smaller and smaller cells. The spermatid cells, with the exception of the spermatozoa, are the smallest in the testis.

2. *Transformations of the spermatid.* Gilson has given a correct genesis of the transformations occurring in the spermatid to form the spermatozoon. Within the cytoplasm of the spermatid a vacuole is formed which transforms into a hyaline vesicle. This presents variations in shape and development in the different species. The rest of the cytoplasm goes to form the prolonged radiating arms, whose number, position and form vary in the different species studied.

The nucleus changes its spherical shape and migrates to the pole opposite the vacuole. It may assume various forms, such as a disc, a concavo-convex lens, a slender rod-like form, etc. The contents of the nucleus becomes modified, losing its staining power and appears homogeneous in aspect. A central body (tigelle), was also observed in the mature spermatozoon.

Herrmann ('09) has given a meager description of the testicular elements of *Astacus fluviatilis*, *Maja squinado*, *Eupagurus bernhardus*, *Homarus vulgaris*, *Galathea strigosa*, and *Crangon vulgaris*. Most of his time was devoted to a description of the development of the spermatozoa in *Astacus*. Herrmann, like Grobben, derives the spermatogonia from the nutritive cells.

St. George ('92) found an interesting case of hermaphroditism in the testis of *Astacus fluviatilis* Fabr., which he collected during the months of July and August. The testis of *Astacus fluviatilis*, as already described by Huxley, consists of an innumerable number of spheroidal vesicles, the so-called acini, which are attached like grapes to the ends of short stalks, formed by the ultimate ramifications of the vasa deferentia. The cavity of each vesicle is at first filled with large nucleated cells near the walls, the spermatogonia, which, during the period of active proliferation in July undergo rapid development, so that the testis grades from spermatogonia in the upper region to spermatozoa in the lower extremity near the vas deferens. Generally, each acinus or vesicle bears cells in the same stage of proliferation.

Nutritive cells are also to be found which stain heavily and lie in a syncytium of protoplasm near the spermatogonial cells. They are also found scattered promiscuously among the other cells of the testis.

Interspersed between the spermatogonia, St. George found very large cells containing yolk material. These were egg cells, and he regards them as having originated from primordial germ cells which have forsaken their normal path of spermatogenesis, thus growing enormously in size and forming oogonia. St. George also discusses the origin of the nutritive cells, and states that these are derived from the spermatogonial cells.

Brandes ('97) studied the spermatozoa of *Galathea strigosa* and found that the nucleus contained two substances, (1) an erythrophyllic substance and (2) a cyanophyllic substance. The latter stains blue with methylin blue, while the other (erythrophyllic), forms a plate within the cytoplasm and stains red with acid fuchsin.

Mrázek ('01) described interesting abnormalities in the mitotic divisions of the testicular cells of *Astacus*. Tripolar and even eight-polar spindles were found to occur. In normal mitotic division ninety-six chromosomes were found distributed over the whole equatorial plate of the spindle.

Prowazek ('02 a) briefly discusses the formation of tetrads in the rhinoceros beetle, in *Astacus* and a land snail. In *Astacus* the resting stage of the nucleus shows a finely granular structure. Soon the chromatin aggregates into irregular islands, and from these the chromosomal threads make their appearance. These threads join one behind the other, in pairs, giving rise to the double or paired chromosomes, and appear snake-like or S-shaped. (It becomes evident from this that Prowazek regards the chromosomes in this form to be joined end to end or telosynaptically). The paired chromosomes now thicken and a longitudinal split occurs, dividing the paired threads into four, from which the tetrads arise.

In another article, Prowazek ('02 b) traced the spermatogenesis of *Astacus fluviatilis* through the maturation divisions, laying particular emphasis on the transformations undergone by the mitochondria through these stages. During July the nurse cells in the tubules were found to divide amitotically, and right next to them, primordial spermatogonial, as well as oogonial cells could be found. Within the nucleus of the spermatogonia, U-shaped chromatin rods were seen, their free ends facing towards the nuclear wall, while inside the cytoplasm an idiozome and a dense mass of mitochondria, lying off at one pole, could be distinguished.

The oogonial cells were very much larger than the spermatogonial cells. They also contained denser zones of cytoplasm surrounding the nucleus, as well as along the periphery of the cell wall. These oogonial cells soon degenerate, while the spermatogonia undergo development and give rise successively to the primary and secondary spermatocytes.

Between the first and second maturation divisions, no distinct resting stage was found. During these divisions, the mitochondrial mass becomes scattered through the spindle fibers, and, when cell division occurs, equal portions of it are given to each of the resulting two cells.

Labbé ('03) in a preliminary paper, discusses the spermatogenesis of *Homarus*, *Palinurus*, *Galathea*, *Eupagurus*, *Porcellana*, *Maja*, *Stenorhynchus*, *Inachus*, *Carcinus*, *Cancer*, etc. In these

forms the maturation divisions follow each other very rapidly, but are preceded by a long period of synapsis, in which the chromatin filament uncoils and drifts to one pole, where it condenses. The tetrads originate by two longitudinal splits. During the first division, the nucleolus is liberated into the cytoplasm and persists there until the spermatid stage. The centrosomes are not visible at the poles of the spindles. Labbé next takes up the transformation of the spermatid into the spermatozoon. According to him, this occurs in the following way:

1. In the cytoplasm of the spermatid a vesicle makes its appearance opposite the nucleus. This grows and soon presses against the nucleus. Two openings are found in the vesicle, one distal and the other proximal. The latter rests against the nucleus. The shape assumed by the vesicle varies with the species. It may be spherical, cup-like, cylindrical or irregular in appearance.

2. The cytoplasm proper is transformed into radiating arms, leaving from behind the nucleus.

3. The cellular membrane of the spermatid persists as an outer vesicle.

4. Mitochondrial masses persist in the internal vesicle.

5. Between the nucleus and the internal vesicle a central body makes its appearance, and this rises somewhat into the canal of the internal vesicle. It stains more heavily than the chromatin elements.

Labbé regards the internal vesicle as nutritive in its function because of the accumulation of albuminous matter within it. He also regards osmotic phenomena as important in the operation of the outer vesicle.

Labbé ('04 a) contributed another short paper on the process of spermatogenesis, and especially the tetrad formation in the lobster. After the last spermatogonial division the nucleus contains a coiling spireme and one or two nucleoli. During synapsis, the spireme superficially splits longitudinally and then fragments into segments, each corresponding to one or more chromosomes. Now the longitudinal split becomes effaced and the chromatin threads thicken, and then unite in pairs. The

double filaments resulting from this union correspond in some cases to two chromosomes, in others to more chromosomes.

Synapsis occurs in the forms of V's, parallel threads, rings, spindles, crosses, twisted X's, etc. The figures that are most common are spindle-like in which two chromosomes are united at their two extremities and spread apart in the center. These figures are called 'protetrads.' All in all Labbé found eighteen protetrads. These form tetrads by becoming separated into four large densely staining granules united by linin threads. The eighteen tetrads therefore represent thirty-six chromosomes.

The tetrads assume an equatorial position during the first maturation division. In the metaphase stage of this division each chromatin granule of the tetrad shows indications of a division perpendicular to the plane of the equator. Labbé does not regard this as a trace of the superficial division which occurred in the spireme previous to tetrad formation, but regards it as a second longitudinal division preparatory to the second maturation stage.

In the second maturation division the dyads arrange themselves in the equatorial plane of the spindle with their longitudinal axes perpendicular to the equatorial plane. Since each granule is divided into two, these dyads resemble microtetrads. In the anaphase stage, the chromosomes are separated equatorially and the enclosing nuclei of the telophase stage, therefore, contain chromosomes that are microdyads, each microdyad representing one-half of a chromosome.

During the first maturation division, a chromatin nucleolus is found within each cell, but in the second spermatocyte division this does not seem to divide, but passes over entirely to one of the spermatids. Therefore one-half of the spermatids contain this nucleolus while the others are without it.

In the same year ('04 b) Labbé also published another paper in which he deals at greater length with the transformations of the spermatozoa of *Homarus vulgaris* and *Maja squinado*.

Keppen ('06), in a Russian paper on the spermatogenesis of *Astacus fluviatilis*, lays special emphasis on the relation of the spermatogonia and nutritive cells to the germinal epithelium.

He finds that when the early histology and embryology of the testis are studied, three types of cells can be discerned, (1) nutritive cells, (2) spermatogonial cells, and (3) cells which are intermediate between these two. From a study of these cells Keppen concludes that they have a common origin, being derived from the germinal epithelium of the testis.

Nutritive cells may often be derived from spermatogonial cells by a fragmentation of the nucleus and a disintegration of the nuclear wall. The nutritive cells then lie within a syncytial mass of testicular protoplasm. All nutritive cells divide amitotically.

Koltzoff ('06) produced a long paper in which he discusses the sperm of *Galthea squamifera*, *Pagurus striatus*, *Homarus vulgaris* and *Portunus corrugatus*. His paper contains three portions dealing with, (1) the general structure of the spermatozoon, (2) the biophysics of the structures in the sperm, and (3) the physiology of the spermatozoon.

(1) General structure of spermatozoon. The sperm is similar to that found in other decapods. There is a capsule or a tail piece derived from the cytoplasm, and a middle piece produced from the mitochondria of the cytoplasm and giving rise to the flagellated arms of the sperm. The nucleus forms the head piece of the spermatozoon. A central body, derived from the centrosome, is also present.

After going fully into the details of the various parts of the spermatozoon, Koltzoff then tries to show how, upon a cytological basis, a classification of the decapods may be attempted, and actually gives such a classification.

(2) Biophysics of the spermatozoon. Here Koltzoff discusses the action of various salts on the spermatozoon. By the use of solutions of K, Na, Mg, etc., the opening of the sperm could be carefully studied, especially the explosion of the tail vesicle. Koltzoff regards this explosion as due to osmotic changes going on within the spermatozoon.

(3) Physiology of the spermatozoon. In this section the process of fertilization is discussed. When the spermatozoon comes in contact with the egg it opens up and the flagellated

arms adhere to the ovum. Then the explosion of the capsule occurs, thus tending to drive the nucleus through the chorion into the egg. Koltzoff observed only a single case of such fertilization.

Spitschakoff ('09) worked on the spermatozoa of the Caridea, mainly on the species *Leander adpersus* Rath., and *L. squilla*. He goes fully into the transformations undergone by the spermatid to form the spermatozoon and finds that they are similar in many respects to those of other decapods. Spitschakoff also studied the fertilization process in these forms and believes, with Koltzoff, that, during fertilization, the nucleus of the sperm is driven through the chorion of the egg by means of the changes undergone in the vesicle of the spermatozoon.

Retzius ('09), worked on the spermatozoa of *Palaemon squilla*, *Crangon vulgaris*, *Hippolyte*, *Nephrops norvegicus*, *Homarus vulgaris*, *Astacus fluviatilis*, *Calocaris macandreae*, *Eupagurus bernhardus*, *Galathea squamifera*, *Porcellana longicornis*, *Lithodes maja*, *Stenorhynchus rostratus*, *Hyas coarctatus*, *H. araneus*, *Eurynome aspera*, *Portunus depurator*, *P. pusillus*, *Ebalia tumefacta*, *Carcinus maenas* and *Cancer pagurus*.

In general, the structure of the sperm of these forms is about the same, and its genesis is similar to that described by Grobben, Gilson, and Koltzoff. The head piece is derived from the nucleus and it may be round, hemispherical, lens-shaped, or of another form. The vesicle, the so-called 'Schwanzkapsel' of the German investigators, assumes various forms, being rod-like, needle-like, spiral-like, cone-like, spherical, hemispherical or lens-like in its shape. Within this vesicle an axial tube is always discerned, and this contains numerous structures, among which is the central body. Also a *nebenkern* organ is often found which sometimes produces the radial arms, and may assume other forms.

Binford ('13) described the male germ cells and the process of fertilization in the sea-crab *Menippe mercenaria*. He briefly touches on the maturation divisions, but describes the transformations of the spermatozoon rather fully.

In the testis a single row of cells persists along one side of the tubule and these give rise successively to batches of spermatozoa.

The spermatogonia divide without the formation of a spireme, and the maturation divisions follow one another quickly. Binford states that there is a long period of synapsis, but does not go into any of the details of this important process.

During the transformations of the spermatid the nucleus becomes reduced in size and uniform in consistency. It becomes oriented to one end of the cell, while at the opposite end a clear vacuole appears in the cytoplasm, which gives rise to the capsule of the spermatozoon. The flagella-like arms arise from a mitochondrial mass, which becomes segregated from the cytoplasm and is deposited as a ring between the nucleus and the capsule. The central body lying in the center of the capsule arises from a granule (probably the centrosome) on the proximal side of the capsule. At the distal end of the central body a vesicle arises which soon changes into an inner tubule.

In hypotonic solutions of various salts the central body becomes lengthened and there occurs an eversion of the inner tubule, with the result that it is turned inside out. Binford believes that this is the force that tends to drive the spermatozoon into the egg, for he found that when the sperm comes in contact with the egg, under normal conditions, such an eversion occurred. The capsule of the spermatozoon is applied to the shell of the egg, and the everted inner tubule and the capsule with its contents are then driven through the covering into the interior of the egg. The nuclear cap remains behind and does not penetrate the egg at all.

To bring this method of fertilization in line with the chromosomal basis of heredity, he postulates that the contents of the capsule may be derived from the nucleus of the spermatid, and is probably oxychromatin, which deposits basichromatin after it enters the egg and so gives rise to the chromosomes in the male pronucleus.

Reinhard ('13) studied the development of the spermatozoon in *Astacus leptodactylus* and found that the head consisted mainly of the nucleus; the middle piece, or the flagellated arms resulted from the mitochondria, while the tail vesicle arose from the rest of the cytoplasm.

He says that although the spermatozoa of different species of *Astacus* have been studied, a comparison of them soon shows that they are dissimilar in appearance. Reinhard believes that these differences are due to the different methods of fixation and staining employed by the various investigators.

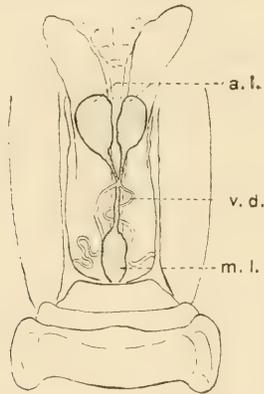
In *Astacus leptodactylus* there are from eight to twenty flagellated arms. The tail vesicle is round or barrel-shaped in appearance and has openings at the proximal and distal ends. The proximal opening leads into a secondary depression of the nucleus, where the central body lies. Here the central body presents the appearance of a flat band instead of a vertical rod so common in other decapods. The walls of the vesicle are rather thick and, when viewed from the side, they appear like two concavo-convex lenses. It consists of chitin as was shown when the spermatozoa were treated with cold or hot KOH. No explosion of the vesicle was observed when the spermatozoon was treated with concentrations of NaCl and other solutions for many hours.

THE TESTIS OF CAMBARUS VIRILIS

1. *Description of the testis*

The testis of *Cambarus virilis* (text fig. A), lies in the thoracic region, directly below the cardiac chamber. It is a trilobed organ, having two large oval lobes in the anterior portion of the thorax (text fig. A, *a.l.*), while the third (*m.l.*), is situated posteriorly in the median axis of the crayfish's body. Generally the third lobe is approximately of the same shape and dimension as the other two. Anteriorly this third testicular lobe becomes thinned into a slender tubular portion, which runs forward and joins with two similar slender tubules originating at the posterior ends of the anterior lobes. Where these three slender tubules join, the two vasa deferentia (text fig. A, *v.d.*) have their origin, one on either side of the testis. Each vas deferens runs along posteriorly within the thoracic cavity and, after coiling profusely, finally opens at the base of the last pair of walking legs.

Text figure A shows a dorsal view of the testis during the month of July, the period when the most active proliferation occurs on the part of the cells. From this drawing, as well as that of Miss Steele in her paper on the crayfish of Missouri ('02) it is evident that the testis of *Cambarus virilis* differs considerably in shape from the testis of *Astacus fluviatilis* as pictured by Huxley. Structurally they are very similar, being made up of a great number of vesicles or tubules called acini, which are connected by short branches of the ultimate ramifications of the



Text figure A Dorsal view of testis of *Cambarus virilis*; *a. l.*, anterior lobes; *m. l.*, median lobe; *v. d.*, vas deferens.

vas deferens. The testicular lobes thus present the appearance of bunches of grapes, the grapes representing the tubules or acini, and the stalks, the branching ramifications of the vas deferens.

2. Variations in the testis throughout the year

The testis of *Cambarus virilis* was studied monthly and considerable variation was found to exist in its size as well as in the state of the germ cells. During April and May the testis is comparatively small and most of the tubules are either empty or filled with mature spermatozoa, early spermatogonial stages, nurse cells, or germinal epithelium cells. In June, the testis

grows to its largest dimensions. For the study of the various states in spermatogenesis, the testicular lobes are in the best conditions of development from the middle of June to the middle of July. During the latter part of July and in August they go to sperm. Testes secured during the latter part of August and in September showed very few of the earlier stages in the spermatogenesis, nearly all of their tubules were filled with either spermatogonia, spermatids or spermatozoa. In October the tubules are charged with myriads of ripe spermatozoa (fig. 78) and the testis has decreased considerably in size.

Copulation often occurs during the months of September and October and then the sperm of the male is discharged through the vas deferens and stocked in the spermatheca of the female. During the latter part of October, the species goes into hibernation and then the testis undergoes little change, remaining practically dormant until the following spring, when favorable weather brings the animals from their burrows. In the early spring another period of copulation may ensue lasting through April and part of May. After this the testis begins rapid growth. The following is a tabulated list of the months of the year and the corresponding condition of the testis during that period.

TABLE 1

	MONTH	CONDITION OF TESTIS
Copulation occurs	April	Testis small and contains germinal epithelial cells, nutritive cells, spermatogonial stages and ripe spermatozoa
	May	
	June	Testis commences active proliferation and increases in size
	July	Period of greatest activity; all stages in spermatogenesis are obtainable; testis assumes its maximum size
	August	Testis goes to sperm; size remains practically the same as in July
Copulation occurs	September	Testis decreases in size and the tubules become charged with ripe spermatozoa
	October	Tubules are almost entirely changed to spermatozoa; testis has become very small
	November to March	Hibernation period; no change in testis from October condition

3. Zones of proliferation in the testis

An examination of the testis during the period of most active proliferation (July), reveals every stage in the spermatogenesis in each of the three lobes comprising the testis. Proliferation always begins along the blind end of each of the lobes, and from this zone the various stages in the spermatogenesis follow each other in more or less regular succession. First come the spermatogonial stages, then the growth periods, next the maturation divisions and finally the ripening of the spermatozoa. The cells of each tubule are generally in the same stages of development. Figure 79 shows a cross-section through many of the tubules.

SPERMATOGENESIS

The following description is devoted mainly to the spermatogenesis of *Cambarus virilis*. The spermatogenesis of another species of *Cambarus* was also studied and the main stages were found to be similar to those in *Cambarus virilis*. This second species could not be determined with accuracy, for only prepared slides, obtained from Mr. Powers of Lincoln, Nebraska, were available for study. On corresponding with Mr. Powers it was ascertained that the species had not been identified by him at the time the slides were prepared. He supplied me, however, with a few young crayfish taken from the same stream as those from which the slides were made and these were identified as *Cambarus immunis*. Therefore it is highly probable that the sections in question were from that species.

1. Spermatogonial stages

During the period of active proliferation the tubules in the blind ends of the testicular lobes are filled with spermatogonial cells in all stages of development. Many nutritive cells are also found imbedded in a mass of protoplasm representing a syncytium. These last cells (nutritive cells), appear oval, irregular or circular in shape and contain masses of chromatin

distributed throughout the interior (fig. 80). In some cases, the nutritive cells closely resemble the nuclei of the spermatogonia and it appears not at all impossible that the spermatogonia may be derived from the nutritive cells by becoming surrounded with a mass of cytoplasm, as Grobben ('78), and Herrmann ('90) have claimed. In other cases, spermatogonia are seen in which the cytoplasm appears to be disintegrating, thus baring the nucleus and this might lead one to the conclusion of St. George ('92) and Keppen ('06), that some of the spermatogonia do not undergo further development, but degenerate again into nutritive cells.

The investigator cannot assert definitely as to the origin of the nutritive cells and spermatogonia. But he is inclined to the view advocated by Sabatier ('85), and Keppen ('06), that both of these cellular structures originate from the germinal epithelium of the testis, and that some of the nutritive cells may then be derived from a disintegration of the spermatogonial cells.

The germinal epithelium can best be studied in tubules which are filled with ripe spermatozoa. In these it is found lining the walls of the cavity (fig. 78). The component cells are large and stain heavily. They contain huge nuclei which possess a great many irregular masses of chromatin, having strong affinity for the basic dyes. The cytoplasm is more or less consistent throughout, with indications of boundaries here and there. These cells correspond to the 'mother cells' or 'replacement cells' of Sabatier. As the spermatozoa are discharged from the tubule the epithelial cells multiply rapidly, displace the discharged elements and soon give origin to the spermatogonia and nutritive cells.

The division of the germinal epithelium cells strongly suggests amitosis. It is difficult, however, to uphold this with certainty. The earlier investigators on the decapods all claim that these cells undergo such division and the preparations of the writer seem to indicate such a conclusion (fig. 80). But in studies of fixed material there is always a danger of misinterpretation. The only way of accurately determining that such division does

occur in this tissue would be to cultivate it in culture medium and to observe the living cells multiply.

During the prophase of the earliest spermatogonial stage (fig. 1), the nucleus is large, round, and contains two or three huge spherical karyosomes. Thin linin strands, granular in appearance, radiate from these karyosomes to the periphery of the nuclear wall. The cytoplasm has a granular appearance, interspersed with slender fibrillae. Distributed through the cytoplasm are larger, heavier-staining granules (figs. 1-2, *M*), which in many cases become grouped mainly at one pole. These appear to be mitochondrial granules, for with basic dyes they stain almost as heavily as chromatin. Especially good preparations of these structures were obtained through the Meves-Duesberg method. Large chromatoid masses (figs. 1-2, *k*) are also found. The centrosome (figs. 1-2, *C*) can often be seen lying in an open space surrounded by a denser cytoplasmic mass, the idiozome (fig. 2, *I*). Each spermatogonial cell is enclosed by a definite cytoplasmic wall which marks it off distinctly from its neighbors.

As development proceeds, dense masses of chromatin make their appearance in the nucleus along the radiating linin strands, while the karyosomes begin to disappear. It appears as if this increase in chromatin is the result of the fragmentation of the karyosomes and the distribution of the chromatin along the fibrous linin strands which radiate toward the nuclear periphery. The cell in general, and especially the nucleus, stains more heavily with the basic dyes.

The masses of chromatin continue to increase within the nucleus, forming a great many irregular clumps joined by thin linin material. In the last spermatogonial prophases the nucleus has somewhat increased in size and the irregular chromatin masses have become distinct, the linin threads which united them not being apparent any more (fig. 2). These masses vary in numbers, as shown by the following counts in different cells: 86, 79, 74, 85. The centrosomes, which in the meantime have divided, now begin to migrate to opposite poles of the cell and the spindle fibers make their appearance. Binford ('13), has

observed the same things to occur in the spermatogenesis of *Menippe mercenaria*.

The nuclear wall soon breaks down and the chromatin masses arrange themselves at the equator of the spindle (fig. 3). During the metaphase and anaphase, the centrosomes at either pole are often found to be double and the mitochondrial granules are distributed around the very delicate and slender spindle fibers (figs. 3 and 5). This is similar to what Prowazek ('02 b) found in *Astacus*.

A polar view of the metaphase stage is shown in figure 4. The chromosomes are distributed throughout the whole plane of the equator and are so numerous that ordinarily they cannot be counted. But on the customary inference that there are always twice the number of chromosomes in the spermatogonial stage that can be found at the reduction period, there must be two hundred chromosomes in *Cambarus virilis* and two hundred and eight in *Cambarus immunis*. The chromosomes are spherical in shape and situated close to one another. In only one case in *Cambarus virilis* was a good count obtained (fig. 4), showing two hundred chromosomes.

During the anaphase stage (fig. 5) the chromosomes move to opposite poles. The mitochondrial granules and the double nature of the centrosome can still be discerned.

In the early telophase stage the chromosomes are at opposite poles and a constriction in the central portion of the cytoplasm makes its appearance. This grows inward and as it does so it constricts the spindle fibers (fig. 6) towards the center, where a thickened heavily staining *zwischenkörper* (fig. 7, *Zw*) is formed. A nuclear wall now makes its appearance around the chromosomes at either pole (figs. 6-7) and the division of the spermatogonial cell is almost completed. At first the nucleus is crescent-shaped (figs. 6-7), but as the constriction between the mitotic cells becomes more apparent and the spindle fibers disappear, it gradually assumes its normal round appearance and the chromosomes distribute themselves throughout the nuclear territory (fig. 8), in order to begin their growth period. These cells represent primary spermatocytes in their earliest stages.

2. *First maturation stage*

a. *The growth period, including synapsis and tetrad formation.*
The following stages may be readily distinguished during this period:

Stage A (figs. 7-8): Early prophases. The autosomes of the ultimate spermatogonial telophases grow irregular, massed, and then weave out into thin threads.

Stage B (fig. 9): Leptotene stage. Here the autosomes have entirely transformed into very thin threads.

Stages A and B (figs. 7-9) represent the presynaptic stages, and are the most important steps in tracing out the exact process of synapsis. Montgomery ('11) regards them as the 'decisive' stages in truly comprehending the method of chromosomal conjugation.

In the forms studied, there are so many chromosomes at these periods that it requires the most painstaking study to determine what occurs. As already stated, in stage A (fig. 8) the autosomes of the last spermatogonial telophases (figs. 6 and 7) are transformed into leptotene threads. This is not accomplished by an uncoiling process such as Wilson ('12), has described in *Oncopeltus* and *Lygaeus*, but the chromosomal masses seem to diffuse out into threads in a manner similar to that which Montgomery ('11) has described in *Euschistus*, and the Schreiners ('06 a and b) have described in *Tomopteris*, *Spinax* and *Myxine*.

In stage B (fig. 9) these leptotene threads have completely formed and all the chromatin masses have disappeared. But no distinct spireme is formed, for the threads can be clearly identified as separate bodies both in smear preparations and in sections. These threads are so numerous that they cannot be counted. It is therefore impossible to state whether each of them corresponds to one of the chromosomes of the telophase stage in the spermatogonial division. The chromosomes in the latter period are also very numerous and their number could not be determined. From a comparison of figures 6 to 9, however, it is evident that the leptotene threads are formed by an unravelling of the chromosomes of the spermatogonial telophases and

hence it seems justifiable to conclude that the former represent the latter in number as well as in quality. Recently, Wilson ('12) has strongly upheld this point of view in *Oncopeltus* and *Lygaeus*.

Stage C (figs. 10-11): Synizesis stage. In this stage the threads drift to one pole of the nucleus and arrange themselves into more or less parallel groups.

Stage D (fig. 12): Synapsis stage. The chromosomes have become closely grouped side by side at one pole of the nucleus, while the opposite pole contains a clear open space.

Stages C and D (figs. 10-12) have been studied in both living and preserved material. In living cells, teased out of the testis and stained by an intravital stain such as methylin-blue, practically the same figures were seen as those represented in the drawings made from fixed materials. The leptotene threads have all migrated to one pole of the nucleus, leaving in the opposite pole a clear space filled with what appears to be a transparent fluid. Figure 11, from a smear preparation, gives a good view of such a cell. Wilson, Morse and others have also studied the contraction phase in living cells of insects and have obtained similar results. Therefore, it is not an artifact due to fixation, but is an actual process occurring in the spermatogenesis.

During these stages, careful and prolonged study has revealed the paired and parallel arrangement of the leptotene threads in a great number of cases (figs. 10-12). Lerat ('05), has observed a similar arrangement of the leptotene threads in the copepod *Cyclops strenuus* and the Schreiners, as well as others, have observed it in various other forms.

Stage E (fig. 13): Pachytene stage. During all the above stages the cell has increased but little in size. Now it begins to grow rapidly and each pair of parallel threads becomes fused into a geminus. The fusion, however, is not complete, for in many of the gemini the longitudinal furrow between the paired leptotene threads may be partially discerned. These gemini soon emerge from the synaptic pole and spread throughout the nucleus.

It is thus seen that here we have to deal with a side by side conjugation of the chromosomes (parasynapsis), and the synapctic and pachytene stages are very similar to those found in *Euschistus* (Montgomery '11), in *Batrachoseps* (Janssens '05), in *Tomopteris*, *Salamandra*, *Myxine*, *Spinax* (the Schreiners '06 a and b), and in *Cyclops* (Lerat '05).

Stage F (figs. 14-15): Diplotene (strepsineme) stage. Here the fused chromosomes of the pachytene threads unravel. Each geminus, which consists of two single chromosomal threads in parallel union, becomes split longitudinally, along the line of their original union. These threads then open up at one end while remaining attached at the other, taking the shapes of 8, V and U (figs. 14-15). There is no evidence that the arms of the geminus twist about each other as in *Batrachoseps* or in *Euschistus*.

Stage G (figs. 14-20): Postdiplotene stage. The individual arms of each geminus open up still more completely at one end while remaining partially fused at the other end. Each arm then splits longitudinally (the second longitudinal split), producing four thin arms which stain slightly (figs. 14-17). The arms then diverge, forming X's and 8's (figs. 17-20). The centrally constricted point of these figures is the place where the four arms are in fusion. This is particularly well seen in figure 17, from a smear preparation. Wilson ('12), in *Oncopeltus* and *Lygaeus* found a diffuse stage between steps F and G, in which the leptotene threads disappear from view, giving rise to vague, coarse and lightly staining structures within the nucleus. In the species of *Cambarus* under discussion no such diffuse stage was found.

Stage H (figs. 20-22): Tetrad formation. The tetrads are formed in the following way: the four arms of each geminus shorten and thicken. Their central point of fusion partially disappears, giving the geminus the appearance of being traversed by a longitudinal and a transverse split (figs. 20-21). The four thin threads then condense into four round chromosomes, forming tetrads (figs. 21-22). Each pair of chromosomes next unites to form a bivalent type and these are attached to each other by thin linin strands (figs. 22-23). This is the final step in the

growth period and the cell is now ready to undergo mitosis and reduction.

Stages E to H (figs. 13-23) follow each other in regular succession and the cells have increased a good deal in size. These stages may therefore be regarded as the actual period of growth, while stages A to D (figs. 8-12) are the preparatory steps, preparing the cells for growth.

b. The chromatoid bodies. In *Cambarus virilis* two chromatoid bodies (figs. 13-16, 18, *K*, and figs. 82-84) make their appearance in the cytoplasm during the pachytene stage (fig. 13, *K*). Before this, the cytoplasm of the cells contain a distinct idiozome (*I*), with its centrosome (*C*), and larger granular masses of mitochondria (*M*), which stain like chromatin, but somewhat lighter. Traces of chromatoid masses can also be seen (figs. 1-2, 8, *k*). These chromatoid masses and the mitochondrial granules, however, decrease in size and disappear during the early growth period. In the pachytene stage they have almost completely disappeared. At the same time, two circular bodies, surrounded by more or less clear areas, make their appearance in the cytoplasm; these stain deeply, like the chromatin of the nucleus, and are the chromatoid bodies (fig. 13, *K*), and it seems as if they were formed by a flowing together of the above mentioned chromatoid masses (figs. 1-2, 8, *k*). Wilson ('13), has found a similar body in *Pentatoma* and believes it to originate in the same fashion.

c. Reduction division. After the formation of the tetrads the centrosomes are observed to migrate to opposite poles and the nuclear wall breaks down (figs. 22-23). In figure 23 only one of the chromatoid bodies is seen. Next, the tetrads are drawn into the equator of the cell and there arrange themselves for division. During the growth period numerous karyosomes were found in the nucleus, but now these have all disappeared from view.'

In the metaphase condition the tetrads appear as two large bivalents connected in the form of dumb-bells (figs. 24 and 26). No split can be noticed in either of the bivalents, where the equational division of the second maturation stage will occur.

They appear completely fused and twice the size of the spermatogonial chromosomes. A polar view of the chromosomes in *Cambarus virilis* reveals 100 chromosomes, this being the reduced number (figs. 28-29). In *Cambarus immunis*, there are 104 chromosomes, seeming to be composed of 96 ordinary chromosomes and a peculiar group of eight, sometimes enclosed within a clear vacuole (figs. 38 and 40). The chromosomes were outlined in the most careful manner by the aid of the camera lucida and then the counts were made.

During the reduction division, the chromatoid bodies in *Cambarus virilis* may behave in either of the following two ways:

1. They may both pass to one pole of the spindle. This is the usual and common method of their behavior. In this case the secondary spermatocytes produced differ from each other in that one contains the two chromatoid bodies, the other being without them (figs. 24-26, 30-32, and 85-90).

2. One chromatoid body may pass to one pole of the spindle, while the other passes to the opposite pole. This mode of behavior is the rarer of the two. Here the secondary spermatocytes produced each contain one chromatoid body (figs. 33-35 and 91-93).

There are thus produced three types of secondary spermatocytes: (1) Type A, containing two chromatoid bodies (fig. 32); (2) Type B, containing one chromatoid body (fig. 35); and (3) Type C, containing no chromatoid body (fig. 36).

The chromatoid bodies lie outside of the spindle and long before the autosomes have made their way from the equator of the poles, these bodies are already seen to migrate towards them. In many cases they are actually at the poles during the metaphase stage (figs. 24-26, 30-35) and 85-93. Their position is outside of the spindle and in the cytoplasm. This is especially seen in ultimate telophase stages where the nuclear wall has already formed around the chromosomes. In such stages the bodies remain in the cytoplasm, as is shown in figures 31, 32, 35, 89, 90 and 93.

In *Cambarus immunis* these bodies could not be studied, this being due, in all probability, to the fixation of the material.

In this species, however, a peculiar group of eight chromosomes is often seen during the stage. The group stands distinctly apart from the other chromosomes, generally along the periphery of the spindle and seems to be enclosed in a clear vacuole (figs. 38, 39, 111 and 112). During the anaphase stage, this group migrates to one pole undivided, and thus, undoubtedly, giving rise to two kinds of secondary spermatocytes, one type containing eight more chromosomes than the other. Even before the anaphase stage, the group of chromosomes may be seen migrating to one pole (figs. 37, 113 and 114). In the second spermatocyte stage all trace of this chromosomal group is lost.

Mrázek ('01) has described interesting abnormalities in the testis of *Astacus*. But in *Cambarus immunis* the clump of eight chromosomes cannot be regarded as an abnormality, for it is of quite frequent occurrence in the cells. It is also impossible to state whether these chromosomes are accessories, for their behavior could not be traced.

Interesting abnormal mitotic figures were sometimes seen within the tubules of both species studied. These manifested themselves mainly as tripolar spindles during the first spermatocyte division. Figure 27 shows one of these tripolar divisions in *Cambarus virilis* when the chromatoid bodies are seen migrating to opposite poles. Mrázek ('01) and Binford ('13) have also observed similar abnormalities in other decapods.

3. *Second spermatocyte stage*

No distinct rest stage follows the first maturation division. The nucleus of the telophase stage of the reduction division is at first crescent-shaped and then it opens up into almost a circular form, but is pale and granular. Very shortly, however, it disintegrates to form the spindle of the second division. No spireme, nor tetrads, nor leptotene rods make their appearance. Prowazek ('02 b) in *Astacus fluviatilis* and Binford ('13) in *Menippe mercenaria* also found no distinct rest stage between the primary and secondary spermatocyte divisions.

The chromosomes line up in the equator of the spindle and appear like dumb-bells (figs. 43, 46, 47, 51, 54). A polar view

(figs. 41-42), reveals that they are about half the size of the chromosomes in the primary spermatocyte stage. They are more or less round, condensed and distributed over the entire equatorial plate. Numerous counts of these polar views revealed 100 chromosomes in *Cambarus virilis* (fig. 42) and 104 chromosomes (including the clump of eight) in *Cambarus immunis* (fig. 41). These counts were made after careful tracings with the camera lucida.

The three types of secondary spermatocytes produced and enumerated under our discussion of the primary spermatocyte stage, behave in the following manner:

Type A. When this form divides there are two possibilities: (1) the two chromatoid bodies may migrate to one pole, producing a spermatid with both these bodies, while the other is without them (figs. 46-50 and 94-100); or (2) one chromatoid body may go to one pole and the other may go to the opposite pole, giving spermatids containing one of these bodies each (figs. 51-53 and 104-106). The first of these modes of behavior is the most usual.

Type B. In the division of this type, the one chromatoid body goes to one pole, giving rise to a spermatid containing this body and an other spermatid without it (figs. 54-56 and 101-103).

Type C. The spermatids resulting from the division of this form are all without either of the chromatoid bodies (figs. 43-45).

In the anaphase stage the chromosomes divide equationally and drift to opposite poles to form the telophase stage. During the late telophase of this division, as in the other divisions mentioned, a *zwischenkörper* is formed in the center of the spindle (fig. 44). After the cells become constricted from each other, this body remains outside of the cells and soon disintegrates. In this stage, as in the primary spermatocyte division, the chromatoid bodies lie in the cytoplasm outside of the spindle as can be seen in the drawings and in the photographs. Thus three types of spermatids are produced, namely:

(1) Type A', containing two chromatoid elements (figs. 50, 99 and 100).

(2) Type B', containing one chromatoid element (figs. 56 and 107).

(3) Type C', without any chromatoid elements (fig. 45).

Type C' is the most common of the forms produced. Roughly estimated, it is about four or five times as numerous as either of the other two classes of spermatids produced.

4. Transformations of the spermatid to the spermatozoon

The transformations undergone by the spermatid to become the spermatozoon are very interesting. The various types of spermatids produced are small and contain minute, granular nuclei which stain deeply with nuclear dyes. The cytoplasm appears homogeneous throughout and stains but lightly. A centrosome is usually found in it, and in types A' and B', the chromatoid bodies are also visible in the cytoplasm.

After the spermatid begins its transformation, however, the chromatoid bodies cannot be followed. They evidently disintegrate and disappear. The nucleus is now observed to wander to one pole of the cell (figs. 57-58, *N*). At the same time a vacuole begins to form in the cytoplasm at the opposite pole (fig. 57, *V*). This vacuole increases in size and at the same time the nucleus becomes more condensed into a crescent-shaped plate (figs. 57-59, *N*). The centrosome is observed to migrate between the nucleus and the vacuole, where it finally takes a median position on the upper surface of the nucleus (figs. 57-60, *C*).

All these stages were also observed in living cells teased out in Ringer's solution and stained with methylin blue or lichtgrün. In methylin blue the nucleus stains heavily and seems to consist of granules and refractile droplets, thus giving them the appearance of oil droplets. The cytoplasm is uniformly granular and stains a very pale blue, while the vacuole is composed of a liquid which refracts light strongly, but does not seem to take the bluish stain readily. The centrosome stains a deep blue.

In smear preparations stained with iron-hematoxylin and counterstained with acid fuchsin, the nucleus stains black, the cytoplasm reddish, while the vacuole seems to be made up of two substances: (1) a ground substance which stains a heavy black as if it consisted of hyaline or chitin-like substance; and (2) droplets which stain reddish with acid fuchsin (figs. 57-58, *V*). The centrosome stains black.

As the transformation continues, the nucleus condenses still more and migrates down into the cytoplasm (figs. 59-61, *N*). The cell now has the appearance of two parts, an upper half composed of the transparent vesicle (figs. 59-61, *V*) and a lower half made up largely of the cytoplasm (*Z*), in the bottom of which the nucleus (*N*) has become condensed (figs. 59-61). Figure 59, drawn from a smear preparation, gives a side view of the transforming spermatid, with the vesicle, *V*, in the upper extremity and the condensed disc-shaped nucleus, *N*, at the opposite pole of the cell. The centrosome, *C*, has a median position between *V* and *N*. Figure 60 is a similar view of a somewhat later stage. Figure 61 is a top view of a transforming spermatid in which the centrosome, *C*, lying above the nucleus, *N*, is seen through the vesicle, *V*.

Now the walls of the vesicle become somewhat thicker, and at the same time the cytoplasmic wall becomes irregular in outline. The centrosome is observed, in favorable preparations, to undergo marked changes. Fine strands (figs. 62-63, *C*), appear to radiate from it into the cytoplasm in various directions. These strands are thin and stain dark with Heidenhain's iron-hematoxylin, and in safranin-gentian-violet preparations they stain a beautiful purple, like the centrosome. Figures 62 to 64 show these strands radiating like the arms of a starfish in all directions. They form, so to speak, the skeleton along which the flagella-like rays of the spermatozoa will be constructed. Figure 62 shows an oblique section made through the vesicle (*V*) and centrosome (*C*) of the transforming spermatid. The centrosome (*C*) seems to be off to one side of the cell. Figure 63 is a top view, in which the radiating strands of the centrosome (*C*) are seen through the vesicle (*V*). Figure 64 is a side

view of a later stage, showing the vesicle (*V*) beginning its process of invagination. The nucleus (*N*) has now migrated to the bottom of the cell and the strands of the centrosome (*C*) are seen making their way through the cytoplasm (*Z*).

The strands increase in length and, as they do so, the cytoplasm spins out a thin arm along each one of them (figs. 65-66). At the same time, the cytoplasmic cup with the nucleus in the bottom decreases in width, thus being brought somewhat nearer to the upper vesicle. This vesicle has also undergone considerable change and now looks like a hemispherical cup whose mouth has become invaginated into the cavity (figs. 64, 65, 67-68, *V*).

Figure 65 is a side view of the transforming spermatid. The vesicle, *V*, is completely formed, showing its invaginated edges. The cytoplasm, *Z*, has produced the radiating arms along the centrosomal strands, *C*. The nucleus, *N*, forms the head piece, opposite to the vesicle, *V*. Figure 66 is a top view of the same stage showing all of the structures enumerated, except *N*, which is completely hidden by the radiating arms.

A recapitulation of the above description shows that the spermatozoon now consists of: (1) A transparent upper vesicle originating in the cytoplasm of the spermatid (figs. 57 and 65, *V*); (2) Radiating arms, produced from the rest of the cytoplasm (figs. 57 and 65, *Z*), and formed along the thin strands derived from the centrosome (figs. 57 and 65, *C*); and (3) The compact nuclear mass, situated in the bottom of the cytoplasmic cup which is suspended from the upper vesicle (figs. 57 and 65, *N*).

In general, the genesis of the spermatozoa of the two species of *Cambarus* studied are seen to be similar to the genesis of the sperm of the other decapods as worked out by Labbé, Gilson, Koltzoff, Retzius, Binford, Reinhardt and others. The central body, however, the so-called 'tigelle' of the French investigators, was not found in the species studied.

Andrews ('04) worked on the spermatozoa of *Cambarus affinis* and he asserts that "an examination of the stages of spermatogenesis in the crayfish leads to the provisional acceptance of the view that the arms of the sperm are made from the nucleus of the spermatid." My studies on *Cambarus virilis* and *C.*

immunis do not confirm this. On the contrary, all the evidence tends to the conclusion that the arms of the spermatozoon are derived from the cytoplasm and that the nucleus forms the head-like mass in the cytoplasmic bowl, opposite the transparent vesicular cup. These observations, moreover, are in accord with those of most other investigators on decapod spermatogenesis, namely, that the rayed arms of the sperm are of cytoplasmic origin.

The final stage in the development of the spermatozoon is the winding of the radiating arms about the combined cytoplasmic and nuclear cup. The rays become tucked around this cup and conceal it (fig. 67, *R*). They must be supplied with a transparent adhesive substance which attaches them firmly, thereby effacing their outlines. In this stage the entire spermatozoon stains lightly and almost uniformly. Figure 67 gives a side view of a mature spermatozoon within one of the tubules of the testis, showing the invaginated vesicle, *V*, set into a cup consisting of the nucleus and the radiating arms. The nucleus, *N*, cannot be seen because the arms, *R*, surround it and hide it from view.

During the breeding season almost all the tubules of the testis and the ducts of the vasa deferentia contain these ripe spermatozoa in enormous numbers (figs. 78 and 81). They all seem to be translucent, spheroidal bodies (fig. 68), consisting of a refractive vesicular cup (figs. 68-70, *V*), set into a larger watery-looking ellipsoid (figs. 68-70, *R*), which contains a somewhat denser granular mass, with a large globule (figs. 68-70, *X*) in its bottom. Figures 68 to 70 are camera lucida drawings of living spermatozoa studied in Ringer's solution. Figure 68 is a side view, whereas figure 69 is a top view. Figure 70 is a side view showing the opening up of the spermatozoa. After copulation in autumn, the females have their seminal receptacles stocked with these spermatozoa.

One can get a very good idea of the mature, uncoiled spermatozoon by imagining an inverted bowl with its wall invaginated at the mouth and turned back into the cavity, fitted into a football of about twice its size at the laced end. The bowl would correspond to the refractive vesicle (figs. 67-68, *V*), and the foot-

ball would correspond to the rest of the spermatozoon (figs. 67-68, *R*). Andrews ('04), in describing the spermatozoon of *Cambarus affinis* states:

The most conspicuous part of the sperm is the well known vesicle that makes up about one-half the bulk of the sperm. This structure gives the sperm as seen from above or from below, the appearance of being chiefly two concentric elliptical rings. Side views, however, make the character and relationships of the vesicle intelligible. The vesicle is an elliptical bowl, inverted and with thick walls that are invaginated at the mouth and somewhat turned back into the cavity. The walls are highly refractive and so is the material filling the bowl. This bowl is set in the body of the sperm somewhat as a very small inverted cup might be held in the hollow of one's closed hand. The body of the sperm is clear and more watery-looking than the bowl. In the bottom of the sperm are some droplets.

5. *Uncoiling of the mature spermatozoon*

The opening of the spermatozoon was studied by teasing out mature sperm from the vas deferens in hanging drops of Ringer's solution, in weak solutions of various salts such as KCl, NaCl and KNO₃, or in water, and studying these under the 2 mm. oil immersion objective. The best results were obtained in Ringer's solution. When the spermatozoon comes in contact with this fluid, its body, below the vesicle, swells and soon opens up (figs. 68-76). The arms which are tucked around the nucleus unwind rather quickly. At first fine ray-like lines make their appearance, then the needle-like projections which form the ends of the rayed arms. Soon the arms themselves are entirely released and they uncoil in somewhat the manner of a watchspring. Figures 73 to 74 are top views of the spermatozoon showing the uncoiling of the arms. As they uncoil a glue-like substance seems to diffuse from them. This substance, undoubtedly, was the cement that kept the arms coiled about the nucleus.

In the uncoiled state the spermatozoon may be compared to a floating amoeba (figs. 75-76). Figure 75 is a side view showing the vesicle, *V*, on top, the nucleus, *N*, on bottom and the flagellated arms, *R*, radiating out from the juncture of these two structures. Figure 76 is a top view showing all these parts

except the nucleus, which is hidden from view by the vesicle, *V*. In the living state, the nucleus presents more or less of a finely granular appearance.

In low concentrations of various salts, such as NaCl, KCl, and KNO₃ (2 per cent and below), the sperm opened quickly. In higher concentrations, the process of opening was somewhat inhibited, and in strong concentrations (16 per cent) the sperm became crenated and their opening up was very slow. Andrews ('04), observed a similar behavior in the spermatozoon of *Cambarus affinis*.

In many cases an interesting change was also observed in the vesicle. In Ringer's solution and especially in water, the invaginated edges of the vesicle straighten out in the manner indicated in figures 68, 70 and 71. Evidently this transformation is produced by the osmotic changes undergone within the spermatozoon during the opening-up process. Now the question arises, can this be the force which tends to drive the spermatozoon into the egg during the process of fertilization? Koltzoff ('06), Spitschakoff ('09), and Binford ('13), have also observed striking changes undergone by the vesicle of the spermatozoon of other decapods when subjected to various salt solutions. These investigators are of the opinion that this force is the operating factor in bringing about penetration of the ovum.

6. Types of spermatozoa produced

In *Cambarus virilis* a trimorphism of the spermatozoa is found. Here five-, six- and seven-rayed types are produced (figs. 108-110). The six-rayed type is the common and most numerous form seen, being about four times as abundant as either of the other two types. In *Cambarus immunis* a tetramorphism of the spermatozoa exists. Here, besides producing the three types found in *Cambarus virilis*, a fourth type of spermatozoon containing eight rays is developed (fig. 77). This eight-rayed form is very scarce and was observed in only two cases. The six- and seven-rayed types are the forms most abundantly developed in the testis of *Cambarus immunis*.

The possibility suggests itself that the trimorphism of the sperm in *Cambarus virilis* may perhaps be accounted for by the behavior of the chromatoid bodies. This must remain a possibility only, since the chromatoid bodies cannot be followed through the transformations undergone by the resting spermatids to form the spermatozoa. However, it is a striking fact, that three types of spermatids, types A', B' and C' are produced in this species, and also, that type C' is about four or five times as numerous as either of the other two. Since the coincidence of almost an exact trimorphism is noticed in the mature spermatozoa, it seems to point to the possibility that the chromatoid bodies are responsible for this interesting difference. In *Cambarus immunis* the chromatoid bodies were not found. This was probably due, however, to the fixation of the material.

GENERAL CONSIDERATIONS AND CONCLUSIONS

1. Synapsis and reduction of the chromosomes

The problems of synapsis and chromosomal reduction are perhaps the most important in the study of the germ cells. Considerable has been written on these questions, but recently, Grégoire ('10), Montgomery ('11), and Wilson ('12) have given us critical discussions of them. Montgomery ('11, pp. 749-750), has ably summarized the various views in the following way:

I. The actual reduction of the number of chromosomes is effected during the prophase of the maturation mitoses, and both of these divisions are equational. This [view] was originated by Boveri ('87) and Brauer ('93). Today it is held in two forms: (A) Meves ('96, '07a), Fick ('07, '08), and Duesberg ('08) argue that a continuous spirem is produced, that this segments into half the normal number of chromosomes, the cleft along such a bivalent chromosome being a true longitudinal split; they reason there is neither metasyndetic or parasyndetic conjugation of chromosomes. (B) Bonnevie ('06, '08) and Vejdowsky ('07) hold there is a parasyndetic union of chromosomes, but that this conjugation leads to complete and persisting fusion.

II. The reduction of the number of the chromosomes is effected by the maturation mitosis, one at least of which is a reduction division. There are several variants of this, as follows:

A. The univalent chromosomes, without conjugation or pseudo-reduction, double their number during the prophases, then become

quartered in number by two successive reduction divisions. This view was founded especially by O. Hertwig ('90) and Wilcox ('95).

B. The univalent chromosomes undergo neither conjugation nor pseudoreduction during the prophases, but conjugate first in the equator of the first maturation spindle and there separate reductionally. Founded by Henking ('91) and Korschelt ('95).

C. The univalent chromosomes undergo pseudoreduction in the prophases by a continuous chromatin spirem segmenting into half the normal number of segments; these divide equationally in the first mitosis and reductionally in the second; Rückert ('93, '94) Häcker ('95), Vom Rath ('95).

D. There is no continuous chromatin spirem produced in the prophases, but the univalent chromosomes conjugate to form pairs or gemini and these undergo a reductional and an equational division. This view, now shared by the great majority of students exhibits itself under these aspects:

D. 1. The chromosomes conjugate metasyngetically.

(a) The reduction in the number of the chromosomes is effected by the first maturation mitosis (Montgomery, '00; Farmer and Moore, '03).

(b) The reduction in the number of chromosomes is effected by the second maturation mitosis (McClung, '00, and his students).

D. 2. The chromosomes conjugate parasyngetically and the first maturation mitosis is reductional. Winniwarter ('00), Gregoire ('04), Berghs ('04), A. and K. E. Schreiner ('04, '05).

My own studies, as set forth in the present paper, are in harmony with the last of these views, i.e., D 2. No continuous spirem is formed, the chromosomes unite, side by side, into gemini, and reduction occurs in the first maturation division.

Within the last few years the view that chromosomal conjugation is parasynaptic has been strongly advocated by many investigators. Von Winniwarter ('00), first called attention to this type of union in the mammals. But in that period the view mainly gained ground among botanical cytologists, and was strongly advocated by them. The botanists called attention to the stages preceding synapsis, the 'presynaptic stages' in the growth period and showed that these are the most important for a concise study of conjugation.

The Schreiners ('04, '05, '06, '07 and '08), more than anybody else, have worked on this phase of synapsis in animals, and have shown parasynapsis to occur in the following forms:

Vertebrates: Man, rabbit, cat, mouse, rat, pigeon, garden snake, Triton, salamander, Chimaera, Raja, Spinax, Bdellostoma, myxine. *Arthropoda*: *Locusta viridissima*, *Eucheata norvegica*. *Annelids*: *Tomopteris onisciformis*, *Ophryotrocha puerilis*. *Molluscs*: *Enteroxenas östergreni*.

In *Tomopteris* these authors obtained especially fine material and have traced out the parallel conjugation of the chromosomes in the most convincing way. Table 2 gives a list of the investigators who have found such conjugation in animals, with dates and the forms in which it occurs.

In the species of *Cambarus* studied the problem of synapsis is one of especial difficulty due to the immense number of chromosomes possessed by the forms. When the leptotene threads appear, these are so numerous that it is impossible to count them. However, in synizesis these are seen to drift to one pole of the spindle and to become arranged in pairs, the individual threads

TABLE 2

GROUP	AUTHORS AND DATES
(1) Mammals.....	{ Von Winniwarter ('00, '02) Schoenfeld ('01)
(2) Birds.....	A. and K. E. Schreiner ('04)
(3) Reptilia.....	A. and K. E. Schreiner ('04)
(4) Amphibia.....	{ Janssens ('05) A. and K. E. Schreiner ('06)
(5) Fishes.....	{ Maréchal ('04) A. and K. E. Schreiner ('04, '05 and '06)
(6) Insects.....	{ Otte ('06) Morse ('09) Montgomery ('11) Wilson ('12) Wilke ('13) Kornhauser ('14)
(7) Crustaceans.....	Lerat ('05)
(8) Molluscs.....	{ Bonnevie ('05, '06) A. and K. E. Schreiner ('07)
(9) Annelids.....	A. and K. E. Schreiner ('06a, '06c)
(10) Nematodes.....	{ Tretjakoff ('04) Marcus ('06)

being parallel to each other. After emerging from synapsis, the threads are much thicker and double in nature. Since no counts can be made, it is impossible to state absolutely whether there are half the number of gemini that there were of leptotene threads, but certainly the number of individual threads is very appreciably diminished. This fact, together with the much thicker state of the postsynaptic filaments, strongly indicates parasynapsis.

2. Summary

1. The testes of *Cambarus virilis* and *Cambarus immunis* are trilobed organs situated in the thorax, below the cardiac chamber.

2. From the middle of June to the middle of July the testes undergo most rapid proliferation and increase to their maximum size. During this period all the stages in the spermatogenesis may be obtained.

3. The spermatogonia originate from the germinal epithelium lining the walls of the tubules, and during the early prophase stages the nuclei of the spermatogonia contain two or three large chromatin masses, the karyosomes, from which thin linin strands radiate to the nuclear periphery. By the fragmentation of the karyosomes, the spermatogonial chromosomes originate.

4. In *Cambarus virilis*, the spermatogonial number of chromosomes is two hundred, while in *Cambarus immunis*, the number is two hundred and eight.

5. After the spermatogonial divisions the growth period sets in, during which the chromosomes become thread-like, forming thin leptotene rods. No spireme is formed, the rods remaining separate and distinct.

6. In the synizesis stage these leptotene threads migrate to one pole and arrange themselves in pairs, parallel to each other.

7. During synapsis, each pair of parallel threads fuses, forming a geminus. Thus the fusion is one of parasynapsis.

8. In the diplotene (strepsineme) stage the arms of each geminus open up at one end, along the line of their original fusion to form such figures as 8, *V* and *U*.

9. In the postdiplotene stage a second longitudinal split appears along each arm of the gemini, thus forming four thread-like arms. These now open up, and soon thicken into round chromosomes and form the components of the tetrads.

10. The nuclear wall breaks through and the tetrads, in the form of dumb-bells, migrate to the equator of the spindle. Each end branch of the dumb-bell consists of two chromosomes partially fused.

11. The first maturation division is the reduction division. A polar view of the metaphase stage reveals one hundred large chromosomes in *Cambarus virilis* and one hundred and four in *Cambarus immunis*.

12. In *Cambarus immunis* a clump of eight chromosomes is often seen encircled in a clear vacuole of the cytoplasm, standing apart from the other ninety-six chromosomes. The history of this chromosomal clump could not be traced.

13. In *Cambarus virilis* two round, large chromatoid bodies appear in the cytoplasm during the pachytene stage of the growth period. These are enclosed in clear areas of the cytoplasm and are probably formed by a flowing together of the chromatoid masses found in the cytoplasm during the earlier stages in the spermatogenesis.

14. During the reduction division, these chromatoid bodies go off, undivided, to one pole of the cell. This is their common mode of behavior. More rarely one goes to one pole, while the other migrates to the opposite pole of the cell.

15. Three types of secondary spermatocytes are thus produced, type A, containing the two chromatoid bodies, type B, with one chromatoid body, and type C, without any chromatoid bodies. Types A and C are the common ones, whereas type B is comparatively rare.

16. No distinct rest stage follows between the first and second divisions. The second spermatocyte division is the equational division. A polar view of the metaphase stage of this division shows 100 chromosomes in *Cambarus virilis* and 104 chromosomes in *Cambarus immunis*. The chromosomes are round in

shape and about half the size of those found in the polar view of the reduction division.

17. When type A divides, most generally both chromatoid bodies migrate to one pole of the spindle. In rarer cases, each of the chromatoid bodies may migrate to opposite poles of the spindle.

18. During the division of type B, the one chromatoid body migrates to one pole, leaving the opposite pole without any.

19. When type C divides, the spermatids produced do not contain any chromatoid bodies.

20. The resulting spermatids are thus of three types: type A', with two chromatoid elements; type B', containing one chromatoid element; and type C', without any chromatoid elements. Type C' is the most common form, being about four times as numerous as either of the other two types.

21. All traces of these chromatoid bodies are lost when the spermatid begins its transformation to form the spermatozoon.

22. The nucleus of the spermatid becomes disc-shaped and migrates to one pole of the cell. At the opposite pole a vacuole appears which is filled with a glue-like fluid. The centrosome migrates between these two structures.

23. The vacuole forms the refractive vesicle of the sperm; the nucleus forms the head-piece, while the centrosome sends out fine ray-like fibers along which the radial arms of the spermatozoon are developed from the rest of the cytoplasm.

24. The mature spermatozoon is a highly refractive spheroidal body, with the radial arms closely packed around the nucleus in the form of a cup which appears transparent and watery in consistency.

25. In Ringer's solution, in water, and in various Na and K salts, the opening out of the spermatozoon can best be studied. In Ringer's solution the radial arms uncoil slowly and the vesicle swells. In many cases, the vesicle undergoes a transformation by means of which its involuted edges straighten out. In water this was especially well seen.

26. Osmotic phenomena of the sperm are, undoubtedly, responsible for these transformations in the vesicle.

27. In *Cambarus virilis* a trimorphism of the spermatozoa exists. Five-, six- and seven-rayed types of spermatozoa are produced. The six-rayed type are the most numerous, being about four times as abundant as the other two forms.

28. In *Cambarus immunis* a tetramorphism of the spermatozoa exists. Five-, six-, seven- and eight-rayed forms are produced. The six- and seven-rayed spermatozoa are the most abundant types developed.

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DESCRIPTION OF PLATES

All figures on plates 1 to 7 were made with the camera lucida, under a magnification of 1800 times. The figures photographed are shown on plates 8 to 10. All photographs, with the exception of figures 78 to 81 are magnified 770 times. The magnification of figures 78 to 81 will be given next to their description. All photographs were taken by the author and none of them were retouched in any manner whatsoever. Figures 78 to 110 are of *Cambarus virilis*, and figures 111 to 114 are of *Cambarus immunitis*.

ABBREVIATIONS

<i>C</i> , centrosome	<i>R</i> , radial arms
<i>I</i> , idiozome	<i>V</i> , vesicle
<i>k</i> , chromatoid masses	<i>X</i> , globule
<i>K</i> , chromatoid bodies	<i>Z</i> , cytoplasm
<i>M</i> , mitochondrial granules	<i>Zw</i> , zwischenkörper
<i>N</i> , nucleus	

PLATE 1

All figures from *Cambarus virilis*

EXPLANATION OF FIGURES

- 1 Resting spermatogonial stage; within the cytoplasm three chromatoid masses, *k*, are seen.
- 2 Late spermatogonial prophase showing the chromatin of the nucleus broken into a great many clumps, preparatory to division.
- 3 Metaphase, spermatogonial stage.
- 4 Polar view of the metaphase during spermatogonial division showing 200 chromosomes.
- 5 Anaphase, spermatogonial stage; the centrosome at one pole is double in nature.
- 6 and 7 Telophase, spermatogonial stage; in figure 7 the zwischenkörper, *Zw*, is distinctly seen.
- 8 Resting primary spermatocytes.
- 9 Leptotene stage.
- 10 Synzesis stage. The leptotene threads are observed in parallel pairs migrating to one pole of the nucleus.

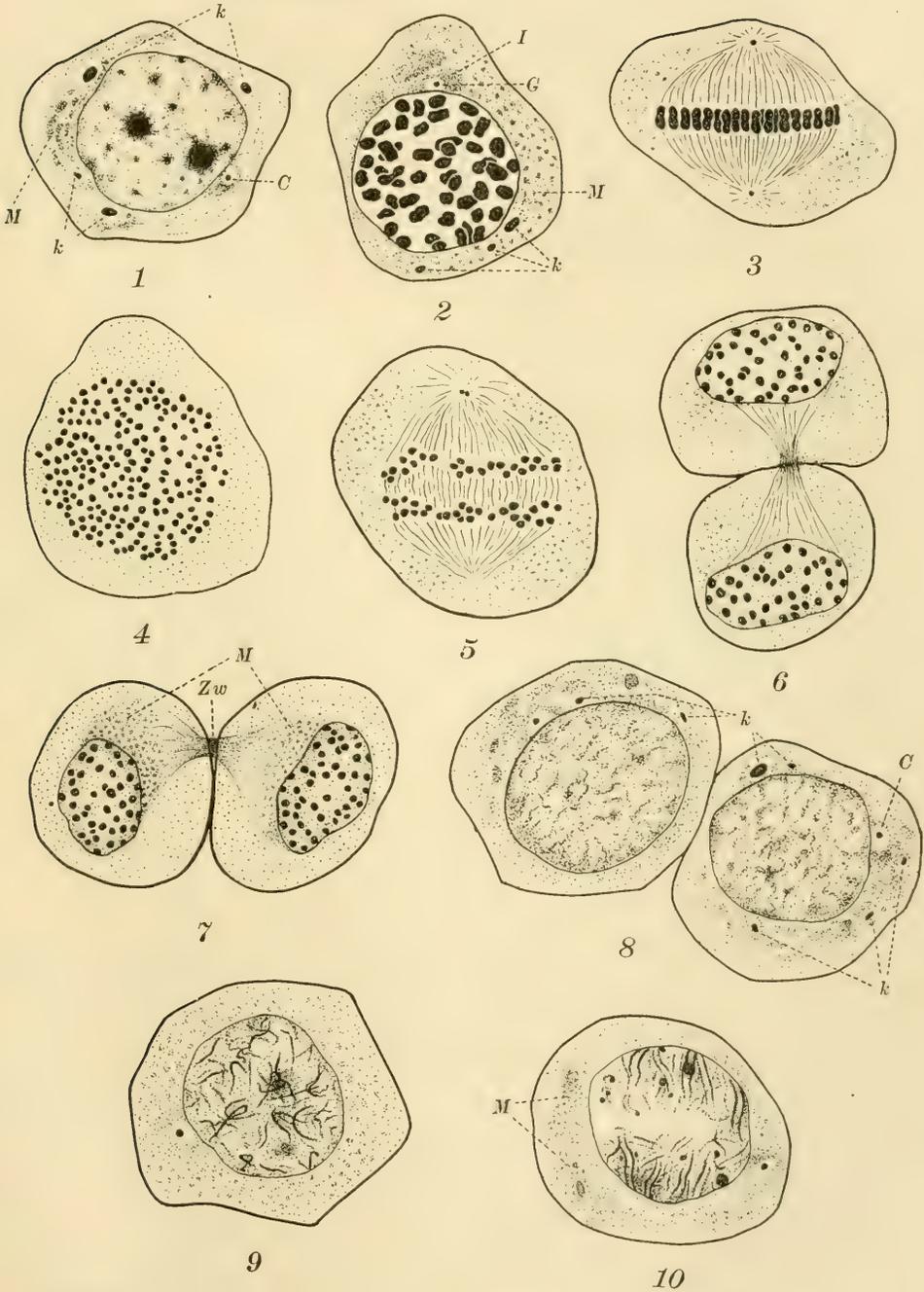
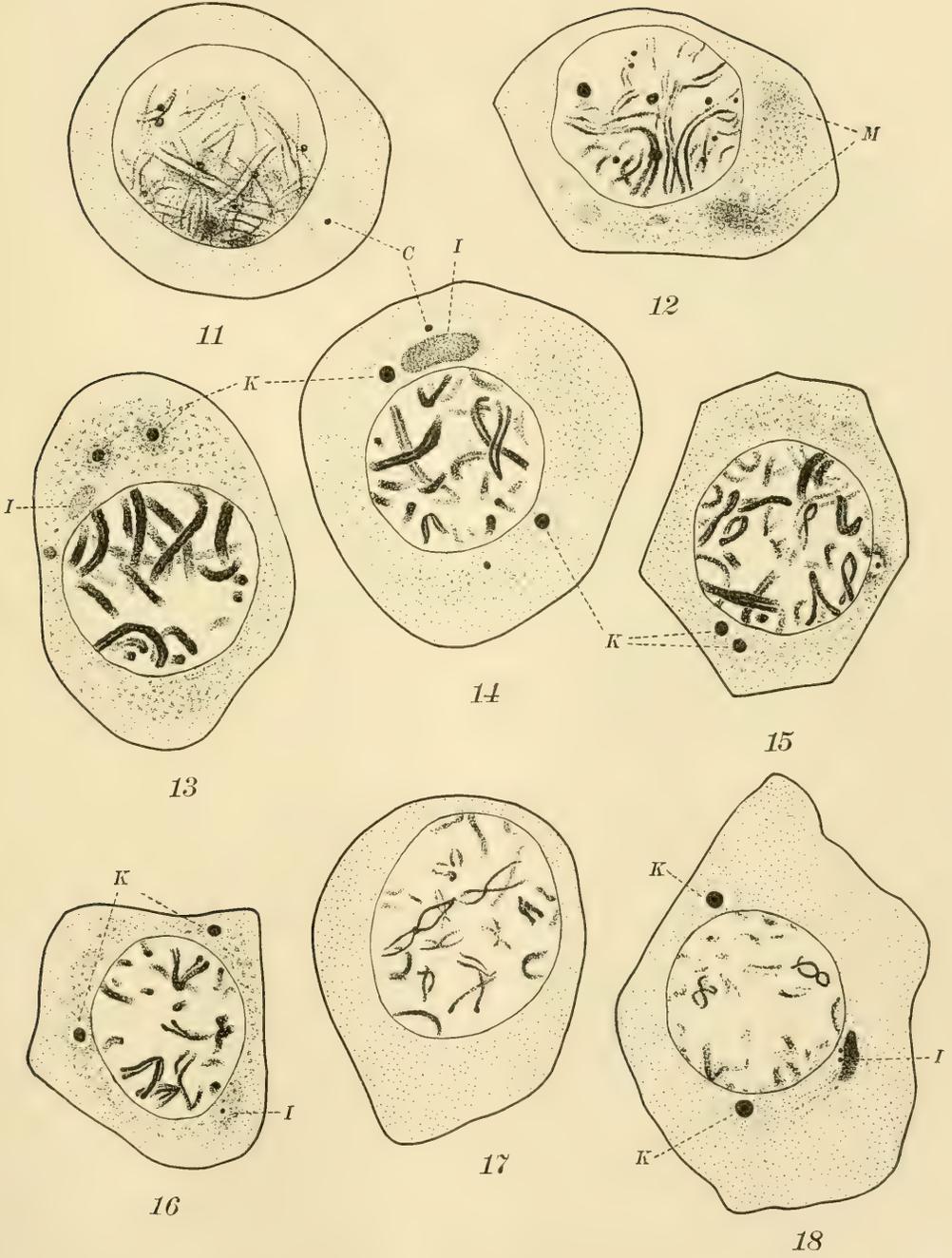


PLATE 2

All figures from *Cambarus virilis*

EXPLANATION OF FIGURES

- 11 Synizesis, primary spermatocyte; smear preparation.
- 12 Synizesis and synapsis stage; here the parallel arrangement of the threads is very clearly seen.
- 13 Pachytene stage. The parallel leptotene threads have united into gemini; the chromatoid bodies, *K*, make their appearance in the cytoplasm.
- 14 to 16 Diplotene (strepsineme) stage. The second longitudinal split is apparent along the arms of some of the gemini.
- 17 and 18 Postdiplotene stage. Figure 17 is from a smear preparation; figure 17 (fig. 84) shows the chromatoid bodies, *K*, in the cytoplasm.



N. Fasten, del.

PLATE 3

All figures from *Cambarus virilis*

EXPLANATION OF FIGURES

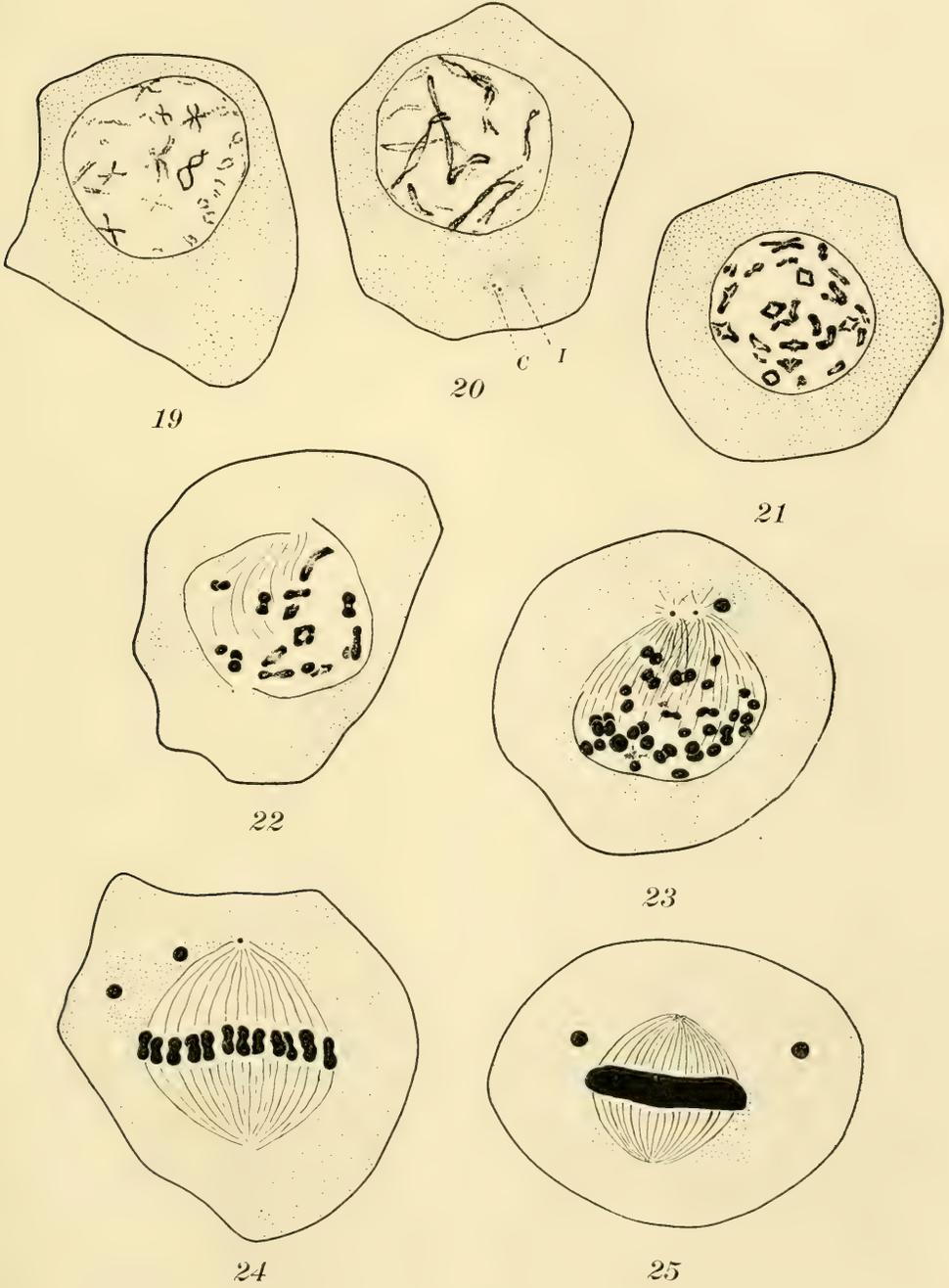
19 and 20 Postdiplotene stage; these are pre-tetrad stages.

21 and 22 Tetrad formation.

23 Tetrads transformed into dumb-bells. The centrosomes are migrating to opposite poles and the nuclear well is disappearing; one chromatoid body lies in the cytoplasm near the centrosomes.

24 Metaphase, primary spermatocyte, (fig. 85); the chromatoid bodies lie in the cytoplasm and are migrating to one pole.

25 Metaphase, primary spermatocyte; smear preparation, showing chromatoid bodies in the cytoplasm at one pole.



N. Fasten, del.

PLATE 4

All figures from *Cambarus virilis*

EXPLANATION OF FIGURES

26 Metaphase, primary spermatocyte, showing the chromatoid bodies at one pole.

27 Abnormal mitosis; tripolar spindle, primary spermatocyte. The chromatoid bodies are seen at opposite poles.

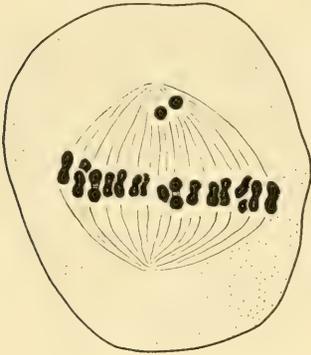
28 and 29 Polar view, primary spermatocyte showing 100 chromosomes.

30 Anaphase stage, primary spermatocyte, with the chromatoid bodies at one pole.

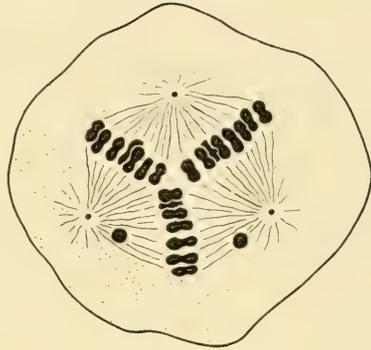
31 Telophase stage (fig. 89); primary spermatocyte, with the chromatoid bodies at one pole.

32 Early second spermatocyte, showing the two chromatoid bodies in the cytoplasm; type A.

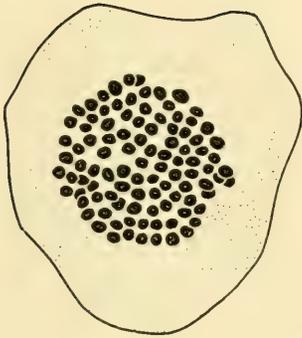
33 Metaphase (fig. 91); primary spermatocyte, with the chromatoid bodies at opposite poles.



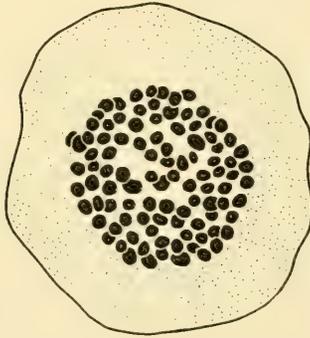
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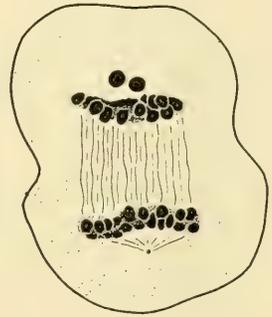
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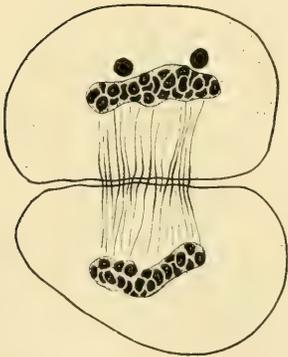
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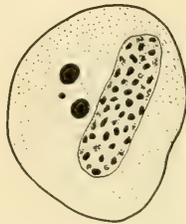
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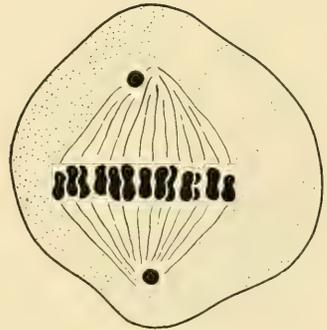
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PLATE 5

Figures 34 to 36, and 42 of *Cambarus virilis*; and figures 37 to 41 of *Cambarus immunis*

EXPLANATION OF FIGURES

34 Metaphase, primary spermatocyte showing the chromatoid bodies at opposite poles; *Cambarus virilis*.

35 Ultimate telophase stage of primary spermatocyte, with a chromatoid body in each cell lying in the cytoplasm (fig. 93). These cells are early secondary spermatocytes; type B; *Cambarus virilis*.

36 Resting secondary spermatocyte without any chromatoid bodies; type C; *Cambarus virilis*.

37 Metaphase, primary spermatocyte, showing the clump of eight chromosomes migrating to one pole (fig. 113); *Cambarus immunis*.

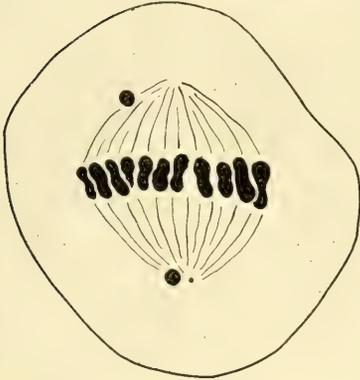
38 Polar view, primary spermatocyte showing 104 chromosomes, 96 ordinary chromosomes and the group of eight enclosed in a clear vacuole of the cytoplasm (fig. 112); *Cambarus immunis*.

39 Polar view, primary spermatocyte showing the clump of eight chromosomes within the clear vacuole (fig. 111); *Cambarus immunis*.

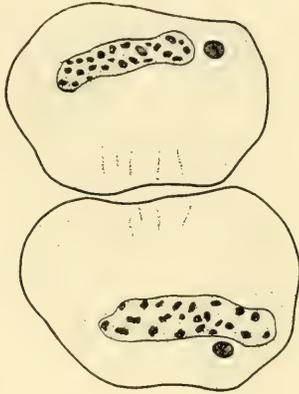
40 Polar view, primary spermatocyte, showing 104 chromosomes. The group of eight is indistinguishable from the other chromosomes; *Cambarus immunis*.

41 Polar view, secondary spermatocyte, showing 104 chromosomes; *Cambarus immunis*.

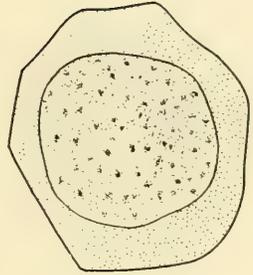
42 Polar view, secondary spermatocyte showing 100 chromosomes; *Cambarus virilis*.



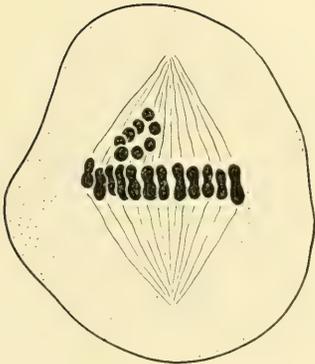
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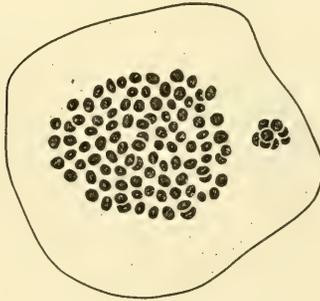
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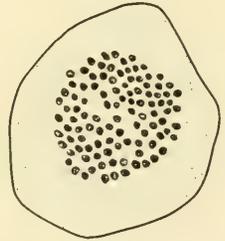
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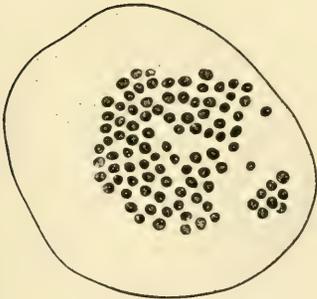
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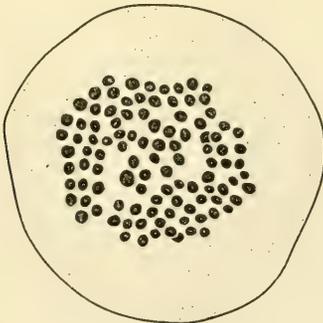
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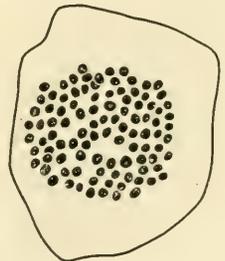
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PLATE 6

All figures from *Cambarus virilis*

EXPLANATION OF FIGURES

43 and 44 Second maturation division stages of a type C, primary spermatocyte.

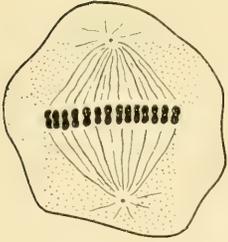
45 Spermatid, type C' containing no chromatoid bodies.

46 to 49 Second maturation stages of a type A primary spermatocyte. Figure 47 is from a smear preparation; figure 48 = figure 97, and figure 49 = figure 98.

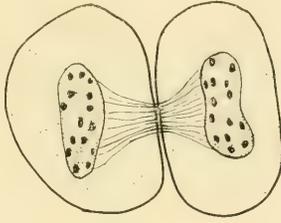
50 Spermatid, type A', with two chromatoid bodies in the cytoplasm (fig. 99).

51 to 53 Second maturation stages of type A primary spermatocyte. Figure 51 = figure 104, and figure 52 = figure 106.

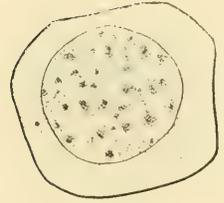
54 Metaphase, second spermatocyte division of a type B primary spermatocyte, showing one chromatoid body at one pole.



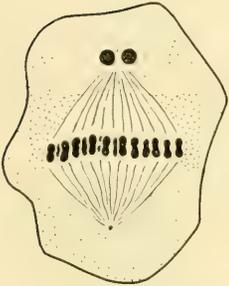
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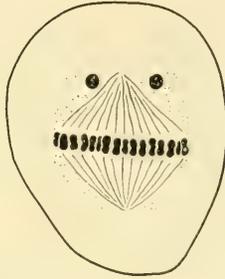
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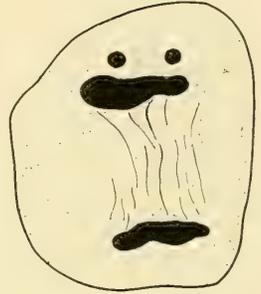
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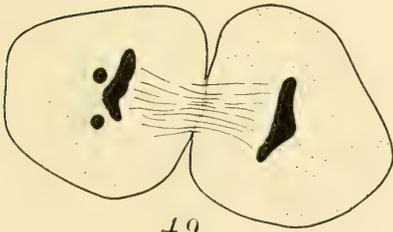
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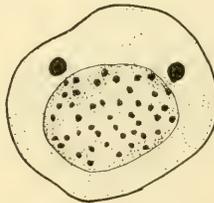
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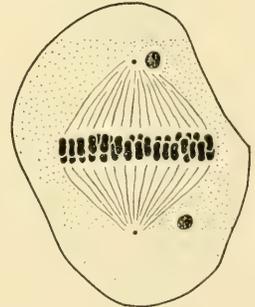
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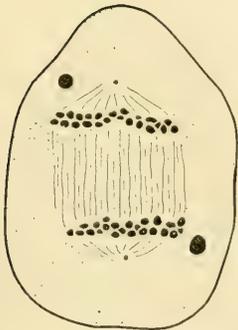
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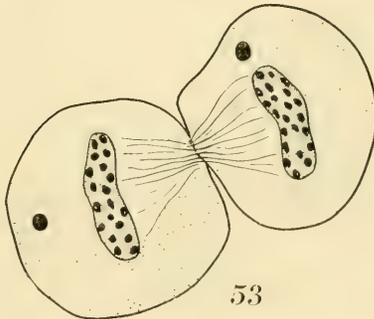
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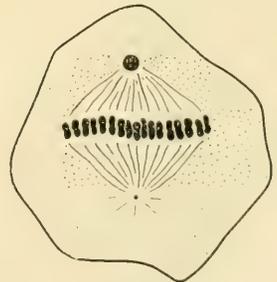
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PLATE 7

All figures, except figure 77, from *Cambarus virilis*; figure 77 from *Cambarus immunis*

EXPLANATION OF FIGURES

55 Telophase, second spermatocyte division of a type B primary spermatocyte showing one chromatoid body at one pole.

56 Spermatid type B', with one chromatoid body in the cytoplasm.

57 to 60 Successive stages in the transformations of the spermatid to form the spermatozoon; side views, drawn from smear preparations.

61 Top view of a transforming spermatid.

62 View of an oblique section of a transforming spermatid, showing the centrosome spinning out its flagella-like strands, *C*.

63 Top view of a transforming spermatid showing the same structure as that shown in figure 62.

64 Side view of a transforming spermatid previous to the formation of the rayed arms.

65 Side view of a transformed spermatid showing the rayed arms, *Z*, formed along the flagella-like strands, *C*, developed from the centrosome. The vesicle, *V*, is completely formed, and the nucleus, *N*, forms the head piece.

66 Top view, showing all the structures mentioned under figure 65, except *N*, which is hidden by the vesicle and the rayed arms.

67 Mature spermatozoon with the arms, *R*, tightly packed around the nucleus.

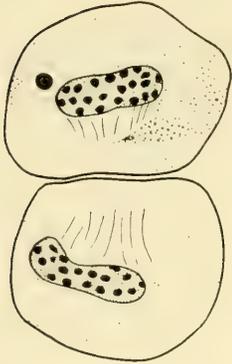
68 to 72 and 74 Uncoiling of the spermatozoon as observed in Ringer's solution H_2O , Na and K salts. Figures 69, 72 and 74 are top views; the others are side views.

73 Top view of an uncoiling spermatozoon, drawn from a stained preparation.

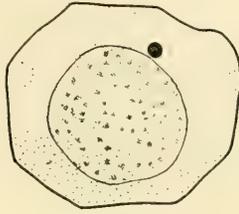
75 Side view of an opened spermatozoon showing the vesicle, *V*, the radiating arms, *R*, and the nucleus, *N*.

76 Top view of an uncoiled spermatozoon.

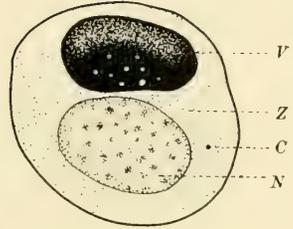
77 Eight-rayed type of spermatozoon, the fourth type found in *Cambarus immunis*.



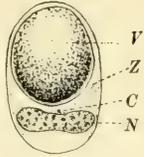
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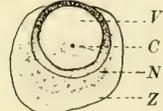
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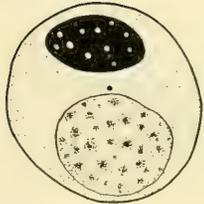
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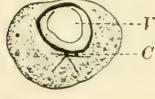
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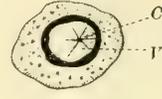
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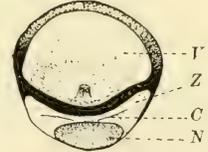
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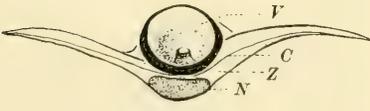
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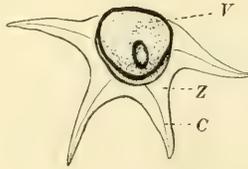
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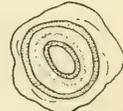
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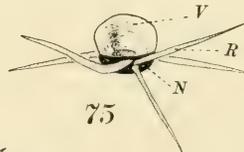
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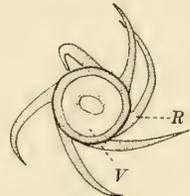
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PLATE S

EXPLANATION OF FIGURES

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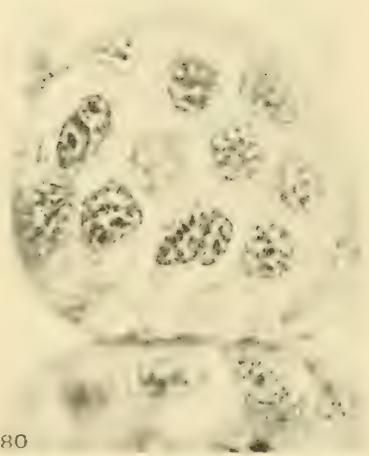
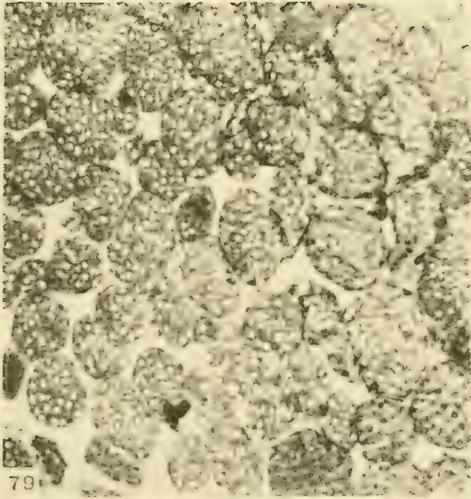
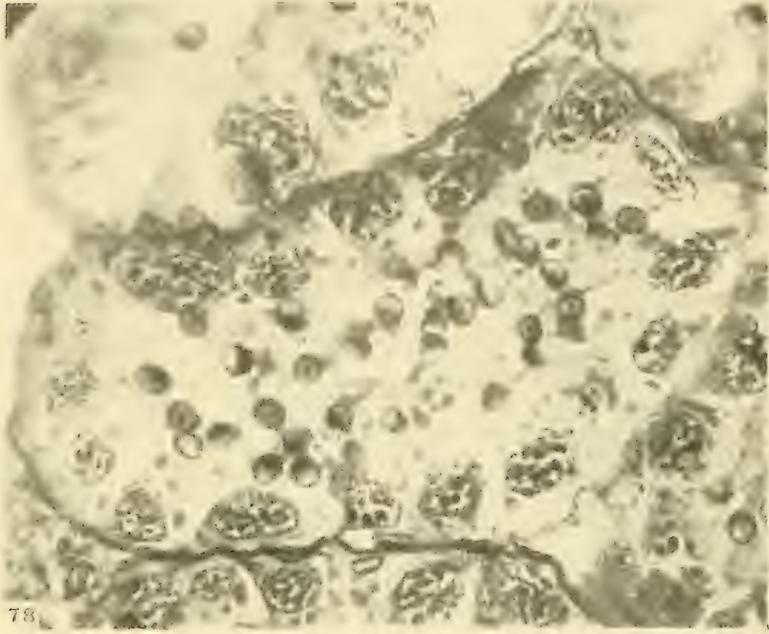
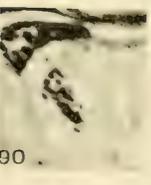
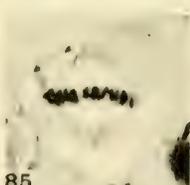
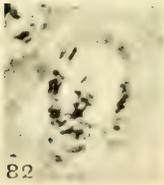
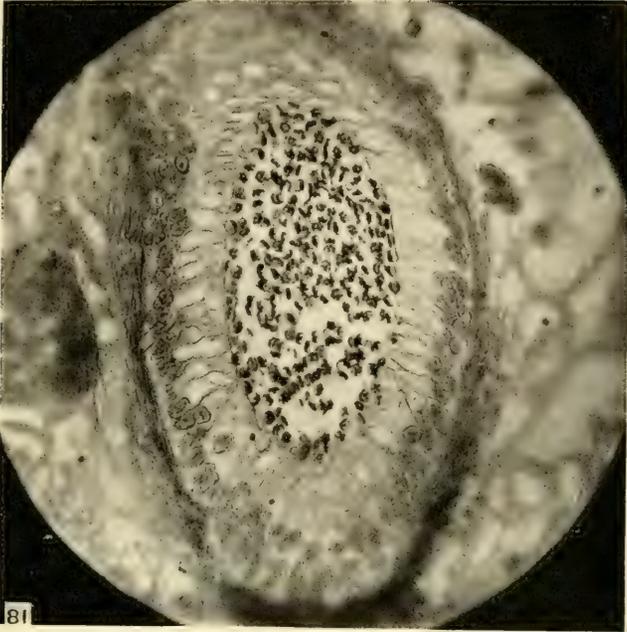


PLATE 9

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N. Fasten, photo.

PLATE 10

EXPLANATION OF FIGURES

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99 and 100 Early spermatids with two chromatoid bodies in the cytoplasm.

101 Metaphase, second spermatocyte, showing one chromatoid body migrating to one pole of the cell.

102 and 103 Telophase stage, second spermatocyte, illustrating the same behavior of the chromatoid body as that found in figure 101.

104 to 106 Division stages of second spermatocyte containing both bodies, in which each migrates to an opposite pole of the spindle.

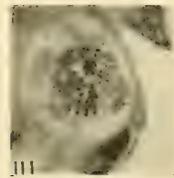
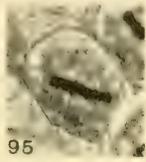
107 Early spermatid stage with one chromatoid body.

108 to 110 Trimorphism of the spermatozoa of *Cambarus virilis*, showing five-rayed (fig. 108), six-rayed (fig. 109), and seven-rayed (fig. 110) types.

111 and 112 Polar view of a metaphase stage, primary spermatocyte division, of *Cambarus immunis*, showing the group of eight chromosomes in a clear vacuole.

113 Metaphase stage, primary spermatocyte, *Cambarus immunis*, showing the group of eight chromosomes migrating to one pole.

114 Anaphase, primary spermatocyte stage, *Cambarus immunis*, showing the group of chromosomes at one pole.



A COMPARATIVE STUDY OF THE CHROMOSOMES IN ORTHOPTERAN SPERMATOGENESIS

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INTRODUCTION

Since the beginning of investigations into the phenomena of the maturation processes there have been conflicting views regarding the nature of the two mitoses which so closely follow each other without the customary resting stage intervening. Almost from the first this period in the history of the germ cells has been assigned great importance and a necessity for a thorough understanding of the facts has been realized. Among those possessed of an intimate knowledge of the complicated changes here taking place there has come to be a general agreement that of all the cell parts the chromatin gives every evidence of being primarily important. Along with this has arisen the conviction

that its organization is very precise and that the most tangible evidence of this is its method of integration into a definite and characteristic series of units in each kind of organism.

From many careful studies of a great number of different animals and plants the following facts have been determined beyond any reasonable doubt: 1. In both male and female—with a significant exception—there occur identical and duplicate series of these chromatin units. 2. During maturation this duplicate series is reduced in number to one-half by the joining of homologous pairs together. 3. In the course of the two maturation mitoses these paired elements are disjoined with the final result that each mature germ cell is provided with one representative of each pair. 4. When the paternal and maternal germ cells thus produced come together in fertilization there is restored again the duplicate series with which the process started.

There is nothing to indicate that any other parts of the cell have such significance as the behavior of these chromatin units indicates is theirs, and accordingly the phenomena of their junction and disjunction requires the most exact analysis. There are many complicated changes taking place here, and the conditions are such as to make it difficult, or even impossible in some cases, to determine exactly what are the facts. Each step in the process must however be understood and no effort should be spared to ascertain all the facts. Of the greatest importance are the stages immediately following the last division with the unreduced number of chromosomes. At this time, undoubtedly, changes of vital significance occur, but unfortunately they are very involved and difficult to determine in detail. However, one fact, which is the important one in connection with the present study, stands out clearly: the series of chromosomes present in the last mitosis with the unreduced number reappears in easily recognizable form in the prophase of the first maturation division. The only difference of importance is that instead of being present in a series of single elements, the chromosomes are now joined together in pairs so that the number of independent chromatin bodies is one-half of that in the preceding generation. But noth-

ing has been lost from the cell and, so far as the morphological identity of the chromosomes is concerned, no difference exists except their pairwise union.

The important phenomena of the synapsis stage will receive consideration in later papers by my students and myself. At the present time I wish merely to consider the comparatively simple conditions apparent in the late prophase of the first maturation division and in the succeeding mitoses. This is an important matter because from studies of these stages, and upon practically identical material, all the possible interpretations of the maturation divisions have been advanced. Diversity of opinion regarding maturation phenomena in unlike materials may therefore be due, as in this case, not to differences in fact but merely to variety of interpretation. Also, regardless of the exact nature of the synaptic changes it is of great importance to know just what takes place in the maturation mitoses, for often a correct interpretation of the prophase chromosomes is followed by a faulty account of their actual division later. No matter how accurately the processes involved in the formation of the tetrads is understood, there could be no solution of the problem of chromosome distribution in the maturation divisions so long as the present uncertainties regarding their movements continue. I therefore desire, in this paper, to discuss the actual movements of the chromatids, regarding it as determined that in the tetrads we have a fusion of homologous chromosome pairs longitudinally divided.

I shall attempt therefore a comparative presentation of the conditions during these later phases of the process, and shall venture a critical consideration of the papers of other authors on the same subject in the hope that a consensus of opinion may be reached. I venture to hope also that my long study of this subject, my extensive comparative acquaintance with Orthopteran material and the consistent results of my students may be taken into consideration in judging my effort to arrive at some conclusion in these disputed matters.

THE SPERMATOGONIAL CHROMOSOMES

a. Hippiscus type

It is aside from the purpose of the present paper to enter far into a discussion of conditions in this generation of the germ cells. In order that the facts may be before us it will however be necessary to state briefly what is known about the chromosomes at this time. For details reference should be had to Sutton's ('00) paper. A polar view of a spermatogonial metaphase shows 23 elongated, rod-shaped chromosomes, arranged radially about the spindle axis with fiber attachment at the inner end (figs. 20, 44, 77, 94, 98). In the anaphase the chromosomes separate, beginning at the *inner* end, and move to the poles of the spindle as simple straight rods. Later they lie side by side, approximately parallel, and become vesicular, with the chromatin on the periphery. During the prophase of the succeeding mitosis the chromatin contracts to the center of each vesicle and the definitive chromosomes of the late prophase reappear in the same relative positions they occupied in the preceding telophase. The cells of one young Chortophaga nymph showed an interesting modification of this condition. The largest four pairs, instead of being separate, were joined together at their ends and appeared in the form of *tetrads* with the fiber attachment at the point of junction (fig. 94). In the anaphase each chromosome commenced to divide, as usual, at this point and in these cases the anaphase chromosomes were V-shaped with the apex (synaptic end) directed towards the pole of the spindle. All the cells of this individual showed the same conditions.

b. Stenobothrus type

Exceptions to many general conditions are found in the appearances presented by the cells of *Stenobothrus*. This is a genus that has been extensively studied and, in general, correctly interpreted. Because of its exceptional nature many generalizations based upon it are wrong. This will appear clearly as the discussion proceeds. To avoid confusion I shall, throughout this

paper, consider *Stenobothrus* separately and it will be excluded from general statements except as indicated otherwise.

The spermatogonia of *Stenobothrus* show two important exceptions to the general statement already made. These are (1) the number of chromosomes is 21 instead of 23 (17 in some species, reported by others); (2) the point of fiber attachment may be terminal, subterminal or median instead of always terminal. For this latter reason the chromosomes are not always rod-shaped but may also be V-shaped or J-shaped. The important thing to note here is that the point of fiber attachment is a definite part of the organization of the chromosomes and is *constant*, not only for the cells of this generation but also for those of the first and second spermatocyte. I have material from only one individual of this species,¹ with 21 chromosomes, and in spermatogonia of this there are always 12 chromosomes with terminal fiber attachment and 9 with subterminal and median. One of these with median attachment is the accessory chromosome, so that there are four pairs with non-terminal fiber connections. The exact point at which this relation to the fibers is established may vary somewhat, but as may be seen in figures 59, 60, 61 and 62 it is easy to distinguish these elements from the ones with terminal attachment. What relation there may be between the difference in number of chromosomes and the shifting of fiber attachment I do not know, but the thought at once suggests itself that this may be due to an altered cross segmentation of a continuous spireme thread which, if there were continued chromosome individuality, would produce multiple chromosomes. Whatever may be the cause it is fortunate for our study that it operates, because it gives us a variation from the typical conditions in the group which is very helpful in coming to an understanding of the movements of the chromosomes in the maturation divisions.

¹I am not sure of the identification of this material. It was among a large collection of specimens put up for me by a student on an early expedition into western Kansas, and the specimen from which it came was not preserved. At the time I made my first study of it no other form but *Stenobothrus* had been reported with such chromosomes, but since then I have found them also in *Chorthippus* and *Chloëaltis* and Miss Carothers in some species of *Trimerotropis*. For this reason I can refer it to no genus with certainty.

c. Comparisons

1. *Acrididae*. The points of interest for this study regarding the chromosome condition in the spermatogonia concern (1) the number of chromosomes, (2) their sizes and forms, (3) the positions of their fiber attachments, (4) their arrangement in the metaphase plate and (5) their behavior in mitosis. I will briefly give the results of other investigators and then compare them with my own.

Buchner ('09) reports 23 chromosomes in *Oedipoda*, all rod-shaped, with terminal fiber attachment, and arranged in the typical way. He is however unable to recognize homologous pairs. Brunelli ('10) in *Tryxalis* finds 23 rod-like, radially arranged chromosomes of various sizes with terminal fiber attachments, but no recognizable pairs. Davis ('08), from studies on *Dissosteira*, *Arphia*, *Hippiscus*, *Chortophaga* and *Melanoplus*, distinguishes the 23 typical chromosomes showing the paired condition, radial arrangement and terminal fiber attachment. Granata ('10), in *Pamphagus*, is able to find only 19 chromosomes, all of which are typically Acridian in their form and behavior. Montgomery ('05) is the only investigator who reports an even number of chromosomes in any member of this family, and, in addition, a variation of the number within the genus. This number is 20 or 24 in *Syrbula acuticornis*. In other respects Montgomery's results are typical for the *Acrididae*, but the even number requires the accessory chromosome to be bivalent. My own students have invariably reached the same general conclusions regarding the nature and behavior of the spermatogonial chromosomes. Thus, Carothers in *Arphia*, *Brachystola* and *Trimerotropis*; Hartman in *Schistocerca*; Nowlin in *Melanoplus*; Pinnéy in *Phrynotettix*; Sutton in *Brachystola* and Wenrich in *Phrynotettix* find typical Acridian conditions throughout. Robertson ('08) also, in *Syrbula admirabilis*, after a very careful study, is quite unable to agree with Montgomery regarding the number of chromosomes and the paired condition of the accessory chromosome, and finds the chromosome behavior in this genus typical of the group.

The conditions in *Stenobothrus* have been reported with much uniformity by Davis ('08), Gerard ('09), Meek ('12) and de Sinéty ('01). Davis and Meek find definitely 17 chromosomes and Gerard suggests the same as his best enumeration. De Sinéty does not give definite evidence on this point. According to Davis the largest 3 pairs are bent rods and have non-terminal fiber attachments at the points of curvature; the remaining 5 pairs are normal rod-shaped elements with terminal attachments. In these matters Meek agrees, and, while the descriptions of Gerard and de Sinéty are not definite and specific, their general statements and figures substantiate Davis' findings. The accessory chromosome is not unusual in its behavior.

My own studies have extended over more than forty genera of Acrididae as follows: *Acrolophitus*, *Aeoloplus*, *Amphitornus*, *Arphia*, *Aulocara*, *Boopedon*, *Brachystola*, *Camnula*, *Chloëaltis*, *Clinocephalus*, *Dactylotum*, *Dissosteira*, *Encoptolophus*, *Eremnus*, *Hadrotettix*, *Hesperotettix*, *Hippiscus*, *Mecostethus*, *Melanoplus*, *Mermiria*, *Mestobregma*, *Orphulella*, *Paroxya*, *Philbotroma*, *Phrynotettix*, *Phaetaliotes*, *Proracorypha*, *Pseudopomala*, *Psinidia*, *Rhomaleum*, *Schistocerca*, *Scirtettica*, *Spharagemon*, *Stenobothrus*, *Syrbula*, *Trimerotropis*, *Tropidolophus*, *Tryxalis*. In all these the same conditions were found as have been described for *Hippiscus* regarding the number of chromosomes, their sizes and forms, the attachment of the fibers, arrangement in the metaphase and behavior during division, with the exception of *Stenobothrus*, *Chorthippus*, *Mermiria*, *Hesperotettix*, *Chloëaltis*, some species of *Trimerotropis* and, occasionally, *Chortophaga*. In *Stenobothrus* there is the same apparent difference in number, shifting of fiber attachment in certain chromosomes and difference in form and behavior in these elements as have been described by other investigators. *Hesperotettix* and *Mermiria* have the association between the accessory chromosome and certain of the euchromosomes that I have elsewhere described ('05) which produces an apparent change in the behavior of the complex, while a certain individual of *Chortophaga* in the early nymph condition showed a spermatogonial synapsis of 4 pairs of chromosomes.

It would seem very evident therefore that in the spermatogonia of the Acrididae we are dealing with a chromosome complex of a very definite and precise organization which, in the great majority of cases, presents itself without essential variation in number, size and form, fiber attachment, arrangement in the metaphase and behavior during division of its elements. *Stenobothrus* and *Pamphagus* seem to be definite exceptions in some of these respects, but doubtless were we more familiar with all the facts in the case a larger harmony would become manifest.

2. *Locustidae*. I desire to consider with much detail only the conditions in the Acrididae, but since exceptions to the general behavior of the chromosomes in the Orthoptera have been taken from studies on other families I shall be obliged to give them consideration. This I shall do as briefly as possible, going into details only when it is necessary.

My own work began on the *Locustidae* and I made a comparative study of *Xiphidium*, *Anabrus*, *Orchesticus*, *Scudderia*, *Conocephalus* and some others which I did not identify. So far as I was able to go at the time there seemed to be a uniformity here also, but of a somewhat different order. Instead of 23 chromosomes there appeared 33 and the accessory chromosome was very large and conspicuous. The chromosomes were rod-shaped, arranged radially in the metaphase with terminal fiber attachments, and they divided regularly into two equivalent straight rods by a cleft extending from the center outward in the plane of the equatorial plate—all except one chromosome in *Anabrus* which at the time I could not explain, but later recognized as a multiple. None of my students have worked on this family but other investigators have, and the result has been less uniformity in chromosome numbers than in the Acrididae but in other respects no essential differences. Thus Stevens ('05) reported 46 chromosomes in *Stenopelmatus*, but later recognized this as an error and gave the correct count as 47. In *Ceuthophilus* ('12) she found 37 chromosomes but in both cases no exceptional behavior. Davis ('08) and Meek ('12 a) in *Steiroxys* agree on 29 as the number present with no atypical behavior. De Sinéty ('01) studied *Decticus*, *Platyceles* and *Orphania* but gives the

chromosome number for only the latter where he found 31. Buchner ('09) however also gives 31 for *Decticus*, while Vejdovský ('12) reports but 23 for the same species. Taking these results at their face value it is evident that in the Locustidae there is an unequal diploid chromosome group with an unpaired accessory chromosome and all the other characters that have been considered in connection with the Acrididae.

3. *Gryllidae*. Careful detailed studies of the chromosome relations in the Gryllidae are few. Baumgartner ('04) reports 29 chromosomes in *Gryllus assimilis* and 21 in *G. domesticus*. This latter number was also found in the same species by Guthertz ('07) and in *G. desertus* by Brunelli ('09). Buchner ('09) and Voinov ('04) have both studied *G. campestris* but without giving any results of value on chromosome relations. *Gryllotalpa* has been studied by Buchner, Payne, vom Rath and Voinov. Vom Rath ('91) reported at length on the spermatogonia of this animal and his figures were widely copied, but it is very evident that he was fundamentally in error regarding the most essential part of his work. He announces 12 as the diploid chromosome number but the internal evidence and the results of other investigators show that this is really the haploid condition.

4. *Phasmidae*. Studies on the Phasmidae are not numerous. Practically the only ones from a modern standpoint are those of de Sinéty and Jordan and in the former case the work is lacking in needed detail. De Sinéty finds 36 chromosomes in *Leptynia* with a typical accessory chromosome joined to a tetrad in the spermatocyte. If this be counted a separate chromosome for each univalent part there is an odd number. Jordan ('08) seems to determine definitely the number 35 in *Aplopus*. Since de Sinéty's enumerations are evidently not made with great care it is possible that in *Leptynia* the same number is present. *Dixipus* and *Menexenus* were also studied by the French investigator but no chromosome counts are given.

5. *Blattidae*. Of the cursorial Orthoptera the Blattidae have been most studied. Morse ('09) investigated four genera and found in *Blatta*, *Leucophaea* and *Stylopyga* a diploid group of 23 paired elements of typical Orthopteran behavior, while in Peri-

planeta there were 33. *Blatta* has also been studied by Stevens ('05) and Wassilieff ('07) with the same numerical results. Farmer and Moore ('05) report 32 chromosomes for *Periplaneta* plus a conspicuous nucleolus which the authors believe is lost during each mitosis. The work of the other students of the Blattidae makes it certain however that the English investigators incorrectly interpreted the behavior of the accessory chromosome which is characteristically Orthopteran in this family.

6. *Forficulidae*. The Forficulidae, taxonomically, are not now regarded as a family of the Orthoptera and their germ cells show a characteristic difference in the nature of the accessory chromosome. According to Randolph ('08) in *Anisolabis*; and Stevens, de Sinéty, Zweiger, and possibly St. George, in *Forficula*, there is an even number of chromosomes, 24, in the spermatogonia. Of these, according to Stevens, one pair constitutes an unequal tetrad in the first spermatocyte and has all the characters of an idiochromosome pair of the Hemiptera and Coleoptera.

d. Summary of results on spermatogonia of Orthoptera

From the foregoing statements I believe the following conclusions are justified: (1) The diploid number of chromosomes in the Orthopteran male is uneven; (2) The diploid complex in each case may be resolved into a duplicate series characterized by differences in relative size; (3) an odd element, sometimes distinguishable by excessive size, may not thus be grouped with a mate and is further marked by occasional peculiarities of behavior; (4) the typical form of the spermatogonial chromosome is a rod which takes a radial position in the equatorial plate of the metaphase with the fiber attached at the inner end; (5) in division the longitudinal halves of the chromosome separate in the plane of the equatorial plate, beginning at the inner end, and pass to the pole as straight rods; (6) an exception to (4) and (5) is furnished by certain chromosomes of *Stenobothrus*, *Chorthippus*, *Chloëaltis* and *Trimerotropis* which are J- or V-shaped with the fiber attachment at the bend.

SYNOPSIS PERIOD

As has already been stated, we have no exact knowledge of the changes taking place at this time. Their significance is unquestionably great, and no pains should be spared to determine their character fully. But even were they completely understood we should still be lacking a continuous picture of well defined, individual chromosomes throughout their entire history for, succeeding the synaptic phase, comes a period when the chromosomes become very diffuse and their outlines with difficulty distinguishable. We may not therefore hope to be able to recognize the chromosomes in a compact and sharply defined form through the various stages of their development. That they are however morphologically continuous seems to me unquestionably evidenced by the fact that, succeeding all these involved and complicated changes, there is finally a reappearance of the same series of elements in the same cell, with the one difference that instead of twenty-three single chromosomes in the *Acerididae* there are now eleven pairs, plus one chromosome that continues on unchanged. I believe, therefore, that we have every reason to consider that in the prophase of the first spermatocyte we are dealing with the same chromosomes that came into the cell when it was produced by the division of the last spermatogonium. Whether union was made side by side or end to end in the synaptic period has not been determined, but it is very clear that in the last prophase the chromosomes show undoubted telosynapsis.

CHROMOSOMES OF THE FIRST SPERMATOCYTE PROPHASE

a. Evidence for chromosome individuality

Fundamental to the entire conception of chromosome relations is the belief that we are dealing with persistent morphological entities. If this be not true then the whole question of the constitution of the chromosomes and the method of their division is of no theoretical importance. That they are of this definite and significant character is however evidenced in many ways. In

a later paper I hope to enter into this matter more fully, but for the present I will merely indicate the facts relating to Orthopteran spermatogenesis which support this view.

(1) There is the fact of greatest significance that in each cell division they perpetuate themselves, and by a process suggestive of the utmost accuracy and importance. The means for conserving the continuity of each chromosome is therefore clear. (2) The result of the operation of this process is demonstrated by the observation that not only does the individual animal show in each of its cells the same series of elements but all the members of the species, genus and even family, with few exceptions, have it. The observed process and the results of its operation through countless cell generations is thus demonstrated. Accordingly, any one chromosome has the form, size and behavior which characterize it for the reason that it is the direct material descendent of a line, each member of which possessed these same characteristics.

It ought not to be necessary, in writing for biological readers, to say that in making such a statement I do not in any sense impute omnipotence, omniscience or immutability to the chromosomes. But the absurd lengths to which some opponents of chromosome investigations have gone in attempting to break down the facts regarding them perhaps make it desirable for me to add that I have always considered the chromosomes as a part of the organic mechanism, reacting to each other and to their environment, as all living substance must do in order to exist. They neither make the organism nor are they made by it, but they do perpetuate themselves and perform functions of vital importance to each cell and to the entire organism. My position in this matter is more fully outlined in a former paper ('08).

In addition to these two facts regarding the reproduction of the chromosomes there are others which speak strongly for their individuality. These concern questions of organization and behavior and will be referred to more at length throughout the paper. At this point I will briefly mention them: (3) Constancy in relation to the spindle fibers. In almost every case the attach-

ment in the spermatogonia is at the end of the chromosome and when they join in pairs these ends are in contact, so that in the first spermatocyte the tetrads have the form of V's with the same orientation to the spindle as in the previous generation (figs. 32-58). In *Stenobothrus* however there are median and subterminal attachments and *these are the same in both generations of spermatocytes as in the spermatogonia* (figs. 59-76).

(4) Occasionally there are peculiarities of behavior which are constant and uniform. In *Hesperotettix* there is a union between the accessory chromosome and one of the tetrads. The members of this combination are always identical and their union never fails. Moreover in different species the proportionate size of the parts varies in conformity with the structure of the body. Thus figure 79 shows the condition in *H. speciosus*, figure 80 in *H. viridis* and figure 81 in *H. pratensis*. In *Brachystola*, as Miss Carothers has discovered, there is a pair, the members of which are unequal, and this is a constant condition accompanying their separation in the first spermatocyte. In *Phrynotettix* Mr. D. H. Wenrich has found an unequal pair also, one member of which is distinguishable from the other by its granular condition in the metaphase. In this case however the reduction division occurs in the second spermatocyte. This element in the first spermatocyte is shown in figures 88 to 91 and will be described in detail by Mr. Wenrich.

Supporting in every way these observations upon Orthopteran cells is the consistent background of similar facts in other groups of animal and plants and the significant parallel between their behavior and character development in breeding experiments. The persistence of individual chromosomes in multiple complexes of giant cells and heterospermized eggs is conclusive evidence of their real individuality and continuity. There are many other facts of this character to demonstrate the individuality and morphological continuity of the chromosome, but for the present purpose these will serve to show the foundation for my belief regarding them.

b. Relation of first spermatocyte chromosomes to those of the spermatogonium

(1) If then we are dealing with a morphologically continuous series we must recognize in the twelve chromosomes of the first spermatocyte the twenty-three chromosomes of the last spermatogonial division. Regarding one of these, the accessory chromosome, there can be no question. After the last spermatogonial division it removes itself from the others, often into a separate vesicle, and, during all the complicated changes of the synaptic period, may be recognized as a separate and distinct structure. It does not divide in the first spermatocyte but, in the form characterizing it in the spermatogonial metaphase, it passes to one pole of the spindle and is included in but one of the two daughter cells (figs. 36 and 45). In the second spermatocyte, showing the same form as in the two preceding generations, it takes its place in the equatorial plate and is divided like its fellows. In this one case the chromosome may be traced with little change through the whole of this period.

(2) There remain to be considered the twenty-two other chromosomes. It may be seen in the spermatogonial metaphase that these are not of the same size but form a series, the largest of which is many times the length of the smallest. When these are studied carefully it is found that for each size there are two representatives (figs. 44 and 98). In the first spermatocyte there are eleven chromosomes, each composed of two approximately equal halves, the point of contact being in the middle of the length of the elongated rod. If the chromosomes are morphologically continuous there is no other conclusion possible than that we have here the twenty-two spermatogonial chromosomes united into eleven pairs. Still more conclusive on this point are the conditions in *Stenobothrus* where not only the duplicate series of chromosome sizes appears, but where, because of variations in fiber attachment, there is found a similar duplication according to form.

It is probably true that until the relations of the chromosomes during the synaptic phase are definitely determined it will not

be possible to assert unequivocally that the longitudinal axes of the paired chromosomes of the first spermatocyte represents the coincident axes of the spermatogonial chromosomes constituting it, for if there be a parasynapsis at any period there is a possibility that the doubly split thread may open out along the plane of the equational cleavage instead of along the space between chromosomes. In either event the form of the resulting chromosome would be the same. There are however some criteria for judging such relations and these, taken in consideration with the movements of the chromosomes in the spermatogonial divisions, make it appear highly probable that the evident telosynapsis of the late prophase actually exists as such.

Two observations strongly support this conclusion. There is first the case of the unequal tetrad, where two spermatogonial chromosomes of unlike size are joined together end to end. If the chromosomes are persistent individuals there can be no questioning this relation. Again, in the multiple chromosome, we have established the coincidence of the longitudinal cleft of the accessory chromosome with that of the tetrad to which it is joined. Because the accessory chromosome is a simple spermatogonial element its plane of cleavage is determined beyond doubt, and since in the spermatogonia it is joined end to end with one-half of the tetrad, the latter's cleft is identified. The remaining member must, of necessity, be joined by its end to its mate and so continue its division plane with that of the other members of the multiple. An additional, though less obvious piece of evidence, is furnished by a comparison between the chromosomes, especially the annular ones, of *Hippiscus* and *Stenobothrus*. It seems evident that there are here two types of division for certain chromosomes, provided the earlier history of each is the same. All the structural relations are similar in these two cases except the position of the fiber attachment and it is difficult to believe that their early history is different. If they are similarly constituted then the presumption is strong that those which lie with their length in the equatorial plate of the first spermatocyte are divided along their longitudinal cleft while those placed in the axis of the spindle with fiber attachment midway between

the ends of the univalent components would then suffer a reduction division.

To this comparatively direct observational evidence must be added the strong presumptive proof that the sister halves of a chromosome remain closely united here as in other generations of cells, and that separation between the parts of a tetrad is much more likely to occur along spaces between whole chromosomes. If this were not true, and if the annular space of the ring chromosome and the angle of the V-shaped elements should be the longitudinal cleft, then there exists a fundamental alteration in the composition of the chromosome and a wide diversity in the behavior of daughter chromatids not only in different species but also in the cells of one organism. From all these considerations it therefore appears highly probable that the rod-shaped tetrad of the late prophase and metaphase of the first spermatocyte represents two spermatogonial chromosomes joined end to end with their longitudinal clefts continuous. We have therefore to determine the relations of the various forms of the first spermatocyte prophase chromosomes to this typical element, and then to trace these into the metaphase of this division, and through the anaphase, in order to establish the fate of the parts of the tetrads.

c. Forms of prophase tetrads

There is apparently a great diversity of form in the prophase chromosomes, but while this is true, so far as external configuration is concerned, in the fundamental matter of organization the rule is uniformity. In discussing this organization certain elements and relations must be regarded. They may be summarized as follows: number of parts—four chromatids; relationship of parts—one pair of maternal, one pair of paternal chromatids, each pair produced by the longitudinal division of a mother chromosome, all four homologous; spatial relation of parts—daughter chromatids more or less in contact along the plane by which they were produced from the mother element, homologous chromatids united endwise; attachment of spindle fiber—at the same point as in previous generations, as a rule at the point where

homologous chromosomes are joined together, but occasionally at the opposite end or between the extremities. All the features enumerated are constants except the one involving the spatial relations, and to the variations here occurring are due the diverse forms of chromosomes found in the first spermatocyte. The problem is therefore to determine how it is possible to derive from a chromosome consisting of four similar chromatids, with constant and uniform fiber insertion, the various rods, rings, crosses, V's, double V's, K's, X's, U's, I's, S's, and other less common forms, without alteration of structure and merely by change of the spatial relations of the four elements.

A written description of these permutations is not easy, but it is a very simple matter with a clay model to produce every form to be found merely by changing the relative positions of its four parts. These changes fall into three general types of movement: (1) flexures in the plane of the longitudinal cleft, (2) movements of the chromatids in a plane normal to that of the longitudinal cleft, (3) divergence of the four chromatids at the center while the ends remain in contact. In addition to these simple movements there are (a) combinations of these and (b) rearrangements of the four chromatids resulting in various complicated forms. As mitosis proceeds these combinations, especially of the first (a) kind, become more common and in the metaphase and anaphase, the rule. The point at which movement occurs in classes (1) and (2) is at the center of the rod where homologous chromosomes are joined together, and this fact cannot be devoid of significance.

The simplest relations are those found in the rod-shaped chromosome where the four parts lie extended along one axis. These I-shaped tetrads are commonly the smaller ones of the complex and do not often occur without some enlargement at their middle where the homologous chromosomes are fused. They represent the final step in the divergence of the joined parental chromosomes which, if there be an earlier parasynapsis, finds its opposite in the side to side position of these units (fig. 117).

Related to this simple form are those of the first category produced by flexure in the plane of the longitudinal cleft, and which

may be grouped into those having free ends and into those with the ends joined. In most species the majority of the chromosomes belong to the first of these types, but in some cases more of them are joined terminally. With but few exceptions the members of any complex will be found included in these two categories of the first group. As a result of these flexures the chromosomes have the form of V's, U's, incomplete rings and complete rings. There is no essential difference between the V-shaped chromosome and the annular form except the extent to which the bending has taken place, or, if there was a previous parasynapsis, the degree of separation of the ends. Movement of the chromatids in a plane normal to the plane of the longitudinal cleft results in the formation of various cruciform tetrads. These changes are likely to occur in the smaller members of the complex and result in the gradual separation of daughter chromatids and the approximation of homologous derivatives. If carried to the extreme the final result is the production of the same I-shaped chromosome as that present at the beginning of the process but with a transposition of the two axes, the shorter, transverse one coinciding with the original longitudinal cleft of the two homologous chromosomes.

Commonly there are flexures of the longer arms of the cross producing a variety of forms depending in part upon the point of view. When placed obliquely the cross may appear X- or K-shaped. Divergence of the four chromatids, except at the ends, constituting the third class of movements, produces the double V-shaped element which appears under a variety of forms when viewed from different angles. One of the included spaces represents the longitudinal cleft of the homologous chromosomes, now bent at an acute angle to each other; the remaining one, lying in a plane at right angles to the first, is the space between the homologous conjugants. It seems impossible to distinguish these apart in most cases. Such a separation of the chromatids is only temporary and a double-V chromosome is never seen in the metaphase. All the tetrad forms are most clearly differentiated in the late prophase, just before going over into the homogeneous condition of the metaphase, for at this time there is less of irregu-

larity in direction and contour of the parts. Nevertheless the various forms of this late period may be traced back by gradual steps into the earliest stages of the prophase where all the chromatin elements are in the form of very elongated, delicate threads such as are shown in figures 99, 101 and 105. These stages are best seen in smear preparations in which the nuclear elements are more isolated and where their parts are more distinct. There can be no doubt of the composition and movements of these tetrads and the only uncertainty relates to the early stages where, for a time, it is impossible to trace the individual chromatids. Whether telosynapsis alone, or parasynapsis in connection with it, is the history of chromosome association has not yet been definitely determined, but however this may be, it is certain that the four parts of each chromosome are derived by the longitudinal splitting of two homologous spermatogonial chromosomes.

CHROMOSOMES OF FIRST SPERMATOCYTE METAPHASE

a. Identification of first spermatocyte metaphase chromosomes with those of the prophase

At the end of the prophase the forms of the chromosomes have been established and, upon the dissolution of the karyotheca, they lose their granular character and become homogeneous. Aside from this they suffer no important change and all the forms seen in the prophase, with the exception of those resulting from wide separation of the chromatids at the middle of the tetrad, may be traced into the equatorial plate. Since however there have been extensive and continued movements of the chromatids with relation to each other, it is only reasonable to expect their continuance during the metaphase, and this, in fact, occurs. Regarding however the late prophase and early metaphase it may be stated that the chromosome forms of the early condition may be traced directly into the later, and what changes there are may be ascribed to a continuation of processes already in operation. It is essential to note this, for by some the bipolar condition of the cell is considered to inaugurate entirely new conditions and the separa-

tion of the chromatids to follow from these changed circumstances. While there may be a period of equilibrium established at the metaphase the character of the chromosomal movements does not materially alter.

b. Movements of chromatids in metaphase

The metaphase movements of the chromosomes have given rise to the most contradictory accounts, due often to the lack of an exact acquaintance with the real structure of the tetrads, but more frequently to erroneous interpretation of the optical images produced by the homogeneous and more or less translucent chromosomes. I shall therefore first consider the various forms of chromosomes, the movements of their chromatids, their relation to the spindle fibers, and will then attempt an interpretation of the accounts given by other investigators.

The simple, unaltered, rod-shaped form is rarely seen in the metaphase for the reason that, as the chromosomes take their position in the equatorial plate, the points to which the fibers attach approach nearer the center, leaving the free ends to be drawn closer together at the periphery of the cell, as is shown typically in figures 2, 33, 46 and 92. While the statement is true regarding the infrequency of this form of chromosome viewed from the pole of the spindle in early metaphase, the opposite is the fact when chromosomes are regarded laterally in late metaphase or early anaphase. Under these conditions practically all the elements of the equatorial plate are long, rod-shaped chromosomes extended in the axis of the spindle. No matter what may have been the shapes of the chromosomes at the beginning of the metaphase they all are drawn out into rods just before they separate on their way to the opposite poles of the spindle. Lateral views of these conditions are represented in figures 8, 9, 10, 78, 96 and 97 where the complexes are extended in a line. That the same chromosome of two complexes may show itself lying in the equatorial plate in one and elongated at right angles to this position in the other may be seen by comparison of these groups.

While therefore the unmodified rod lying in the equatorial plate is rare, the form produced by bending in the plane of the longitudinal cleft to form V's and U's is very common. In *Mecostethus*, as may be seen by inspection of figures 32 to 34, 37, 41 to 44 and 46, practically all of the long, slender chromosomes are V- or U-shaped except such as extend across the plate, in which case they are more or less straight, simple rods. It is rare to find so much uniformity of shape as appears in this genus, but its significance with regard to the movements of the chromatids is great. Lateral views of the *Mecostethus* chromosomes at this time show that while the small ones are extended in a plane of the spindle axis (fig. 39) as in other species, the larger ones suffer an earlier separation of the chromatids and become diamond shaped with an open center (fig. 35) instead of linear and solid as in most cases. This is merely the precocious appearance of a chromatid separation which occurs in the early anaphase of all species.

Should the outer, divergent ends of the V-shaped chromosomes become approximated (or should they remain united, if the reverse is the character of the movement), annular chromosomes are produced. Much confusion regarding these structures exists needlessly in the literature. The method of their formation is clear and unmistakable and transitional forms are so common as to point definitely to the relations existing between them and the V- and U-shaped forms. In figure 2, for instance, the upper chromosome is a ring but the outer ends are only slightly joined. With just a little wider separation the form would be similar to the next chromosome to the left, a little more space and it would be shaped like the second one to the left, and with wider divergence of the ends would approach the type of the one below and to the right. In figure 2 there are at most two ringed-shaped chromosomes, while in figure 3, from the same animal, there appear six without divergent ends. It is evident that some of the chromosomes which in figure 2 are V's are, in figure 3, rings. Similar evidence is afforded by the chromosome groups of *Tropidolophus* shown in figures 21 to 30, in which the annular chromosomes vary in number from three to seven. As a rule the larger

elements tend to become ring-shaped, but sometimes fail to pass the V-condition and then may be identified with the same sized element in other cells. Such relations appear in the cells of *Tripodolophus*, just referred to, and also in cells from *Mestobregma* shown in figures 92 and 93.

In many cases the approximation of the outer ends of the chromosomes does not pass beyond their mere endwise fusion, but in some species there is a marked tendency for this portion of the chromosome to take on the same form as the inner end, or to exceed this and to form a second ring. In such cases the outer ring has its plane at right angles to that of the inner. Elements of this type appear in figures 21 to 26, 28 and 30. They are lacking in the cells represented in figures 27 and 29 from the same animal. A lateral view of an incomplete second ring is shown in the chromosomes at figure 25 b and at the right of the row in figure 97.

All the possibilities of form, due to movements of the chromatids within the plane of separation corresponding to the longitudinal cleft of the homologous chromosomes, have been considered, and mention has been made of the movement of these elements when they pass from the plane of the equatorial plate to that of the spindle axis. The latter condition is added to the other changes in form in varying degrees depending upon the extent to which it has already taken place in the prophase. During one or the other of these periods this gliding of daughter chromatids along the plane of their cleft and the subsequent approximation of homologous chromatids in a plane at right angles to this must occur. In *Mecostethus* all this transposition falls in the metaphase, for the elements start this period as rods or V's extended in the equatorial plate. Polar views of such stages are shown in figures 32 to 34, 37, 41 to 43, and 46, and in figures 35, 36, 39, 40 and 45 are lateral views. Figure 38 shows individual chromosomes from different angles with a prophase chromosome at the bottom for comparison. In this case it is demonstrated beyond any question that the separation of the elements is along the length of the chromosomes and that they recombine, for a time, by the approximation of homologous chromatids in the

plane of the spindle axis. The extremes of such a process are shown by two chromosomes lying side by side in one cell as represented in figure 64 e.

While for the sake of convenience in analysis the conditions of the prophase have been considered separately from those of the metaphase, the facts indicate that the changes in form are continuous and may proceed in varying degrees in different chromosomes during any one phase. For the same reasons the different chromatid movements have been reduced to types, but it is observed that they are rarely simple and that they occur in different combinations so that in most cases, in any one chromosome, it is necessary to resolve the changes into their components. When this is done for the metaphase chromosomes it is found that in general there is more or less bending or flexure in the plane of the longitudinal division combined with varying degrees of extension of homologous chromatids side to side at right angles to this. These two movements are sufficient to account for by far the greater number of metaphase chromosome forms and exclude the possibility of the occurrence of fundamentally different chromatid arrangements.

c. Relations of chromosomes to archoplasmic fibers

(1) As a part of the relations in the metaphase there must be considered those between the chromosomes and the archoplasmic fibers. It is not pertinent, in Orthopteran cells, to speak of the fibers attaching to the chromosomes as 'mantle fibers' for the reason that the spindle encloses the area occupied by the chromosomes and of itself constitutes a mantle (fig. 129). Nevertheless there are definite fibers attaching to the chromosomes and the relation thus established is most precise and constant. With but few exceptions fiber attachment is at the center of the rod or V-shaped chromosomes or, in the various modifications of this simple form, at the corresponding place, which is the point of fusion between homologous spermatogonial chromosomes. Directly related to the form of the tetrad and to the character of its movements is this fiber attachment, for it fixes the position of the chromosome

in the equatorial plate and guides the passage of the chromatids in their divergence. The result is that all of the chromosomes take up such a position that the synaptic points lie toward, or near, the spindle axis, and all of the chromatids execute like convergent movements toward their respective spindle poles. The position that the chromosomes take in the first spermatocyte metaphase and the position of their fiber attachment are seen, by a comparison with an equatorial plate of the spermatogonium, to be the same in both generations, the sole difference being that in the earlier generation each chromosome is free and independent while in the later they are joined by their corresponding inner ends into pairs. These conditions are clearly evident from a consideration of the two stages in a form like *Mecostethus*, as may be seen by comparing figure 44, representing a polar view of a spermatogonial complex, and figure 43 showing a like aspect of a first spermatocyte group. There are the same twenty-three elements in the same relative positions to the spindle and fibers, the one difference being the pairwise union.

From all these facts it is reasonable and fair to conclude that there is a typical organization of the tetrad which extends even to the relation which it bears to the archoplasm. In *Hippiscus* and most other Acridians this is of the character just described, but there are a few exceptions and these are of particular interest because of the strong evidence they give regarding precision of chromosome organization.

(2) In *Stenobothrus*, *Chorthippus*, *Chloëaltis* and *Trimerotropis* are found conditions that differ somewhat from the account which I have just given as characteristic of the Acrididae. These differences, at first suggestive of underlying, essential changes in type, are found on careful study not to be such, but only individual modifications of certain features by a few chromosomes. The same precision of organization obtains, but details are modified. Apparently the differences are due to changes in the position of the fiber attachments, but whether this is the result of inner structural modification of the chromosomes themselves or whether it is an archoplasmic change does not appear with certainty. Considering all the intrinsic permutations of the chromosomes, it

would seem probable that this changed external relation is the result of internal organization. Whatever be the cause the results are definite and exact.

During the prophase, indications of change are wanting until in the late stages, but upon the establishment of the full mitotic figure marked differences in appearance of certain chromosomes are at once apparent. These are perhaps not so prominent in polar views, although the familiar clear rings are wanting and the common V-shaped elements are few, but regarded laterally most striking differences at once appear. The prophase rings, which later were missing in their customary position in the equatorial plate when viewed from the pole, are very evident now, extended instead, at right angles to this position (figs. 63 and 64 a, 66), and there are unfamiliar E-shaped chromosomes (fig. 65) and asymmetrical rings (fig. 64 g). Occasionally there are rings lying in the plane of the equatorial plate where they form part of an E-shaped element, as is represented by the large chromosomes at the left of figure 63. There are also elongated rods with bent ends (fig. 64 b) and among these unusual forms appear the common rods and crosses (figs. 63, 64 e, 64 f, 65). The accessory chromosome, instead of being rod-shaped and going thus to the pole of the spindle, is V-shaped with fiber attachment at the center (fig. 66).

An analysis of these conditions, in comparison with the spermatogonial chromosomes which enter into the first spermatocyte, and with the second spermatocyte chromosomes which emerge from it, gives definite evidence of the cause underlying the changed conditions in these species. Primarily they seem to be due to a shifting of the fiber attachment from the end of the chromosome to the middle or to some intermediate point, as was described for the spermatogonia. Whatever the position of the fiber insertion in the earlier generation it is carried over into the first spermatocyte and appears again in the second spermatocyte. If this be at the center of the chromosomes when they join in synapsis, fusion takes place at both ends producing a ring with a possible divergence of the chromatids at each contact end. If such lateral extensions occur they must disappear later when the halves

of the chromosomes separate. The two conditions are shown in figure 64 a, being a drawing of chromosomes from the same cell, and in figure 66. Occasionally rings are formed when the fiber insertion is not median, and in that event divarication of the chromatids takes place only on the side of the larger members (fig. 65, next the E-chromosome) or it may be that the opposite result is reached (fig. 64 g). More commonly however, when the fiber attachment is subterminal, rings are not formed and synapsis occurs between the longer limbs only. This is commonly true of the larger pair of spermatogonial chromosomes and the result is the E-shaped chromosome or some modification of it. In this element it not infrequently happens that the divergent ends, at the point of fusion, become much extended in the equatorial plate at the expense of the arms attached to the fibers, and swing round in the form of a ring. This part when viewed laterally appears as a much extended middle bar of the E, but when seen from above exhibits the ring form modified in appearance by the bent arms above and below.

All the evidence indicates that the composition of the tetrads is the same in all of the Acrididae studied. There are the same elements of the tetrads to be distributed in each case. It is therefore apparent that the method of this distribution must differ in *Stenobothrus* and other similar forms, with rings extended in the axis of the spindle, from that in such as have rings lying in the equatorial plate. A comparison of the annular chromosomes of these two types is most suggestive and seems to offer unquestionable evidence of the distinction between chromosomes that suffer a reduction division in the first spermatocyte and those that are divided equationally. In considering the work of different investigators upon the question it will be necessary to enter into this comparison more fully, but in order to give a basis for this I will here briefly note what appear to be essential elements involved in the discussion.

(1) The composition of the rings is the same in all cases and the point of fiber attachment is fixed for each chromosome.

(2) Rings that divide longitudinally in the first spermatocyte lie extended in the equatorial plate and have the fiber attachment

at the synaptic end, which lies toward the spindle axis. In the anaphase the daughter chromosomes go to the pole as simple V's (figs. 5, 6, 7 and 35).

(3) Annular chromosomes dividing transversely lie extended parallel to the spindle axis and have the fiber attachment at the middle of the chromosome or near the middle. The anaphase chromosomes are double V's (fig. 67).

It is necessary also to consider those other chromosomes with median or subterminal attachment of fibers which do not take on the form of rings. For our present purpose it is sufficient to note that they differ only in the degree of their chromatid movements from the rings and in the late prophase are indistinguishable from them. The chromosomes shown in figures 64 b, c, d and h may have been rings like figure 64 g or their parts may never have come into the ring association. The points of interest to note here is that such chromosomes, whether rings or not, always show in the anaphase double V-shaped forms.

CHROMOSOMES OF SECOND SPERMATOCYTE METAPHASE

The conditions of most interest for this study with regard to the second spermatocyte reveal themselves fully only in the metaphase condition, but it is necessary to note briefly some of the earlier phases. Of course, so far as the accessory chromosome is concerned, it is found in but half this generation of cells, but, since this does not otherwise influence the chromosome relations, it will not be necessary to consider the matter further at this time. The distinction between the first spermatocyte telophase and the second spermatocyte prophase cannot be sharply drawn, for the reason that in some species there is no loss of form by the chromosomes, and the only indication of the prophase condition is the presence of the chromosomal group within a nuclear membrane and the forming archoplasmic spindle. This period is very brief and it is possible to find exceptionally in one cyst first spermatocyte telophases and second spermatocyte metaphases.

A second spermatocyte metaphase figure distinguishes itself at once from that of either of the two preceding cell generations,

and it is most unlike that of the first spermatocyte. In the size and form of the spindle and shape of the chromosomes it most resembles the spermatogonial mitotic figure, but differs in the possession of the haploid instead of diploid number of chromosomes. While it is like the first spermatocyte in number of chromosomes it is most unlike in the form of the chromosomes and spindle. When viewed from the pole the second spermatocyte metaphase figure shows all the elements of like form—simple rods with terminal fiber attachment and arranged radially in the equatorial plate (figs. 12, 13, 16 to 19, 31, 47, 49, 50, 53 to 58). A lateral view reveals each chromosome as two rods superimposed with the inner ends, where the fibers attach, in close contact, while the outer ends may be widely divergent (figs. 48, 51 and 52). Occasionally chromosomes lying in the center of the group may swing widely apart, remaining united only at the point of fiber attachment and may then appear almost as straight rods. Such instances are represented in figures 18, 49, 50, 53, 55, 57 and 95.

In the cases of *Stenobothrus*, *Chorthippus*, *Chloëaltis* and *Trimerotropis*, where some of the chromosomes have median and subterminal fiber attachment in the spermatogonia, a similar series of such chromosomes reappears in the second spermatocytes. Under these conditions polar views of the second spermatocyte metaphase (figs. 68 to 76) instead of presenting all the chromosomes as simple rods will show V's with equal or with unequal arms, depending upon the point of fiber attachment. Divergence of the outer ends also occurs to some extent in such chromosomes.

DISCUSSION OF RESULTS ON THE SPERMATOCYTES

In weighing the evidence presented in the different accounts of Orthopteran maturation phenomena it will be necessary to take into consideration the following items; (1) the relation of the chromosomes of the first spermatocyte to those of the spermatogonium, (2) the composition and form of the first spermatocyte chromosomes, (3) the relation of the chromosomes of the first spermatocyte metaphase to those of the prophase, (4) the position of the chromosomes in the spindle, noting the *Hippiscus* type and

the *Stenobothrus* type of chromosomes, (5) the movement of the chromatids in the metaphase in these two types, (6) the relation of the chromosomes to the archoplasmic fibers in the two types, (7) the form of the anaphase chromosomes in *Hippiscus* and *Stenobothrus* types, (8) the results of the first spermatocyte mitosis under the conditions of the two types, in terms of the chromosomes, (9) the results of the second spermatocyte divisions in the two types.

Because of the involved and contradictory nature of the various accounts it has seemed best to review briefly the work of the different authors, individually, according to the analysis proposed, and then to consider the general evidence on each of the points. By this means it will be possible to exclude the palpably incorrect portions of the various descriptions and to present for judgment those possibly correct ones upon which there is difference of opinion. The papers on each family will be grouped together.

a. Acrididae

Wilcox ('95) in his paper upon the spermatogenesis of *Melanoplus* (*Caloptenus*) *femur-rubrum* describes the behavior of the chromosomes in such a way that it is quite impossible to compare his account in detail with more exact studies. The attempt to do this will nevertheless indicate somewhat the advance that has been made since his work was done. (1) The 12 univalent chromosomes of the spermatogonium become extended into a granular thread upon which the chromatin collects at twenty-four points, and then the thread breaks transversely into 12 dumb-bells. (2) By joining these dumb-bells in pairs, 6 tetrads are produced, making 24 chromosomes. These are all in the form of rings. (3) Contraction results in the production of metaphase chromosomes having also an annular shape. (4) The rings are placed on the spindle parallel to its axis. (5) By the first spermatocyte mitosis they are separated into half rings. (6) No clear statement of fiber attachment is given. (7) Chromosomes in the anaphase are not described. (8) The first spermatocyte mitosis is a reduction division. (9) Also the second spermatocyte division is reductional.

The work of de Sinéty ('01) upon *Oedipoda*, *Stenobothrus*, and certain Phasmids was not done with a detailed consideration of chromosome relationships and so it is not possible to bring it strictly into line with more recent studies. Such a comparison will however be valuable in showing the greater precision of our present knowledge: (1) No adequate attempt is made to analyse the members of the first spermatocyte complex in terms of the spermatogonial chromosomes. (2) The first spermatocyte chromosomes are formed by the transverse division of the chromatin thread, which then divides twice longitudinally in planes perpendicular to each other. There result, by different degrees of separation and movement of these four parts, chromosomes in the shapes of rings, loops, crosses, etc. (3) Through concentration these become the final metaphase chromosomes in which the second longitudinal split is lost. (4) Because no distinction is made between the *Stenobothrus* and *Hippiscus* types it is not possible to compare the behavior of the various chromosomes in the spindle. (5) No uniformity in the movements of the chromatids in metaphase can be deduced from de Sinéty's account except that they separate along the first longitudinal cleft. (6) The fiber attachment may be terminal, median, or subterminal. Differentiation between conditions in *Stenobothrus* and *Oedipoda* is not made. (7) Forms of chromosomes in the first spermatocyte anaphase depend upon the position of fiber attachment and may be V-shaped with terminal attachment, double V-shaped with median and double J-shaped with subterminal. (8) The first spermatocyte division is longitudinal in the plane of the first split. (9) Along the second longitudinal cleft the chromosomes separate in the second spermatocyte. There are accordingly two longitudinal divisions and no reduction.

Montgomery ('05) confined his Orthopteran studies to a single species, *Syrbula acuticornis*, and came to a number of conclusions very different from those reached by other investigators, although in general the processes were conceived to be similar to those in other Acrididae: (1) Twenty chromosomes were reported for the spermatogonium, of which one pair constitutes the heterochromosomes. These pairs might be distinguished by differences in

size and form. By end to end synapsis these 20 chromosomes become reduced to 10, among which the heterochromosomes are indistinguishable from the tetrads. There is a persistent chromosome individuality manifested. (2) The various forms of prophase chromosomes reported by other cytologists appear, according to Montgomery, but X's are rare. Rings are not uncommon at this stage. (3) Forms of chromosomes recognizable in the prophase may appear in the metaphase of the first spermatocyte, but such forms as rings, common in the prophase, are rare in the metaphase. Whatever the form, however, a common type is recognizable, i.e., two spermatogonial chromosomes united end to end and having fibers attached to the opposite ends. (4) In the mid period of mitosis the chromosomes take up such a position that the fused ends lie in the equatorial plate. Rings are found only exceptionally, as are X's. (5) The first maturation division sees only a separation of whole, univalent spermatogonial chromosomes. Such as lie extended in the equatorial plate are pulled past each other to an opposite pole of the spindle. (6) Fiber attachment is at the ends of the chromosomes opposite the synaptic fusion. (7) In the anaphase the chromosomes all have the form of V's in which the enclosed space represents the earlier longitudinal split. (8) A reduction division of all the chromosomes is accomplished in the first spermatocyte where the heterochromosomes behave as do the other tetrads. (9) Also in the second spermatocyte all the chromosomes divide in the same way, so that a mere equational division results. This includes the heterochromosomes.

Davis ('08) has made a careful and extended study of a number of Acrididae and Locustidae and finds a common type of maturation processes in all. For the Acrididae he chooses, as representative, the conditions found in *Dissosteira*, and includes also a consideration of *Stenobothrus*. For the Locustidae *Steiroxys* is employed. From this diverse material he concludes that (1) the spermatogonial chromosomes may be recognized in pairs, with the exception of the accessory chromosome, and these reappear in the first spermatocyte, joined end to end, to form the tetrads. (2) Chromosomes of the first spermatocyte are all of the

same type of construction, but show a variety of shapes which are constant in number. Thus in the Acrididae there are always three rods, two very small and one medium in size; three crosses, two of medium size and one large; and five rings or loops, four of which are large and one of medium size. In *Stenobothrus* similar constant shapes, but in different numbers, occur. In every case the chromosome is formed by the end union of spermatogonial chromosomes and, if unmodified, produces the simplest type—the rod. If, at the point of union in such an element, the parts diverge and become extended at right angles to the length of the rod, the cross is formed. Rings represent a union of the two spermatogonial elements at both ends instead of at one as in the other types. When the fiber attachment is not terminal, as in *Stenobothrus*, there may be a lateral extension of the parts, as in the rods, joined to a flexure at the point of fiber attachment forming E-shaped chromosomes. (3) A gradual condensation of the chromosomes carries the forms of the prophase over into the metaphase. (4) In the first spermatocyte spindle the rods are placed parallel to its length, the crosses with one arm in the direction of the spindle axis and the other in the equatorial plate, while the rings and loops are in such a position that their free ends lie central and crossed with the synaptic ends directed peripherally. According to the description homologous chromosomes should lie one above the other so that the opening of the ring would be apparent from a lateral view, but in the few metaphase figures shown (text fig. N) they appear clearly in polar views. The figures of *Stenobothrus*, upon which form Davis clearly has established his views, are much more numerous at this stage and the rings are accurately shown lying extended in the direction of the spindle axis. The other chromosomes appear as in *Dissosteira*. (5) At the time of chromatid separation the halves of the extended rods merely move apart, showing first a constriction at the point of division; the crosses suffer a reduction of the lateral arms, due to an extension of their substance into the vertical arms, and at length come to have the same shape as the rods. The rings have their curved halves pulled past each other in the axis of the spindle and at the end of the

movement are extended in this direction as split rods. Where the fiber insertion is not terminal, as in *Stenobothrus*, the rings separate, as longitudinally divided half rings. (6) Davis conceives the point of fiber attachment to be permanent from generation to generation of cells, and finds strong confirmation for this idea in the chromosomes of *Stenobothrus* where there are terminal, median and submedian attachments. In *Steiroxys* there are also median attachments, but in the other forms studied apparently they are all terminal. (7) Where the fiber attachment is terminal the anaphase chromosomes of the first spermatocyte appear as simple V's, but in those cases with median or subterminal attachment the chromosomes are double V's or J's. (8) As a result of his study on the different species of Orthoptera, Davis concludes that the first spermatocyte mitosis witnesses a separation of homologous chromosomes and is therefore a reduction division in Weismann's sense. (9) Further he concludes that in the second spermatocyte the halves of chromosomes, longitudinally split in the first spermatocyte prophase, are separated by an equatorial division.

Buchner ('09), from his investigation of Oedipoda, reaches the following conclusions: (1) The twenty-three chromosomes of the spermatogonium are recognizable as eleven tetrads, plus the accessory chromosome, in the first spermatocyte. (2) The planes of the future longitudinal and cross divisions are recognizable in the prophase chromosome, which is an elongated thread with a central cleft representing the position of the future reduction division. This extended thread may be subjected to unequal pressure on the two sides and then takes the form of a U. The free ends of such a tetrad may come into contact and thus produce a ring. In oblique view these rings, which do not lie in one plane, have the appearance of a figure 8. By approximation of the two elements lengthwise and by breaking apart at the ends, parallel rods result. If the forces exert themselves equally on both sides of the tetrad it remains extended. Short elements under these conditions are straight rods, but longer ones may be drawn out at the center and produce crosses with arms of various proportionate lengths. Combinations of the two types may occur and

rings produced by the first form of movement may have the elements drawn out at the center producing a cross on a ring. Such secondary crosses do not, however, occur on both sides of the ring. (3) By continued contraction the various forms become more uniform and with the loss of the nuclear membrane there may be found round and elongate bodies corresponding to the earlier rings and crosses without any indication of central opening or longitudinal split except in the largest rings. (4) Upon the establishment of the spindle there are found, in lateral view, chromosomes with their longitudinal axis parallel to the spindle and others at right angles to it, with all intermediate forms. The conclusion is that "die Stellung der extremen Chromosomen gestattet uns den Schluss, das die Teilungsebene übereinstimmt mit der Ebene, in der die Chromosomengrenzen liegen; die erste Reifeteilung stellt eine echte Reduktionsteilung (Praereduktion Korschelt-Heider) dar." (5) Buchner fails to observe the movements of the chromatids while in the equatorial plate. (6) One fiber attaches to each chromosome at a clearly marked point, and this varies in position in each chromosome. (7) The form of the anaphase chromosomes is variable depending upon the position of the fiber attachment. No indication of the longitudinal cleft—or plane of second spermatocyte division—is visible, but it runs the length of the arms of the anaphase U's. (8) All chromosomes are divided transversely in the first spermatocyte and the various forms of anaphase chromosomes go over into the second spermatocyte with an invisible longitudinal cleft. (9) After a brief resting period and prophase, in which the anaphase chromosomes may be recognized, the second spermatocyte metaphase figure is formed with the chromosomes, longitudinally divided, lying in the equatorial plate. They are all of one type with terminal fiber attachments and divide along the plane established by the longitudinal division of the first spermatocyte spireme. The anaphase shows no V-shaped chromosomes.

Brunelli ('09, '10, '11) has studied the maturation phenomena in *Gryllus desertus* Pall. and in *Tryxalis* and concludes that, in essentials, they are similar. For *Tryxalis* ('10, '11) he states

that (1) it is difficult to determine exactly the spermatogonial number, but it is probably 21 although his figure 17 shows 23 very clearly. In the first spermatocyte, however, there are 10 tetrads plus the accessory chromosome. There is an individuality of the chromosomes and spermatogonial elements reappear in the first spermatocyte joined in pairs. (2) There are various forms of the chromosomes due to the movements of their parts as well as to the different aspects from which they are viewed. They are conceived to be rods joined at one end and variously twisted and separated. The union is slight and often they lie across each other or one is bent around the other. Rings are loops, although in figure 11 a perfect annular element is shown, while crosses are formed of overlying parts. (3) The forms of the prophase are traced into the metaphase. (4) No clear polar views of the metaphase are given, but in later views the paired elements are shown placed one above the other—'superposition'—with their inner ends crossed. (5) In the dicession of the chromosomes they move past each other going to the opposite pole of the spindle from that side of the equatorial plate upon which they lay in early metaphase. Whole spermatogonial chromosomes are thus separated from each other by simply being pulled apart. (6) Fiber attachment is terminal and to the spermatogonial derivative lying on the *opposite* side of the equatorial plate. (7) The anaphase chromosomes are single V's whose contained space is the longitudinal division established even as early as during the anaphase of the preceding spermatogonial division. (8) By the segregation of whole chromosomes in the first spermatocyte there results a prereduction type of maturation. (9) The V-shaped chromosomes of the anaphase are traced through the interkinesis and into the second spermatocyte metaphase where they are divided along the longitudinal cleavage of each, thus constituting an equatorial division.

Gerard ('09) has studied only *Stenobothrus* and his results may be compared in detail only with those of other investigators upon similar material. Summarized, his findings are that (1) while there is a loss of chromosome outlines after the last spermatogonial division and the formation of a network, there subse-

quently appear two spiremes which, by parasynapsis, become a double one, and this then divides by transverse fission into the reduced number of chromosomes, 17. These are of the sizes found in the spermatogonium and represent them in the paired condition. (2) The forms of chromosomes in the first spermatocyte prophase are rings, figures of 8, etc., in which the open space represents the plane of fusion between the spiremes—it is interchromosomal. (3) By concentration the metaphase chromosomes are formed from those of the prophase. (4) Upon the spindle the rings are so placed as to lie extended in its axis. Rods also occur in the same position. (5) The rings break into half rings or V's whose enclosed space is the interchromosomal cleft between the spiremes. (6) There is no detailed consideration of fiber attachment. (7) In the anaphase there appear double V's whose separation is due to the longitudinal split of the chromosomes. (8) Through the movements of the chromosomes in the first spermatocyte mitosis a reduction division is accomplished. The accessory chromosome does not divide at this time. (9) In the second spermatocyte an equational division occurs.

Granata ('10) has studied for the maturation phenomena of the chromosomes the material employed by Giglio-Tos in his investigations of mitochondria, which was derived from the European genus *Pamphagus*. As has already been noted in considering the spermatogonia, this form appears to show different numerical conditions from most other Acrididae. The conclusions of Granata are as follows: (1) The number of chromosomes is constant and the members appear as paired elements of definite sizes. An unpaired accessory chromosome is present. A spireme is formed in which the spermatogonial chromosomes lose themselves. There is no persistent chromosome individuality since these structures are but the expression of chemical activities. (2) The typical first spermatocyte chromosome is the cross formed from a rod which has been twice divided longitudinally and has then opened out part way along one of these clefts and later, from the opposite end of the rod, along the other. Rings are similar structures which, instead of opening out completely along the second longitudinal cleft, have remained united at the

end while diverging in the center. Figure-8 chromosomes are such rings with a secondary divarication of the end opposite the first producing a second ring at right angles to the primary ring. (3) The definitive metaphase chromosomes reach their forms by a condensation of similar figures in the prophase. (4) No clear statement regarding position of the chromosomes in spindle is given but figures show rings lying in the equatorial plate and the statement is made that the author agrees with the descriptions of McClung and his students. (5) A similar statement may be made regarding the movements of the chromatids in metaphase. (6) The relation of chromosomes to fibers is not clearly stated. (7) In the anaphase the chromosomes pass to the poles of the spindle as simple V's. (8) The result of the first spermatocyte mitosis is a longitudinal division of the chromosomes and no division of the accessory chromosome. (9) The telophase chromosomes may be traced into the second spermatocyte and later are divided along the second longitudinal cleft, while the accessory chromosome divides lengthwise also.

Meek's work ('11) deals with the case of *Stenobothrus* only. His conclusions are that (1) while there appears to be a complete loss of chromosome identity during the growth period, the same series of sizes and shapes reduced to the haploid number 9, reappears in the first spermatocyte metaphase and it is possible that the chromosomes of this stage are formed by earlier conjugation of homologous spermatogonial elements. (2) Each first spermatocyte prophase chromosome is formed of four parts, and may show a variety of shapes—crosses, rings, loops, figures of 8, etc. (3) The metaphase chromosomes appear either as rods, or as V's with more or less unequal arms. (4) On the first spermatocyte spindle the chromosomes are placed principally as extended rods or crosses. (5) No clear account is given of the movement of the chromatids in the metaphase. (6) There is no statement regarding fiber attachment. (7) An account of the anaphase chromosomes is not given. (8) The first spermatocyte division is thought to be longitudinal. (9) In the second spermatocyte a possible reduction division occurs, although it is conceived that both spermatocyte divisions may be longitudinal.

b. Locustidae

Stevens ('05), in connection with a series of studies upon the accessory chromosome, describes briefly the spermatogenesis of *Stenopelmatus*, a Locustid. Several errors appeared in this paper which were later corrected. Such of her final conclusions as relate to this study are indicated in the following: (1) From the 47 chromosomes of the spermatogonium there are formed by telosynapsis in the prophase of the first spermatocyte twenty-three tetrads and an unpaired accessory chromosome. (2) The tetrads thus produced have the form of rings, crosses and rods. (3) In the metaphase the same series of forms may be traced. (4) The chromosomes are so placed in the spindle that their plane of longitudinal cleavage lies in the equatorial plate. This is clearly indicated by persisting linin connections. (5) By movement of the chromatids along the plane of the longitudinal split in the late metaphase, the chromosomes have the form of rods extended in the direction of the spindle axis. (6) Fiber attachment is not described, but by inference it must be at the synaptic points. (7) Anaphase chromosomes are in the form of simple V's or double rods. (8) An equatorial division occurs in the first spermatocyte. (9) Segregation of homologous chromosomes results from the second spermatocyte mitosis.

Otte ('07) has confined himself to a study of the Locustid, *Locusta viridissima*, and comes to some unique conclusions regarding maturation phenomena. His results may be summarized as follows: (1) There is a constant series of chromosomes recognizable by differences of size. The paired elements of the spermatogonium, after becoming elongated in the first spermatocyte prophase, join together side by side to form the haploid number—parasynapsis. (2) Of these, the longer elements become rings by a bending of the double thread until the ends meet, at which point they may, or may not, fuse. These annular chromosomes may continue in this form or they may become compressed so that they break at the point of greatest curvature—opposite the free ends of the loop—and thus produce parallel rods, each of which shows a space representing that produced by the earlier ap-

proximation of the two threads. Smaller elements may fail, or be unable, to form rings and then appear as straight rods. Such rods then break across at the middle and form tetrad-like chromosomes. Sometimes at the middle point the halves of the threads are drawn out to form cruciform chromosomes. (3) The various prophase forms are traced directly into the metaphase through a gradual condensation. (4) The rings lie in the spindle parallel with its axis; the rod-shaped forms are extended in the same manner. (5) A mere separation of the half rings or half rods is all that occurs in the metaphase. (6) In the case of the rings, fiber attachment is at the middle of each half ring, in the rods at opposite ends of the extended structure. (7) Forms shown by the anaphase chromosomes are split half rings from the annular chromosomes and V's from the rods. (8) By the first spermatocyte division one end of each of the spermatogonial chromosomes, joined side by side in the prophase, is separated from the other end and goes into a different second spermatocyte. It is not a reduction or segregation division because homologous elements are not separated; it is not an equation division for the reason that not all parts of each chromosome are represented in the daughter cells. As described by Otte it is an entirely unique form of division. (9) The second division is like the first, even to the forms of the chromosomes. Before the second spermatocyte metaphase the half rings have again become rings and are once more separated as in the preceding mitosis. The rods are again divided transversely and go to the poles as split rods. This also is neither a reduction nor an equation division. The result of the two divisions is to divide each spermatogonial chromosome, joined by parasynapsis to its homologue, twice transversely, one-fourth of its length going into each spermatid.

Stevens ('12) discusses supernumerary chromosomes and the method of synapsis in *Ceuthophilus*. She finds a parasynapsis, subterminal fiber attachment and a reduction division in the first spermatocyte.

Vejdovský ('13), together with studies upon *Ascaris*, *Gordius* and other genera, has reported the results of his investigations upon *Decticus* and *Diestramena*, two Locustid genera. His

results may be stated as follows: (1) There is constancy in number of chromosomes with differential sizes in a duplex series. After the last spermatogonial division the chromosomes lose their linin center and the naked chromatin threads of homologous chromosomes fuse their substance to produce a new mixochromosome. (2) These mixochromosomes early split longitudinally to produce dyads. Just before the first spermatocyte metaphase these dyads again divide longitudinally to produce tetrads, the largest of which are rings or ellipses. (3) These tetrads, by slightly more concentration, pass into the metaphase where the rings are large and conspicuous. (4) These rings are of the Stenobothrus type and lie parallel to the spindle axis. (5) In the metaphase the annular chromosomes are reduced to half rings by dividing across at their middle. (6) The fibers attach at the centers of the half rings. (7) The anaphase chromosomes resulting are double half rings or V's. (8) As a result, the first spermatocyte mitosis represents a longitudinal division. (9) In the second spermatocyte the chromatids are separated along the second longitudinal cleft. There are, therefore, two longitudinal divisions and no reduction.

c. Gryllidae

Vom Rath ('91, '92, '95) has reported the process of maturation in *Gryllotalpa vulgaris*, but, as stated in the discussion of the spermatogonia, his results are so evidently erroneous that it is profitless to attempt a comparison between them and the findings of others. It is only necessary to mention, to indicate the character of his work, that he reported the diploid number of chromosomes to be 12 instead of 23, and that he entirely overlooked the prominent accessory chromosome. For these reasons his work will not be considered further.

Gutherz ('07, '08, '09) in a series of papers on *Gryllus* has discussed the behavior of the accessory chromosome and its relation to sex determination, but his work does not concern itself closely with the problems of this paper. He notes the typical behavior of the accessory chromosome during spermatogenesis

and denies the presence of such an unpaired element in the female cells.

Voinov ('04) deals only with the behavior of nucleolar bodies.

Brunelli ('09) states in this paper many of the views which he elaborated later in his study of *Tryxalis*. Briefly, he finds the 21 spermatogonial chromosomes of *Gryllus* converted into 10 tetrads plus the accessory chromosome. The prophase chromosomes may be interpreted as double rings, which in the metaphase, take on a variety of forms due to the point of fiber attachment and to the fluidity of the chromatin. The form in the metaphase may vary from that of the prophase for these reasons. In the metaphase the chromosomes are arranged in 'superposition,' and the half rings, representing spermatogonial chromosomes, separate. Fibers attach at the center of the half rings and the anaphase chromosomes are split V's. The first spermatocyte mitosis is reductional and the second is equational.

Payne ('13) in a short paper gives an account of the behavior of the accessory chromosome in *Gryllotalpa vulgaris* and describes an unequal tetrad, the large member of which always accompanies the accessory chromosome in its unipolar movement in the first spermatocyte mitosis. For our present purpose we may note that the diploid number, 23, is reduced to the haploid, 12, in the usual manner so as to leave an unpaired accessory chromosome. Baumgartner ('11), in an abstract of a paper read before the American Society of Zoologists, reports similar conditions in *Gryllotalpa borealis*.

d. Blattidae

Farmer and Moore ('05), after a study of a number of plants and animals, conclude that there is a typical series of processes, common to all multicellular organisms, in the reproductive cycle. Among the forms studied was *Periplaneta americana* and the processes of maturation in the male are thus conceived: (1) The chromosomes are morphologically continuous from generation to generation and the reduced number of the first spermatocyte is derived from the diploid spermatogonial series by fusion of homologous pairs. Details of this process are not clearly given

but it would appear from several statements that this must be due to union of chromosomes, and not to a unique cross-splitting of a continuous spireme. On page 527 this statement occurs: "It does not seem to be a matter of any consequence how the bivalent arrangement is produced, since there is so much variability in the process, but the temporary union in pairs of somatic chromosomes is the really important feature." (2) For *Periplaneta* the analysis of the tetrads into their univalent parts is not made clearly, but the idea of variability of association and dissociation is held. How much this means for these authors may be gathered from the description of the different methods by which the common X-shaped chromosome is produced in *Osmunda* (p. 522). At least three variations in constitution are mentioned. In *Periplaneta* the prophase chromosomes are in the form of loops with their free ends polarized at one side of the nucleus. A chromatin nucleolus is present. (3) The metaphase chromosomes are in the form of rings and are produced by approximation of the free ends of the loops. (4) Upon the spindle the rings are so arranged that they lie in its axis. They are therefore of the *Stenobothrus* type. (5) In the metaphase the annular chromosomes are separated into half rings. (6) Fiber attachment is median as in the spermatogonia. (7) The anaphase chromosomes are irregular half rings, or V's, with the original longitudinal cleft of the chromatin thread dividing them into double elements. (8) As a result of the first spermatocyte division the reduction, or segregation, of the biparental chromosome groups is effected. (9) After a very nearly complete rest stage the reduced number of chromosomes reappears in the second spermatocyte metaphase and there they divide "in the ordinary premeiotic manner."

Stevens ('05) describes the maturation phenomena of (*Blattella*) *Blatta germanica* with these results: (1) The 23 chromosomes of the spermatogonium become converted by telosynapsis into 11 tetrads, leaving the unpaired accessory chromosome. (2) Rings, crosses, U's and rods occur in the first spermatocyte prophase but they are of essentially the same type of construction. (3) Variation disappears in the metaphase and all the

tetrads are dumb-bells; (fig. 125 seems, however, to show rings very clearly). (4) All chromosomes lie with their longitudinal axes parallel to that of the spindle. (5) A mere separation of the dumb-bells marks the changes in the metaphase. (6) Fiber attachment is not discussed, but from the figures it appears to be terminal. (7) The forms of the anaphase chromosomes are not described or clearly figured. (8) In the first spermatocyte a segregation, or reduction, division occurs. (9) After a complete rest stage the chromosomes of the second spermatocyte appear and divide, presumably equationally.

Wassilieff ('07) says that in *Blatta germanica* the unreduced number of chromosomes is 23, of which one is the unpaired accessory chromosome. By some means, not fully explained, the haploid group of 12 chromosomes is produced from the chromatin of the nucleus resulting from the last spermatogonial division; (in explanation of fig. 37, rods are described as being chromatin segments conjugated end to end). One of the twelve behaves in many ways like a nucleolus and later passes undivided to one pole of the first spermatocyte spindle. The first spermatocyte prophase chromosomes have the form of rings or V's which are produced by the bending of a longitudinally split, transversely cleft thread upon itself. The two segments are separated at this cross division in the first spermatocyte, which is therefore a reduction division. Along the longitudinal split occurs an equation division in the second spermatocyte.

Morse ('09) records the consistent phenomena apparent in four genera of Blattidae. Among his observations the following of interest in the present study may be noted: (1) The chromosomes of the first spermatocyte are derived from the spermatogonial series by a parasynapsis of homologous members, leaving an unpaired accessory chromosome. (2) By an enlargement of the interchromosomal space rings are produced, but persistent rods also occur. (3) Condensation and contraction of these result later in the production of similar forms in the metaphase. (4) In the spindle the rings are arranged parallel with its axis; rods lie in the equatorial plate. (5) Annular chromosomes divide directly into half rings or V's after more or less approximation of the two

extremities in the equatorial plate; rods are pulled apart by gliding along each other in the equatorial plate until separated. (6) Fiber attachment is either median or subterminal. (7) Anaphase chromosomes are U's or V's for median fiber attachment, or J's in subterminal. (8) The first spermatocyte division is reductional. (9) In the second spermatocyte an equational division occurs.

e. Phasmidae

Jordan ('08 a, '08 b) presents a study of the spermatogenesis of the Phasmid, *Aplopus mayeri*, in which he deals at length only with the accessory chromosome. This is typically Orthopteran in its behavior. Additional facts having relation to maturation processes are that the 35 chromosomes of the spermatogonium become reduced during synapsis to 18, of which one is the accessory chromosome, and that these undergo a prereduction.

As a part of a comprehensive study of Phasmid structure de Sinéty ('01) reports the maturation phenomena in the male of several species. Since his conclusions have been discussed in connection with Oedipoda they will not be taken up here.

f. Forficulidae

Zweiger ('07) reaches the following conclusion from his study of the spermatogenesis of *Forficula auricularia*: (1) The haploid number of chromosomes, 12 or 13, is reached through a telosynapsis of the 24 or 26 chromosomes of the spermatogonium. (2) Tetrads in the form of rings are produced by the folding of longitudinally split bivalents joined end to end. The enclosed space is interchromosomal. (3) Concentration of the prophase rings results in the formation of dumb-bell shaped metaphase chromosomes. (4) These are placed on the spindle so that homologous chromosomes lie superimposed. (5) In the metaphase these are merely separated from each other. (6) Position of fiber attachment is not described but it must be median. (7) The anaphase chromosomes are spherical bodies. (8) By separation of whole spermatogonial chromosomes the first spermatocyte mitosis becomes a reduction division. (9) In the second

spermatocyte homologous chromosomes divide longitudinally and so undergo an equational division.

Randolph ('08) gives an outline account of the spermatogenesis in the earwig, *Anisolabis maritima*, dealing principally with the accessory chromosomes which are regarded as similar to an equal pair of idiochromosomes. No detailed description of chromosome behavior is given except that the 24 spermatogonial chromosomes become reduced to 12.

Stevens ('10) gives the results of her studies upon the spermatogenesis of *Forficula auricularia*. Briefly stated, her conclusions are that the diploid chromosome number, including an unequal idiochromosome pair, is 24. This, by a telosynapsis of the typical insect character, produces a haploid number of 12. The first spermatocyte prophase shows the chromosomes in the form of loops, U's, rings and figures of 8, with an occasional cross. A reduction division follows after the concentration of the chromosomes. Occasional variations in the number of second spermatocyte chromosomes are ascribed to precocious division of the small idiochromosome.

Sutton ('00, '02, '03) Baumgartner ('02), Nowlin ('08), Pinney ('08), Robertson ('08) and Carothers ('13) have studied various species of Orthoptera in my laboratory and their interpretations do not differ from mine in any essential respects. For this reason I shall not enter further into a consideration of their work here.

CONCLUSIONS

With the results of the various investigators before us it is now possible to draw comparisons and to arrive at conclusions. Such comparisons may advantageously be made under the various headings that have been used in outlining the results of individual investigations.

a. Relation between chromosomes of the first spermatocyte prophase and those of the spermatogonium

1. Concerning the relation existing between the chromosomes of the first spermatocyte and of the spermatogonium there are three theoretical possibilities. (a) The chromosomes of the

first spermatocyte may be entirely new structures, having no relation either in material or in form to those of the spermatogonium, (b) they may be derived in substance only from the spermatogonial elements, or (c) they may individually be descended from similar structures without loss of identity other than that involved in the processes of chromatin metabolism. All these conceptions have found expression in studies on Orthopteran material.

Granata ('10), adopting the views of Giglio-Tos, argues for a purely chemical explanation of chromosome behavior and so removes his interpretations from a direct comparison with the work of others. Despite this extreme view he is constrained to admit the presence of a chromosome series, constant in number and in size relations, with an exact reduction of the euchromosomes² to the haploid number of maturation. No other investigator has made an appeal from morphological conceptions and since Granata's interpretation is purely theoretical it need not be discussed further, while so much remains to be done by observation.

For the second possibility there are more advocates, although it is often rather by inference than by exact statement that this appears in the accounts. For while some profess a belief in the genetic continuity of the chromosomes they yet describe a double longitudinal division or a double cross division. Persistent chromosome individuality is, however, possible only in the presence of a segregation, or reduction division during maturation. With this understanding of the case Wilcox and Otte, conceiv-

² The word 'euchromosome' I shall employ to distinguish those without any marked peculiarities of form or behavior. Montgomery's term 'heterochromosome' might advantageously be used as an antonym, signifying any general variation of structure or behavior. Subclasses within the general term heterochromosomes could be constituted under the headings 'idiochromosomes' (Wilson) to include paired sex chromosomes; 'accessory chromosome' (McClung) to distinguish the unpaired sex element; 'microchromosomes' (Wilson) to designate small compact chromosomes not connected with the sex chromosomes. I believe it much preferable to use the genetic word 'chromosomes' in all terms denoting such cell elements and to mark their specific differences by qualifying terms, rather than to coin entirely new names for them in which no intimation of their relations is included.

ing the presence of a double cross division, and de Sinéty and Vejdovský a double longitudinal division, must be recorded as upholders of a mere substance relation between the chromosomes of the two generations of germ cells. Wilcox and de Sinéty fail to effect a careful analysis of these relations and a detailed criticism of their accounts will not be undertaken.

Otte expresses his belief in the genetic continuity of the chromosomes and describes a parasynapsis of spermatogonial derivatives in the first spermatocyte, and yet believes that there are two cross divisions without any reduction. Vejdovský, on the contrary, from studies upon other Locustidae, argues for a complete fusion of homologous spermatogonial chromosomes to form a new mixochromosome, which is later divided twice longitudinally. It is thus seen that the two recent workers upon Locustid material, who doubt the occurrence of a reduction division, both believe in the preservation of integrity of the chromosomes, but in one case through a double cross division and in the other through a double longitudinal cleavage.

Opposed to these few contradictory advocates of doubtful or impossible processes for the preservation of chromosome continuity are the large number who perceive and describe the only possible means for ensuring it—through a temporary union of homologous chromosomes and their later segregation in one of the spermatocyte divisions. While there are differences of opinion about the details of processes, there is agreement regarding the end results. Always in some form the opinion is expressed by the other students of Orthopteran spermatogenesis that because of their organization the chromosomes may be identified in the first spermatocyte as the descendants of the ones found in the preceding spermatogonium. All find a pseudoreduction which they ascribe to associations between the members of the diploid group. There is a constant specific number and there are recognizable, in most cases, either individual chromosomes, or groups of them, which may be traced from one cell generation to the next.

Criteria for such identifications are furnished by differences in size, form and behavior and they apply with great exactness

in the studies of different investigators, working upon similar materials secured from widely different sources, and through a great variety of technical methods. As a most marked instance of such concordance of results may be mentioned the accounts of spermatogenesis in *Stenobothrus*. This genus has been studied very generally and by cytologists of France, England, and America—Carnoy, de Sinéty, Girard, Meek, Davis—and yet the published accounts upon the same species show practical agreement upon the uniformity of number, sizes, forms and behavior of the chromosomes in different generations. If there were not genetic relations of the most exact character such agreements would be impossible. In a similar way, with the exception of the *Stenobothrus*-like species, and *Pamphagus*, the students of the *Acrididae* have reported a reduction of the 23 spermatogonial chromosomes to 12. Among this haploid number may be recognized the unpaired accessory chromosome and 11 paired elements whose members correspond in size, at least, with those of the previous generation.

Regarding the details of such a relation there is not a complete uniformity of opinion, but there is agreement upon the fact that by an association between definite members of the spermatogonial complex there are produced the bivalent structures of the first spermatocyte. For the *Acrididae* this is the opinion of Montgomery, Davis, Buchner, Brunelli, Sutton, Pinney, Nowlin, Robertson, Carothers and myself. Although the other families have been less studied these genetic relations appear to exist in the *Locustidae* according to Davis, Buchner, Stevens, and by both Otte and Vejdovský despite their views upon the character of the maturation divisions. For the *Gryllidae* Baumgartner and Payne; for the *Blattidae* Farmer and Moore, Stevens, and Wassilieff; and for the *Phasmidae* Jordan, are in agreement on the direct relation between the spermatogonial chromosomes and those of the first spermatocyte.

Of the greatest importance in determining the relations of chromosomes to each other in successive cell generations is the behavior of the accessory chromosome. There is almost uniform agreement regarding its direct and evident continuity, without

any loss of identity, from the spermatogonium over into the first spermatocyte. It was at first regarded as very different from the other chromosomes and was described as a nucleolus by many, but longer study has taught that it parallels the euchromosomes, although in a modified form, in every part of its history except in actual synapsis. That one of the spermatogonial complex, in most cases slightly distinguishable from its fellows throughout the 'division period,' should pass into the first spermatocyte without loss of form or identity and there perform much the same evolutions as the others, while still remaining distinctly delimited, is a very strong piece of evidence for the individual continuity of the others.

I believe therefore that it may fairly be said that the evidence from Orthopteran studies is strongly in favor of the view that the chromosomes of the last spermatogonial mitosis pass over as such into the first spermatocyte where they appear joined together in pairs. As I have stated elsewhere this seems to me a fundamental conception, for unless we are dealing with structures of definite and characteristic organization their manoeuvres in maturation are of small moment. In the further discussion it will therefore be assumed that the establishment of the concept of chromosome continuity has been made with a reasonable degree of certainty. This being done we have next to consider the method of association set up in the first spermatocyte chromosomes and the forms through which it is manifested.

b. Composition of chromosomes in the first spermatocyte prophase

A thoroughgoing consideration of the composition and form of the first spermatocyte chromosomes would require a full analysis of the complicated series of changes through which the chromosomes pass after the last spermatogonial division, when they enter into the tetrad condition. This cannot be done, for we are yet lacking a sufficient knowledge of these stages. We can but regard the haploid group of the first spermatocyte prophase and find therein the recognizable members of the unreduced complex, which, as I have shown elsewhere, may be accomplished very

definitely in many cases. The problem before us therefore, according to the terms of the argument set down, is to analyse the associated spermatogonial derivatives and to determine their relations as they prepare for, and undergo, division in the first spermatocyte.

In comparing the figures of the different investigators a striking uniformity in the outlines of the chromosomes is apparent. Also in the written descriptions there is always mention of the occurrence of chromosomes in the form of rods, crosses, rings, V's, X's, U's, etc. The practical uniformity of chromosome structure throughout the group seems to exist beyond question. Variation inheres therefore in the interpretations and not in the structures themselves.

It will suffice for the present comparison to begin a consideration of tetrad formation with the stage wherein the chromosomes have taken on their definitive condition and yet show their constituent parts. For convenience of discussion also the different chromosome forms will be taken up individually and the opinions of various authors regarding them compared.

1. *Rod-shaped tetrads.* Of all those appearing at this time the rod-shaped chromosome is the most simple, and, according to my view, is the fundamental type. It seems without doubt to be constituted of two spermatogonial chromosomes united end to end with their longitudinal clefts coincident and with their points of contact indicated by more or less divergence of their chromatids. This interpretation is given to the structure by Sutton, Montgomery, Davis, Buchner, Robertson, Pinney, Nowlin, Stevens and Wassilieff.

So far as the actual composition of the rod is concerned it is of no consequence whether there has been an earlier parasynapsis or whether, later, there is pre- or post-reductuon. Should parasynapsis occur, however, there is a possibility that the plane of separation may be along the longitudinal cleft of the homologous elements instead of through the space between them. Extended rods in the metaphase would then be almost completely divided longitudinally at this time. Involved in this is also the

question of the position of fiber attachment, for, in case there should be such an early separation of the chromatids in this plane, the point of fiber attachment would still be at the synaptic ends, whereas if there were a segregation division at this time it would mean a shifting of the fiber attachment to the opposite ends.

It is not possible to come to a complete understanding of all these changes and it may be that each of these conditions is met in different forms. There are, however, a number of significant facts which point to the interpretation I have advocated from the first, i.e., of longitudinally split rods lying extended in the equatorial plate with the median synaptic ends directed toward the center of the plate and with fiber attachment at this point. One of these is the case of the unequal tetrad discovered by Wenrich in *Phrynotettix*, showing differential ends and dividing so that each daughter cell receives equivalent parts. Another is an also unpublished series of circumstances in *Trimerotropis*, worked out by Miss Carothers, showing the attachment of the so-called plasmasomes in such a way as to identify daughter chromatids. Here also the parts are recognized according to my description. The polar granules of Miss Pinney, the constancy of fiber attachment in *Stenobothrus*, the occurrence of cross-shaped chromosomes in the metaphase with one-half the separation accomplished, and finally the appearance in *Mecostethus* of a group of chromosomes, all but the smallest of which lie extended in the equatorial plate, form a mass of evidence which is most convincing when taken into consideration with that furnished by other chromosomal forms.

2. *V-shaped tetrads*. This form represents only a slight and unimportant modification of the simple rod. It is, as has been stated, such a chromosome bent at the synaptic point in the plane of the longitudinal cleft with more or less separation of the chromatids at the center. Such chromosomes appear in the late prophase and are easily recognizable in the polar view of the metaphase. They are shown in the figures of Sutton, Baumgartner, Davis, Nowlin, Granata, Payne and Robertson.

An occasional and interesting prophase modification of the V occurs when the chromatids separate along the longitudinal

split and at the same time show the space between homologous chromosomes. This produces a double V, the enclosed spaces of which are in planes perpendicular to each other. In such a structure it is not possible to distinguish these apart and it is theoretically possible that, in the subsequent concentration, either of these might represent the plane of division in the first spermatocyte. Such forms do not appear in the metaphase and they are comparatively rare in the prophase. In principle all the tetrads would be of this type if there were parasynapsis and equal separation of the chromatids.

3. *Cross-shaped tetrads.* This simple form of chromosome has been subjected to the most inexplicable misinterpretation possible, ranging from a practical denial of its presence by Wilcox, Otte, Vejdovský and Montgomery to the strange accounts of its formation by de Sinéty and Brunelli. It is correctly represented in prophase by Stevens, Sutton, Davis, Otte and Robertson, and, in a modified form, due to the shortness of the chromatids, by Jordan. Failure to appreciate the true composition of this element can be due only to very imperfect or superficial observation. Nothing is clearer than the fact that the four arms, at the middle, lie in the same plane and that each is split along its length. Such appearances as are shown by de Sinéty in figures 123 and 124, and by Brunelli ('11) in figures 12 and 13 never occur, and Montgomery's supposed X-shaped chromosomes in figure 31 is in reality a V with the synaptic ends extended. Both de Sinéty and Brunelli are inexcusable for describing the cross as two superimposed chromosomes. Even the most casual inspection will show that the limbs lie in the same plane and have a clear diamond-shaped opening where their clefts intersect. Such figures are shown in the first spermatocyte of *Brachystola* by Sutton ('02, figs. 6 and 7); by Stevens ('05 in *Stenopelmatus*, figs. 56, 58, 59 and 64); by Robertson ('08 fig. 29); by Davis ('08, figs. 60, 166 to 168); by Nowlin ('08, figs. 3, 5 and 7, pl. 29, and figs. 3, 4, 13, 14, pl. 32); and by Carothers ('13, fig. 28).

4. *Ring-shaped tetrads.* Such tetrads I have always regarded as of the greatest significance, and I am now even more firmly of the opinion, if possible, that they present most clearly the real

composition of the first spermatocyte chromosomes. Objection has been made by Montgomery that they are complicated and difficult to interpret, but I do not believe that this is justified. He also states that they are transitory prophase stages which rarely persist into the metaphase. This is certainly an error, as Robertson has shown in the same genus studied by Montgomery, where there are always at least two in each metaphase complex and may be as many as seven. A fruitful source of error in determining the method of division in the ring chromosomes lies in the failure to discriminate between such rings as appear in *Hippiscus* and most other Acrididae, from those of *Stenobothrus*. In general it may be said that the mere structure of the ring is fairly interpreted, in most cases, but that its relation to the archoplasmic fibers and its actual division in the first spermatocyte mitosis have been much misunderstood. Because of its significance I shall make a careful analysis of this form of tetrad, as reported in the Orthoptera, and hope to make clear the cause for the confusion that has arisen.

That we deal here with a universal form is indicated by the fact that it is described and figured by Wilcox, Sutton, de Sinéty, Baumgartner, Montgomery (prophase), Farmer and Moore, Stevens, Moore and Arnold, Otte, Wassilieff (prophase), Brunelli, Pinney, Davis, Robertson, Gerard, Jordan, Morse, Granata, Meek, Nowlin, Hartman, Vejdovský, Carothers and myself. Indeed it may be said that practically without exception every investigator of recent years who has made a careful study of the maturation stages in the Orthoptera has seen and figured annular chromosomes. It would appear certain, therefore, that if a knowledge of the structure and division of such elements is attained we shall have made an important and significant advance toward a comprehensive understanding of all chromosomes in all the members of the order.

Essential to such an understanding is a knowledge of the exact structure of the ring in the prophase. This necessity I have always felt, and in my first paper on the Acrididae ('00, p. 94) appears this statement in italics: "Too much importance

cannot be laid upon the necessity for a thorough understanding of the early formative periods in the history of the first spermatocyte chromosomes." In my own work, and in that of my students, this stage has been carefully studied. It is nothing less than amusing therefore to read Brunelli's statement that I have not appreciated the real nature of the tetrads because of failure to study their formation in the prophase. On the contrary, it was just through such a study that I was saved from the errors of de Sinéty, Davis, Montgomery, Buchner and Granata in interpreting the ring chromosomes, as I hope to show.

The rings first appear clearly in the late prophase, following a period in which the chromatin is much diffused and almost unstainable. At this time the general forms of the chromosomes may be recognized in the hazy shapes within the nucleus, and among them are irregularly outlined rings. Very shortly after this, sometimes even in other regions of the same cyst, a central thread of darkly staining chromatin, split along its length, appears in each of the diffuse masses. Immediately one recognizes in the outlines thus established the various familiar rods, crosses, V's, rings, etc. Early in this period of their development the four chromatids of each chromosome lie approximately parallel and the rings are therefore much flattened, but with the rapid concentration of the hazy masses around their axial darkly staining threads, separation occurs and the rings spring open as if they were composed of elastic rods released from constraint. Such rings are shown in figures 99 to 105 and 114 to 123.

Misinterpretation begins here. As may be seen by inspection of figures 100, 115, 118 and 119, the rings are not plain bands but on one side there is an enlargement, and not infrequently there may be a corresponding one opposite this (fig. 122). When such structures are viewed laterally it is seen that each elevation from the ring is continuous with the half of the ring upon the same side as that on which it lies. When viewed *en face* (fig. 127) the ring appears as a cross, in which case the upper and lower arms are represented by the elevations which were seen in lateral view. From this it is clear that each lateral half of these elevations is

continuous with the quarter ring from which it arose. In other words, it may be stated that each prophase ring is divided into superimposed half rings by the longitudinal division of the conjoined homologous chromosomes, and that, by a plane at right angles to this, and passing through the points of endwise union of the homologous chromosomes, each half ring is divided into quarter rings. There is, then, no difference between a rod, V or ring except that in the latter the free ends are joined as at the opposite synaptic ends. Uniformity of construction is maintained. Such an interpretation of annular tetrads is given by Sutton ('02, figs. 5 to 7); Baumgartner ('04); Pinney ('08, figs. 18 a, d, e, f, h, 21); Davis ('08, figs. 59 to 61, 85, 170 to 178); Robertson ('08, figs. 26, 29); Buchner ('09, fig. 41); Granata ('10, figs. 27 to 29, text fig. 1 g, h, i, l, m, n, o, p).

A modification of this method of ring production consists in the simple approximation of the two longitudinally split chromosomes without divergence of their synaptic ends. This form of ring has been reported for the Acrididae only by de Sinéty and by Montgomery and their figures are of doubtful character. In the Locustidae, Otte and Vejdovský figure such rings along with others of the first type and it is probable that they overlooked small indications of divergence, for in my own studies of this family no marked differences from the Acrididae appeared.

Opposed to such a conception of ring formation is one in which the elevations just described as being constituted of diverging members of two superimposed half rings, are regarded merely as accidental crossings of the free ends of synaptic mates. No reason is assigned for the maintenance of this chance relation through the subsequent chromosome movements during which extensive adjustments take place which might reasonably be supposed to change or obliterate it. Doubtless such relations do occur in the prophase but upon close analysis it becomes very evident that they are not permanent and do not have a meaning opposed to that of the first interpretation. It is most significant that in all the figures drawn by those who hold to the second type not a single one represents a ring *en face*—the point of view from which the constitution of the ring is indubitable. I think it

may therefore be asserted beyond any reasonable doubt that the annular chromosomes of the first spermatocyte prophase are constituted as the great majority of investigators have described and figured them.

c. Relation of the chromosomes of the first spermatocyte metaphase to those of the prophase

There is little or no difference of opinion regarding the passage into the metaphase of the chromosome forms found in the prophase. With regard to the rings Montgomery states that although they are common in the prophase of *Syrbula* they occur but rarely in the metaphase. Robertson, however, showed clearly that this was an error. It may therefore be confidently asserted that the structural conditions shown by the prophase chromosomes are carried over into the metaphase, suffering only such modifications as result from continued condensation and possible chromatid movements.

d. History of the tetrads in the first spermatocyte mitosis

1. Rod-shaped tetrads. Reference has already been made to the position of the rod-shaped chromosomes in the first spermatocyte metaphase. That rods elongated in the spindle axis occur is not denied by any one; that they occur extended in the equatorial plate and divide in its plane is, however, disputed. My description of the actual occurrence of rods extended in the plane of the future cleavage and of their subsequent movements has been criticised by Montgomery, Davis and Gregoire as an assumption of complicated changes. The case of *Mecostethus* is a sufficient answer to any such criticism, for here nearly all the chromosomes lie in the equatorial plate and, since the fibers attach at the middle synaptic points, all the movements of division have to take place by an extension of the axial arms at the expense of the equatorial. Because of the unusual length of some of these chromosomes, however, instead of the chromatids remaining in contact entirely through this transformation, as usual, they sepa-

rate before the mid period of the movement and so appear as V's instead of as extended rods.

While *Mecostethus* presents an extreme case, it is not at all unique and many species show one or more of the same type of rods in metaphase. Wherever found they show a fiber attachment at the middle point and pass from the rod shape, through that of the cross, to the rod again, but now extended in the spindle axis and with an approximation of homologous chromatids instead of sister elements. This approximation is retained, in most cases, until the complete separation of the metaphase chromosomes, whereupon the chromatids spring apart and move toward the poles as V's. Obviously this is an equatorial division.

2. *The V-shaped tetrad.* As has already been stated the V-shaped chromosomes of the metaphase represents merely the rod type bent at the point of fiber attachment, so that the apex is directed toward the spindle axis. Such forms are rarely reproduced in the figures of investigators for the reason that polar views, from which point only they are visible, are not given. Attention has already been directed to this neglect. *Mecostethus* is the most striking instance of the occurrence of such chromosomes, but, as may be noted in plates accompanying this paper, they are common in *Hippiscus*, *Tropidolophus*, *Hesperotettix* and *Mestobregma*. Robertson shows in a series of complexes the forms of the individual chromosomes in the first spermatocyte metaphase, mainly in polar view, and here it is seen that the ones numbered 3, 4, 6, 7, 8 and 10 may appear as V's. If not in this form they are rings viewed laterally. V-shaped chromosomes may only with some difficulty be distinguished from rods or rings. *En face* they are again similar to these forms, and all of them resemble the crosses. These facts are very suggestive of the fundamental unity of form underlying all the diverse shapes present in the first spermatocyte. In actual division the V's are not to be distinguished from the rods.

3. *The cross-shaped tetrad.* While V's are a feature of the polar aspect of the metaphase figure, crosses appear only in lateral views, and then only when the chromosomes are regarded *en face*. While simple crosses occur, other chromosomes may present

this aspect when studied from the same position. Rings, rods and V's all look alike when viewed from the spindle axis in the equatorial plate. This is not a mere superficial resemblance but represents fundamental community of structure. In every case the cross is the result of the approximation of four chromatids in such a way that each chromatid is bent upon itself at right angles and so set that this angle is one of four grouped about a center. This center appears as a diamond-shaped opening, in favorable cases, but in the metaphase is usually apparently obliterated. The arms of the cross to which the fibers attach are approximately straight but the remaining two may be bent in any degree, even to the formation of rings.

Because the cross comes into view from the lateral aspect of the mitotic figure, it is common in the illustrations of many papers. De Sinéty ('01) shows such chromosomes in figures 87, 88, 110, 124, 131 and 137; Baumgartner ('04) in figures 13, 14, 16, 18 and 19; Moore and Arnold ('06) text figure; Pinney ('08) in figure 24; Buchner ('09) in figures 49, 51, 52 and 55; Granata in his text figures *d* and *e*; Nowlin ('08); ('13), figures 15, 18 of plate 29, figures 3 and 4 of plate 30, figures 25, 26 of plate 31, figures 19 of plate 32; Carothers ('13) in figures 29, 30, 31 and 47.

By most investigators these metaphase tetrads are represented objectively as they present themselves in stained preparations. Their interpretation depends upon a knowledge of their structure gained from the prophase, and by most students of the Orthoptera they are conceived to be the four armed figures with a cleft the length of each arm, apparent in the prophase but obscured by the concentration of the metaphase chromosomes. De Sinéty ('01), however, in his figure 124 a, represents a cross of two superimposed, independent chromosomes lying over each other at right angles. Similarly, Brunelli ('11) in figures 12 and 13 shows crosses thus constituted. Both of these authors unconsciously represent the cross correctly, de Sinéty in his figures 124 c, 131 and 137, Brunelli in his figure 14. The practical unanimity of opinion on this very clear question makes any further discussion of it unnecessary.

The movements of the chromatids, the relation of the spindle fibers to the chromosomes and the form of the anaphase chromosomes is similar to those of the rods and V's, so need not be considered here. The important matter in connection with the crosses is to understand that they represent a mid-stage in the movement of the chromatids, either in prophase or in metaphase.

4. *Ring-shaped tetrads.* If confusion exist in reports of the prophase condition of the rings, it is certainly multiplied when attempts are made to describe the metaphase forms. Here are added a number of factors which complicate the problem, but which yet give meaning to the chromosomes and to their movements. We approach an analysis of the metaphase conditions fortified by a reasonably strong consensus of opinion regarding (1) the relations of the chromosomes of this period to those of the spermatogonium, and (2) concerning the inner structure of the tetrads. Also (3) these are traced into the metaphase, where we have to note (4) their position in the mitotic figure, (5) the movements of the parts, (6) their relation to the archoplasmic fibers, (7) their character after separation. These will first be discussed for the *Hippiscus* type of rings and then for the *Stenobothrus* type.

(1) It is now universally admitted that the chromosomes of the first spermatocyte are made up of four chromatids, and a large number of cytologists believe that two of these are of maternal and two of paternal origin from the homologous pair of spermatogonial chromosomes. In the rings (2) they are so disposed that the maternal pair constitute one-half the periphery of the ring, the paternal pair the other, with the direction of the longitudinal cleavage passing through the arms of the ring parallel to the plane in which they lie, so as to produce two superimposed rings, alike in their derivation. (3) The metaphase sees these same forms placed in the mitotic figure where (4) they lie as rings extended in the equatorial plate, so that they are visible from the pole of the spindle. In most cases they lie perfectly flat in this plane so that the entire ring is sharply in focus at one time, with the exception of the synaptic extension, first seen in the prophase, which now lies at a point nearest the axis of the spindle and

shows as a darker spot at both high and low focus. Usually these two extensions fall within the width of the ring's wall and both are in the same vertical axis. Sometimes the annular body is more pointed at this inner side and the extensions may fall beyond the outer limits of the ring proper. Under such circumstances the enclosed space becomes drawn out in this direction also and passes somewhat into the extended wall of the ring (figs. 21 to 30, 129 to 133). Should the diameter of the curved rods be large in proportion to their length, the enclosed space is small, or may even disappear, and a cleft shows in the outer margin where non-synaptic ends are in contact (figs. 2, 3, 11). Viewed laterally or *en face* the annular chromosomes are with difficulty distinguishable from the V's and crosses (figs. 8, 9, 10, 25, 111), but oblique views are very clear and convincing (figs. 83, 133). (5) In division, during the spermatogonial mitoses, chromatids lying superimposed, are separated from each other, beginning at the inner end, where the fiber attaches, and moving in the same vertical plane to opposite poles of the spindle. *Mecostethus* shows exactly the same conditions in the first spermatocyte except for the fusion in pairs of homologous chromosomes. Ring-shaped chromosomes of the *Hippiscus* type maintain the same spatial relations in the cell and undergo similar movements of the chromatids.

(6) Of much importance is the fiber attachment for determining fixed points on the chromosomes. As has been shown, particularly in *Stenobothrus*, the point at which the fiber attaches remains the same from one generation of cells to the other. Where all the chromosomes are straight rods in the spermatogonium this point is always at the inner ends, and, in the first spermatocyte, the chromosomes occupy a similar position, as in *Mecostethus*, with the single exception that they are now joined in pairs. Where rings appear these relations are again preserved, only fusion has occurred at both ends of the univalent chromosomes. Terminal fiber attachment and rings of the *Hippiscus* type always occur together. (7) On separation all the anaphase chromosomes are simple V's and the former elements of the rings are no longer distinguishable from the other derivatives (figs. 5, 6, 7, 35).

Similar views with regard to the rings are held by Sutton, Pinney, Stevens, Robertson, Granata, Nowlin and Carothers. In the accounts and figures of these investigators is found substantial confirmation on each of the points of observation that I have just discussed. It is important to note here that among these are careful detailed studies of the rings in the prophase and representations of them not only in lateral view but also from above and *en face*. Such aspects of the rings are conspicuously lacking in the figures of those who favor the other explanation of ring formation and division. The numerous tabulated drawings of chromosome complexes in polar view by Robertson demonstrate the almost uniform occurrence of the rings in the equatorial plate—a circumstance that is not mentioned by the advocates of prereduction.

The second form of explanation of the behavior of the rings in the first spermatocyte was advanced by de Sinéty and was later endorsed by Montgomery, Davis, Buchner and Brunelli. It contradicts the one just discussed in these points: (a) The rings lie in the axis of the spindle and are visible on the margin of the complex in lateral view (de Sinéty, fig. 131; Buchner, fig. 55; Montgomery, fig. 23; Davis, figs. 63 and 64); (b) fiber attachment is opposite the synaptic ends (de Sinéty, figs. 123, 131; Buchner, fig. 55; Montgomery, fig. 33; Davis, fig. 63); (c) the synaptic ends are directed away from the spindle axis; (same figs. cited under *b*); (d) the plane of chromosome separation lies perpendicular to the equatorial plate (Sinéty, fig. 312; Montgomery, fig. 33); (e) whole univalent chromosomes separate from each other; (f) they pass across the equatorial plate and go to the pole on the opposite side (Sinéty, fig. 131; Buchner, fig. 55; Montgomery, figs. 32 and 33; Brunelli, figs. 14 and 15); (g) the diverging ends of the chromosomes to which the fibers attach do not lie superimposed but are displaced laterally (no views *en face* are given by any advocates of this method) and there would be no resemblance to the crosses or V's regarded from the same point of view. The two interpretations may fairly be contrasted by saying that, according to the first, uniformity of relations throughout the cell generations, and between elements of the complex, is preserved;

while according to the second diversity must rule under these circumstances. In one instance position of fiber attachment, relation to the spindle, position of daughter chromatids, and method of division remain the same; in the other, these are subject to variation in greater or less degree. It will not be necessary to consider at length the disagreements between the views, for, in presenting my interpretation, I have already gone into these matters. There are, however, some specific objections which I should like to urge against the evidence presented on the various points which I have distinguished as characteristically different in the second account, and these I will take up in order.

a. Position of ring in the first spermatocyte metaphase. Sinéty represented this (fig. 131) as being so placed that the central opening becomes visible in lateral view. In this he was followed by Davis and Buchner, while Montgomery and Brunelli fail to show exactly such appearances, but represent extended chromosomes with their inner ends being pulled past each other. Davis illustrated his interpretation by rope models very clearly. To one familiar with the conditions it is apparent that what de Sinéty and Davis saw were oblique views of rings near the end of their division, while Montgomery and Brunelli were confronted by lateral, edgewise views of rings which they failed to interpret as such, definitely, because they had not sufficiently studied polar views. I believe this criticism is justified for the polar aspect of the chromosomal complex is so striking and so illuminating that if it had been appreciated it would have been figured. (Brunelli shows in fig. 13 a complex before the full establishment of the equatorial plate.) Few or no complete complexes are represented by these authors—a striking contrast to the large series pictured by Robertson. To some extent also this criticism lodges against Davis and Buchner. The former represents no polar views in his carefully prepared plates and only two in outline text figures while Buchner shows but one, in which no attempt is made to indicate the structure of the chromosomes. A mere inspection of the numerous drawings and photographs given in this paper will be sufficient to show

that the rings really lie in the plane of the equatorial plate and not parallel to the spindle axis.

b. Fiber attachment. This is always nearest the axis of the spindle, therefore if it be at the free ends of synaptic mates the point of union must be directed away from the spindle. This means either that the fiber attachment has shifted to the opposite end from that in the spermatogonium, or that synapsis has been at the distal ends. The evidence seems to point strongly to fixity of fiber attachment, and if whole spermatogonial chromosomes were separated in the first spermatocyte, synapsis would have to occur, or at least persist, longest at the distal ends. Either assumption calls for differences between the rings and the other forms of tetrads instead of conformity with them.

c. Position of synaptic ends. If fiber attachment is at the free ends of synaptic pairs then of necessity the fused ends must be directed away from the spindle. This is just the opposite of the conditions which certainly prevail in the case of *Mecostethus* and all other forms with V-shaped tetrads. Not only the prophase structure of the rings but all other considerations of order in the behavior of the chromosomes speak against this view. It should be noted that neither Davis nor Buchner, who figure the rings with divergent cross-shaped synaptic ends in the prophase, show this condition at the outer extremity of the rings in the metaphase. Here again is diversity conceived in place of uniformity.

d. Relation of division plane to equatorial plate. In the division of chromosomes, planes of cleavage lie within that of the equatorial plate, at least at the inner end where are attached the archoplasmic fibers. By the interpretation of de Sinéty and his followers this is not true, for the chromosomes are pulled past each other in the plane of the spindle axis. This is not, in fact, a division of the chromosomes in the proper sense of the term, but is a mere disjunction, but one step removed from the exploded 'primary type' of Goldschmidt. It is to be noted here that in the case of *Stenobothrus*, where a segregation division occurs, the rings show extensive movements of the chromatids during the metaphase. Divergence from normal behavior is again involved in this explanation.

e. Movements of chromatids. Whole univalent chromosomes are separated bodily by this form of division, according to the explanation of Montgomery, Davis and Buchner, but because of his conception of a double longitudinal division, de Sinéty sees in it an equatorial division. The evidence against this has already been given and will not be repeated here.

f. Relation of chromosomes to spindle poles. According to de Sinéty and those who follow him, there is described a most unique movement of the chromosomes involved in the ring. Instead of passing to the spindle pole toward which they lie, they cross over to the opposite side. All explanations of cell division, which involve an element of organization finding expression in the bipolar condition of the cell parts at this time, are controverted by such a process. I feel convinced that there is no such condition of the chromosomes and I do not believe that the reported twisting of the chromosomes in the metaphase described for many forms, particularly plants, is correct. As a pure matter of observation I am sure that it does not occur in the Orthoptera. Perhaps the appearance of the looped or double ring chromosomes in many species of this order has been responsible for the error. By the interpretation of de Sinéty (figs. 124 and 125), Brunelli (fig. 15), Buchner (fig. 42), Davis (figs. 184 and 185) these are merely twisted rings. Although occasionally such conditions are met in the prophase the forms that later appear in the metaphase are not thus constituted. At this time the second ring, the one further removed from the center of the equatorial plate, will always be found to lie perpendicular to the primary ring and to merge symmetrically into it. If these were really twisted rings they would commonly lie more nearly in the same plane and the outer loop would not enter into the inner symmetrically. When prophase structures, corresponding to those of the metaphase, are studied it is found that the secondary ring is really produced by the further development, at the contrasynaptic ends, of occasional divergences of the chromatids of the same character as the ones found commonly at the synaptic ends; and these may even go so far as to start the production of a third ring in the same plane as the primary one. In other words, the chromatids do

not cross from one side to the other but remain constantly superimposed. These conditions have been clearly worked out by my students and myself on all the forms studied, but have not generally been understood. An exception to this statement is furnished by Granata who in his figure 28 and text figure 1, *o*, (fig. 141) shows accurately the structure of the looped chromosomes. All considerations of uniformity in chromatid structure and movement speak against the transposition of structures to the opposite pole of the spindle. Boveri has fully discussed this matter in relation to *Ascaris* chromosomes.

g. Relation of rings to crosses. Crossed ends of chromosomes when viewed across the equatorial plate, *en face*, would necessarily appear displaced laterally—they would not be superimposed in one plane. Despite the number who have advocated this relation not one has shown a figure of a chromosome in such a position. The omission is significant, for appearances of this kind are not to be found. Such an aspect of the ring is a crucial test of de Sinéty's conception. From this point of view, indeed, the rings are of the same appearance as crosses, or, viewed somewhat obliquely, show clearly the extended ends directly over each other and with the lateral parts of the annulus merging symmetrically into each (figs. 130, 133),

So far as the prophase is concerned, the rings in *Stenobothrus*, *Chorthippus*, *Chloëaltis* and the unidentified species which I have figured—which I shall speak of as the *Stenobothrus* type—do not seem to show any differences from the ones found in *Hippiscus* and other similar forms. In the metaphase, however, it is clear that they bear a different relation to the archoplasmic fibers; and, by comparison with the chromosomes of the spermatogonium and second spermatocyte, it appears that this is a constant feature of the organization of certain ones of the complex. This is made sure by the work of all investigators who have studied *Stenobothrus*, but was emphasized by Davis. A failure to appreciate the difference between the rings of *Stenobothrus* and *Hippiscus* types has been responsible for much of the confusion that exists in the literature regarding Orthopteran spermatogenesis. It is very necessary

that this be removed because the *Stenobothrus* form of chromosome, while rare in the Orthoptera, is very common in other animals and in plants. Had these matters been understood many extended reviews, seeking to reduce all maturation phenomena to a type, upon the basis of chromatid relations in the first spermatocyte metaphase, would not have been written. It is barely possible that a complete understanding of synapsis conditions may result in such a harmony, but with our present knowledge of the subject the metaphase relations are very different and point almost certainly to prereduction in *Stenobothrus* rings and to post reduction in those of *Hippiscus*. The difference between these two types of annular chromosomes relate to the fiber attachment and to the position in the spindle, the latter being a consequence of the former. By some chance there is no difference of opinion on these matters, and all observers agree that in *Stenobothrus* the rings lie extended in the axis of the spindle with fibers attaching somewhere near the middle of each of the half rings that later enter the anaphase. There results accordingly a reduction division.

It is difficult to see how these sharply contrasting conditions could be confused, yet neither de Sinéty nor Davis, both of whom studied *Hippiscus*-like chromosomes, differentiated between the two varieties of rings. That this was due entirely to misinterpretation and not to varied conditions is rendered certain by comparing two adjoining figures of de Sinéty, figure 125 showing the *Stenobothrus* ring extended along the spindle with subterminal fiber attachment, and figure 132 of *Oedipoda*, the only polar view, with the rings lying in the equatorial plate with terminal attachment of fibers. Davis also shows clear lateral views of the spindle of *Stenobothrus* with extended rings (figs. 88, 91) and in text figure N sketches of two cells in polar view with rings showing. In both instances mistakes in determining the correct relation of the *Hippiscus* rings to the archoplasmic fibers resulted in an apparent uniformity.

Stevens ('12) shows rings of this type in *Ceuthophilus*, Jordan ('08) in *Aplopus*.

Summarizing the comparison between the rings of *Hippiscus* and of *Stenobothrus* under the various headings used in the consideration of this structure we find that there are in each case (1) four chromatids derived two from the male parent and two from the female parent, (2) joined so that homologous pairs are united end to end and sister pairs are superimposed. (3) These relations of the prophase are carried over into the metaphase where (4) in *Hippiscus* the rings lie in the equatorial plate with sister chromatids directed toward opposite poles of the spindle, while in *Stenobothrus* although the parts of the tetrads are in the same relation to each other the whole chromosomes lie extended in the direction of the spindle axis with sister chromatids toward the same pole. (5) In *Hippiscus* the sister chromatids glide away from each other and become extended in the line of the archoplasmic fiber beside homologous chromatids, thus progressively reducing the size of the ring to supply the increased length of the extensions; in *Stenobothrus*, on the contrary the whole rings separate directly into half rings or V's. (6) The rings in *Hippiscus* have the fiber attachment at the synaptic ends, which are slightly drawn out, directly above each other, and which consist each of homologous chromatids, while in *Stenobothrus* the fiber attachment is approximately median and in each case involves connection with sister chromatids. (7) The dyads of *Hippiscus* in the anaphase are simple V's like all the others of the complex, while the ones in *Stenobothrus* are double V's and unlike the simple V's of the metaphase rods in the same complex. (8) The results of the separation of the rings of *Hippiscus* is an equational division while in *Stenobothrus* it is a reductional one.

e. The chromosomes in the second spermatocyte

There is comparatively little difference of opinion regarding the actual appearance of the chromosomes in this generation, and, so far as interpretation is concerned, it is altogether dependent upon the meaning attached to the first spermatocyte division. While therefore extended discussion is not called for, it will be convenient to keep separate the consideration of the chromosomes of *Hippiscus* and of *Stenobothrus* types.

1. *Hippiscus type*. The striking variation of form appearing among the metaphase chromosomes of the first spermatocyte gives way in the second spermatocyte to as marked a uniformity. The paired, straight rods of the first spermatocyte anaphase reappear in the succeeding metaphase in the same sizes and forms, and between these two stages—through the telophase and prophase—they may often be recognized individually. This visible continuity is a variable matter in different species, but almost always the individual elements may be traced without confusion. Here, as elsewhere, the accessory chromosome comes into prominence during the prophase by reason of its greater density, clearly indicating the cells wherein it lies. To some extent, however, it shows a loosened structure like the euchromosomes and again in this way presents a parallelism with them. The changes between the two generations of spermatocytes take place rapidly, and occasionally cysts are formed in which appear telophases of the first spermatocyte and metaphases of the second spermatocyte. It is evident that no profound changes are accomplished at this time—everything points to a rapid rearrangement of formed chromosomes into a new division figure with the least possible change or movement of the cell elements.

A study of the metaphase figure discloses physical conditions very similar to those of the spermatogonia. The chromosomes are straight rods arranged radially, for the most part, about the spindle axis with fiber attachment at the inner ends (figs. 16 to 19, 31, 49 to 58). The same series of sizes apparent in the earlier generation again presents itself, but now in a single instead of a duplicate grouping. Presence or absence of the accessory chromosome marks the two classes of cells descended from each secondary spermatogonium. One notable difference between the chromosomes of the spermatogonia and of the second spermatocytes manifests itself. During the earlier period the chromosomes were much extended and the chromatids were closely apposed, while in the second spermatocyte the chromosomes are shorter and show a marked divergence of their halves. This tendency of the chromatids to separate may go to the extreme of extension in a straight line if the chromosome happens to lie toward the

center of the equatorial plate (fig. 57). Only at the point of fiber attachment do the chromatids remain in contact. Separation of the chromosome halves already exists at this time, and movement to the spindle poles as simple, straight rods easily follows. The accessory chromosome, being also a dyad, divides like the other chromosomes. Upon these points there is practical agreement by all who have studied the *Acrididae*, so a further discussion is not called for; but whether the two chromatids are sister elements or merely homologues is a mooted question. It entirely depends upon what has happened in the first spermatocyte and the evidence of the earlier generation is much clearer. According to my belief, in most of the chromosomes they are homologous elements and are separated, through a segregation³ division by this mitosis.

2. *Stenobothrus type*. In this, as in the *Hippiscus* type, the sizes of chromosomes in the spermatogonium reappear in the second spermatocyte but, in addition to this, also the forms recognizable in the earlier generation are found. Indeed, aside from the haploid condition of the chromosome group, all the relations of the spermatogonium are preserved. As may be seen from figures 68 to 76, there are, in the *Stenobothrus*-like form I have studied, always six *Hippiscus*-like rods in each second spermatocyte and four J- or V-shaped dyads. When the accessory chromosome is present there is added a fifth chromosome with non-terminal fiber attachment. In *Stenobothrus curtippennis* Davis reports a similar constancy of forms and his conclusions are borne out by the work of Meek and supported by the less detailed studies of de Sinéty and Gerard. It seems established beyond question that organization is so exact that even the form of the chromosomes is

³ In this paper and others I have used the term 'segregation division' synonymously with 'reduction division.' It seems to me a preferable term with our present knowledge of cellular processes, since it indicates the nature of the separation in concordance with our understanding of the composition of the tetrads out of elements derived from the two parents. There is no 'reduction' in Weismann's sense, only a segregation of paternal and maternal derivatives. Of course this involves the conception of such a biparental composition of the tetrads, but practically the same idea is at the bottom of any 'reduction' scheme and so it would not hold more against one than the other.

repeated in every cell of every individual of a species, and, in the case of *Stenobothrus*, in every species of the genus. Further, this question of form finds its explanation in the relation which each chromosome bears to the archoplasm of the cell—it represents a fundamental matter of cellular organization.

Apparently, in interkineses, all this is lost, but the history of the *Stenobothrus* chromosomes shows that this is not so. The forms of the spermatogonial chromosomes reappear in the grand-daughter cells, and in these two derivatives may be found all the chromosomes of the single cell of the earlier generation, each recognizable by its size and form. This is the common experience of different investigators working upon material derived from the most remote sources and from specimens classified into four or more species. Since the actual act of reproduction is seen in each mitosis, the only conclusion that can be drawn is that these chromosomes of certain definite sizes and forms possess these attributes in the second spermatocytes for the reason that they are lineal descendants of the like series in the spermatogonia. It is justifiable to conclude further that the like series in various species of the genus is the result of the same laws operating over a longer period of time. The animals now in existence within this genus are here because they descended from similar organisms in the past, and their common characters are due to their common ancestry: the chromosomes within the cells of these animals bear the stamp of resemblance in series for the same reason. Indeed the processes of reproduction in the greater and in the less are coincident and form a common problem.

It causes no confusion therefore to find interposed between the two cell generations with similar formed chromosomes, an intermediate one in which these configurations are not present, for the index of these variations is furnished by the position of the fiber attachment. In each case it is possible to identify the particular element by this feature of its organization, in connection with its relative size in the complex. We are just beginning to understand something of the exactness of chromosome organization, and the conditions in *Stenobothrus* furnish one of the most generally recognized cases of it. Attention has been directed

to the very obvious conditions in the formed chromosomes of the metaphase, but more extensive knowledge must come from an intimate analysis of structure, which can only be made when the elements exhibit their finer details within the nuclear membrane. What has already been accomplished in this way lends great encouragement to the belief that we may expect to know much more about the structures, relations and functions of the chromosomes than we do at the present time. It may justly be said here that the evidence for persistent individuality afforded by the relations between the second spermatocyte chromosomes and those of the spermatogonia is strengthened and made more exact the further the study is pushed back into the entire history of the chromosomes.

According to many investigators, the result of the second spermatocyte mitosis is to separate daughter chromatids through an equatorial division. So far as the chromosomes with median or subterminal fiber attachment are concerned, I am inclined to agree, although it must be admitted that with parasynapsis the opposite might be the case. Chromosomes with terminal fiber attachment, however, show the same relations and behavior as similar ones do in *Hippiscus*. Figures 64, e, f, show such elements extended in the equatorial plate and they must divide along their length if at all. The evidence in support of this view is stronger in *Stenobothrus* than elsewhere, for the reason that the position of fiber attachment demonstrably remains constant. If this be the case then in such chromosomes as the one shown in figure 64, e, lying in the equatorial plate, the synaptic ends are also the ones to which the fibers attach. Division must separate daughter chromatids from each other, along the longitudinal split of the chromosomes. Beside this chromosome, in the same complex, appears a rod-shaped chromosome extended in the direction of the spindle axis. This I conceive to have already passed through the changes which its neighbor is just beginning. Under any other assumption it must be that synapsis has taken place at the distal ends of the chromosomes or that fiber attachment has shifted after synapsis. The evidence is all in favor of constancy in position of fiber attachment, and actual movement

of chromatids is shown in cross-shaped chromosomes such as are found in many species including the *Stenobothrus*-like form I have studied (fig. 64, f).

The result of the second spermatocyte mitosis is therefore neither entirely equational or segregative, for certain ones of the chromosome complex are separated in one way, others in the opposite way.

SPECIES OF ORTHOPTERA WHOSE SPERMATOGENESIS HAS BEEN DESCRIBED

1. *Acrididae*

- Artom, C. ('09) *Stauronotus*
 Brunelli, G. ('10) and ('11) *Tryxalis nasuta*
 Buchner, P. ('09) *Hippiscus* sp.
 Bütchli, O. ('71) *Aeridia*
 Carothers, E. ('13) *Brachystola magna*, *Arphia simplex*, *Trimerotropis suffusa*, *T. schaefferi*, *T. agrestus*, *T. maritima*
 Carnoy, J. B. ('85) *Oedipoda coerulea*, *Aeridium lineola*, *Stenobothrus viridulus* or *bicolor*
 Davis, H. S. ('08) *Arphia tenebrosa*
 Chortophaga viridifasciata
 Dissosteira carolina
 Hippiscus tuberculatus
 Stenobothrus curtippennis
 Gerard, Pol ('09) *Stenobothrus biguttulus*
 Giglio-Tos, S. ('08) *Pamphagus marmoratus*
 Granata, L. ('10) *Pamphagus marmoratus*
 Hartman, Frank ('13) *Schistocerca alutacea*
 McClung, C. E. ('00) *Hippiscus phoenicopterus*
 ('05) *Hesperottetix speciosus*
 Hesperottetix viridis
 Hesperottetix pratensis
 Mermiria bivittata
 Chortophaga viridifasciata
 Meek, C. F. U. ('11) *Stenobothrus viridulus*
 ('12a) *Stenobothrus viridulus*. *S. bicolor*, *S. parallelus*
 ('12b) *Stenobothrus curtippennis*
 Stenobothrus parallelus
 Stenobothrus viridulus
 Stenobothrus bicolor
 Minot, C. S. ('80) *Melanoplus femur rubrum*
 Montgomery, T. H. ('05) *Syrbula acuticornis*
 Nowlin, Nadine ('08) *Melanoplus bivittatus*
 Pinney, Edith ('08) *Phrynotettix magnus*
 Robertson, W. R. B. ('08) *Syrbula admirabilis*

- de Sinéty, R. ('01) *Oedipoda miniata*
Stenobothrus parallelus
- Veselý, J. ('13) *Chrysochraon dispar*
- Sutton, W. S. ('00) ('02) *Brachystola magna*
- Wenrich, D. H. — *Phrynotettix magnus*
- Wilcox, E. V. *Melanoplus femur rubrum*
2. *Locustidae*
- Buchner, P. ('09) *Decticus verrucosus*
- Davis, H. S. ('08) *Steiroxys trilineata*
- Gilson, G. ('84) *Locusta viridissima*
Decticus verrucivorus
- McClung, C. E. ('99) *Xiphidium fasciatum*
 ('02) *Anabrus*
Orchesticus
Scudderia
Microcentrum
- Meek, C. F. U. ('12) *Steiroxys trilineata*
- Minot, C. S. ('86) *Anabrus*
- Otte, H. ('96) *Locusta viridissima*
- Sabatier, A. ('90) *Locusta*
- Schellenberg, A. ('13) *Diestramena marmorata*
- de Sinéty, R. ('01) *Orphanina denticauda* C.
Platyceles grisea Fab.
- Stevens, N. M. ('05) *Stenopelmatus*
 ('12) *Ceuthophilus*
- Veselý, J. ('13) *Locusta viridissima*
- Vejdovský, F. ('12) *Decticus verrucivorus*
Diestramena marmorata
3. *Gryllidae*
- Baumgartner, W. J. ('02) *Gryllus assimilus*
 ('04) *Gryllus domesticus*
- Brunelli, G. ('09) *Gryllus desertus*
- Buchner, P. ('09) *Gryllus campestris*
- Carnoy, J. B. ('85) *Gryllotalpa vulgaris*
- Gutherz, S. ('06) *Gryllus domesticus*
- Meek, C. F. U. ('08) ('12) *Gryllus domesticus*
- Payne, F. ('12) *Gryllotalpa borealis*
- vom Rath, O. ('91) ('92) *Gryllotalpa vulgaris*
- de Sinéty, R. ('01) *Gryllus domesticus*, *Nemobius sylvestris*
- Voinov, D. N. ('04) *Gryllus campestris*
4. *Blattidae*
- Carnoy, J. B. ('85) *Blatta germanica*
Periplaneta orientalis
- Erlanger, R. v. ('97) *Blatta germanica*
- Moore, J. E. S., and Robinson, L. E. ('05) *Periplaneta americana*
- Morse, Max ('09) *Blatta germanica*
Periplaneta americana
Leucophaea maderiae
Stylopyga orientalis

Wassilieff, A. ('07) *Blatta germanica*

5. *Phasmidae*

Carnoy, J. B. ('85) *Bacillus linearis*

Jordan, H. E. ('08) *Aplopus mayeri*

de Sinéty, R. ('01) *Leptynia attenuata*

Menexenus obtusespinosus

Dixippus morosus

6. *Forficulidae*

Carnoy, J. B. ('85) *Forficula auricularia*

Meek, C. F. U. ('12) *Forficula auricularia*

Randolph, H. ('08) *Anisolabis maritima*

St. George, v. la V. ('87) *Forficula auricularia*

Stevens, N. M. ('10) *Forficula auricularia*

de Sinéty, R. ('01) *Forficula auricularia*

Labidura riparia

Zweiger, H. ('06) *Forficula auricularia*

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EXPLANATION OF PLATES

Figures are drawn with a camera lucida at an original magnification of 2860 diameters, which, in reproduction, is reduced to 1800. The photomicrographs were made with a Zeiss apochromatic 2 mm. N. A. 1.40, projection ocular 4 and Watson 'Parachromatic' oil immersion condenser. The original magnifications were 600 and 1000 diameters which are here reduced to 525 and 875 respectively

PLATE 1

EXPLANATION OF FIGURES

1 to 19 Spermatocyte chromosome complexes from *Hippiscus*.

1 to 4 Polar views of first spermatocyte metaphase, with varying number of rings. The accessory chromosome, marked X, appears within the outer circle of chromosomes and is rough in outline.

5 and 6 Daughter groups in first spermatocyte anaphase. Former associated members of tetrads are seen in the same relative positions in the two groups. In figure 6 the accessory chromosome, X, appears, for which there is no mate in the opposite group.

7 Lateral view of an early first spermatocyte anaphase. In figures 5, 6 and 7 the dyad character of the anaphase chromosome appears clearly.

8 to 10 Lateral views of the first spermatocyte metaphase complexes, in which the members have been extended in rows with homologous elements in the same vertical column and with the smaller chromosomes at the left and the larger of the series at the right. The variable shape of the same element at different stages of division is shown.

12, 13, 16 to 19 Polar views of second spermatocyte metaphase chromosome complexes. The accessory chromosome, X, is present in figures 12, 16, 17 and 19. In figure 17 it is shown surrounded by a vesicle.

14 and 15 Somewhat oblique lateral views of first spermatocyte metaphase.

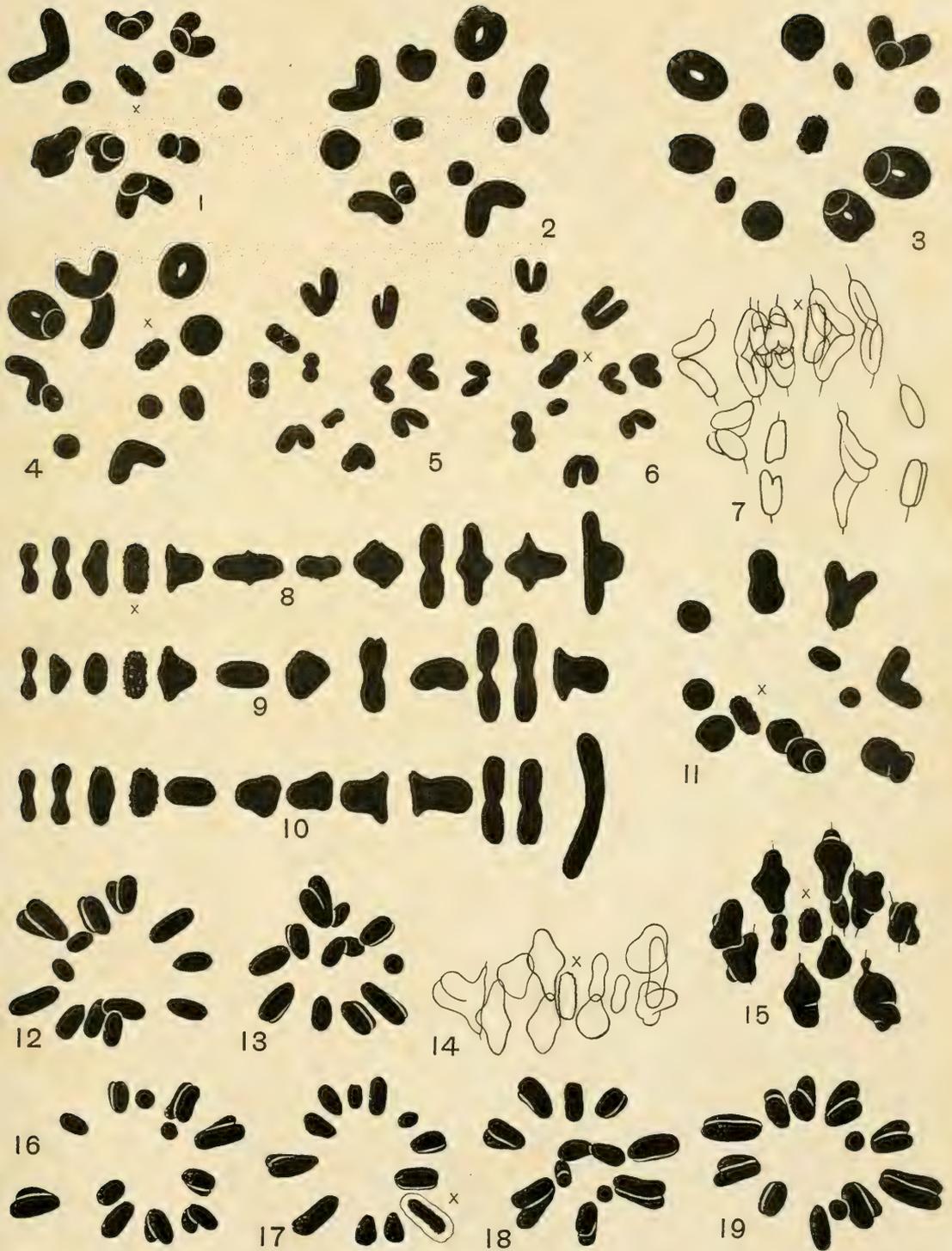


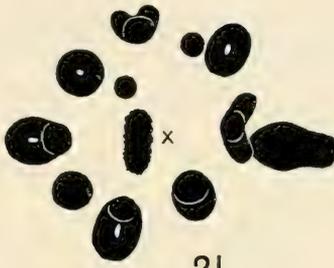
PLATE 2

EXPLANATION OF FIGURES

- 20 to 31 From *Tropidolophus*.
- 20 Polar view of spermatogonial complex.
- 21 to 24 Polar views of metaphase groups in first spermatocyte. The accessory chromosome is centrally placed and is irregular in outline, as in *Hippiscus*.
- 25 Lateral view of first spermatocyte metaphase complex with the members extended in rows. At *a* is a ring, *en face*; at *b* a lateral view of a ring with the contrasynaptic ends also drawn out.
- 26 to 30 Polar views of first spermatocyte metaphase complexes.
- 29 A complex, six members of which are plain rings. A photomicrograph of this group is shown in figure 110 and in figure 140 appears a photograph of wax models of the group arranged as in the cell. Similar rings are seen in the remaining polar views, but in addition there are others, evidently rings, which are not circular in outline but show various enlargements on the other side. These modifications are due either to a simple divergence of the contrasynaptic ends or to a further extension of these so as to complete a secondary ring at right angles to the first.
- 31 Polar view of second spermatocyte metaphase with a complex of 12 chromosomes. The uniformity in shape of the chromosomes in this cell generation is well shown.



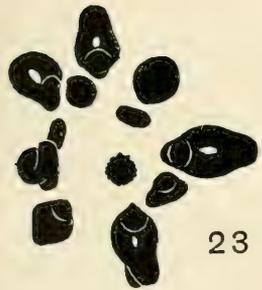
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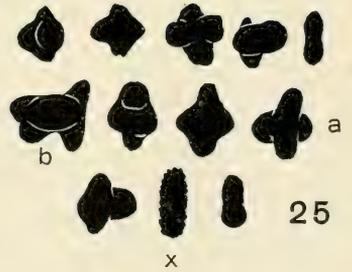
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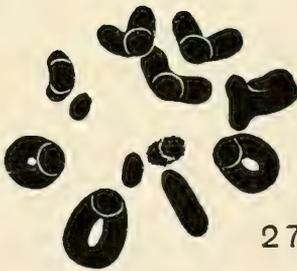
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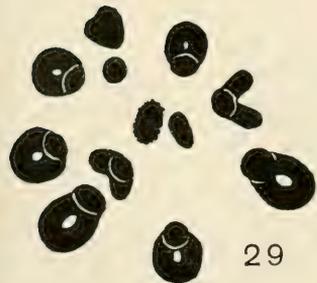
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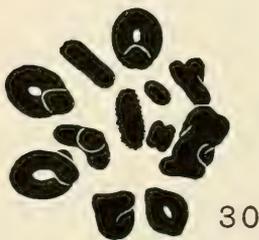
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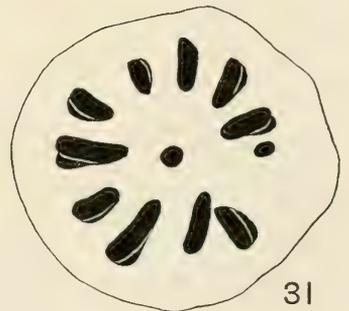
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PLATE 3

EXPLANATION OF FIGURES

32 to 58 From *Mecostethus lineatus*.

32 to 34 Polar views of first spermatocyte metaphase complexes. All the chromosomes are in the form of rods, straight or, more commonly, bent at the synaptic ends into V-shaped figures. The entire absence of the common rings is noteworthy in this species (see fig. 109 for photomicrograph of the cell drawn in fig. 34).

35 Beginning of the first spermatocyte anaphase. The chromosomes here are all of the common V-shape, the same as in complexes with rings (cf. figs. 5-7). The only unusual behavior is the divergence of the limbs of the V's before separation of daughter chromatids in the equatorial plate.

36 Oblique view of three euchromosomes and the accessory chromosome in the first spermatocyte metaphase.

37 Polar view of the first spermatocyte metaphase.

38 Various metaphase chromosomes of first spermatocyte (except the lowest chromosome which is late prophase). The character of the chromosomes at this period is shown in the different views.

39 Four first spermatocyte metaphase euchromosomes. The chromatids of the two small euchromosomes remain in contact until the end of the metaphase as in most other species.

40 Oblique view of three euchromosomes in early anaphase.

41 to 43 Polar views of first spermatocyte metaphase. Twelve is the haploid number, but because of the early poleward movement of the accessory chromosome it does not often appear in the plate. One other chromosome is missing from figure 37.

44 Polar view of spermatogonial complex in metaphase.

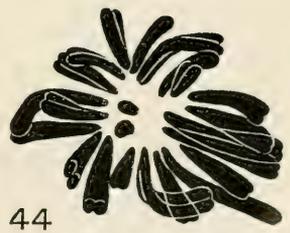
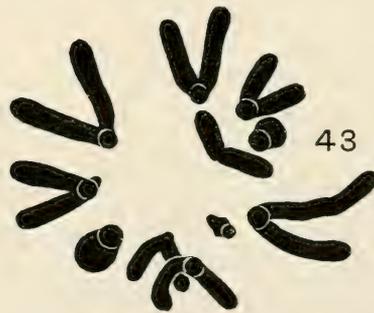
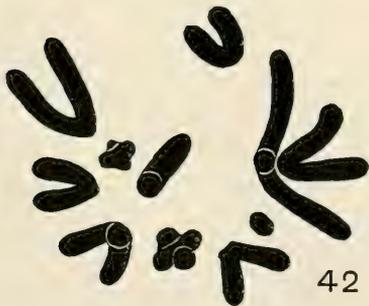
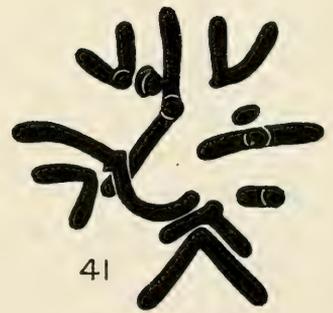
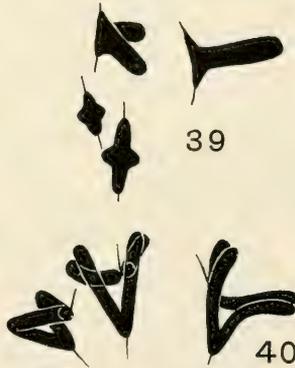
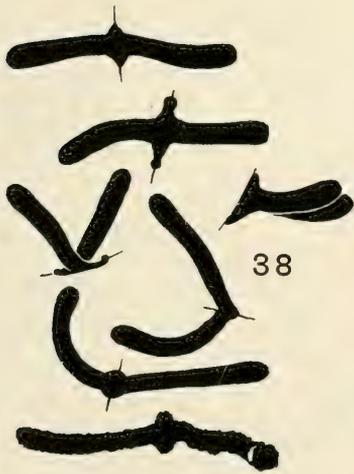
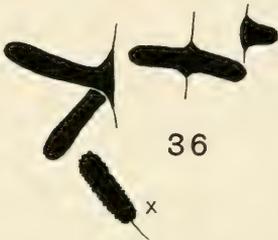
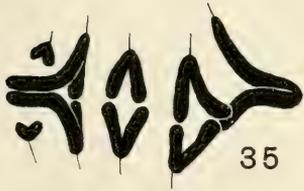
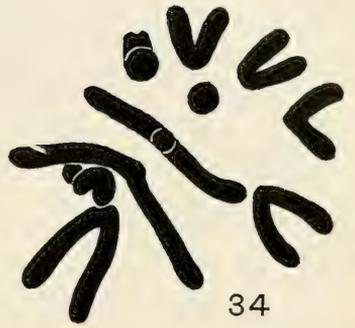
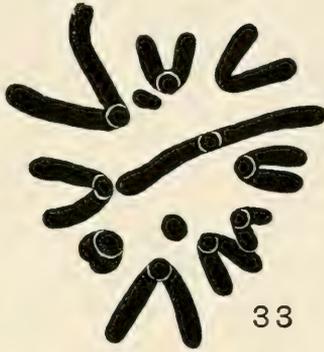
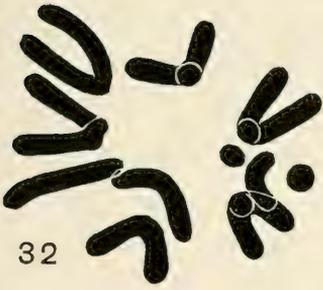


PLATE 4

EXPLANATION OF FIGURES

45 Lateral view of four euchromosomes and the accessory chromosome in first spermatocyte metaphase.

46 Polar view of first spermatocyte metaphase.

47 to 58 Second spermatocyte metaphase complexes.

47 Polar view with the accessory chromosome at the center of the group. The series of sizes is well shown, but it is worthy of note that all the members are only approximately two-thirds the size they were in the first spermatocyte.

48 Lateral view showing superposition of the chromatids and their wide divergence.

49 and 50 Polar views.

51 and 52 Lateral views.

53 to 58 Polar views; in figures 47, 50, 53, and 58 twelve chromosomes appear, in the others the accessory chromosome is lacking.

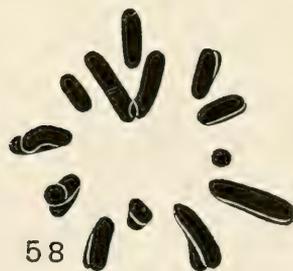
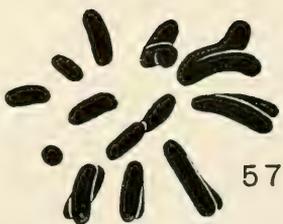
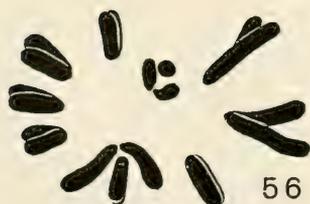
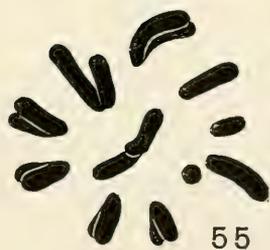
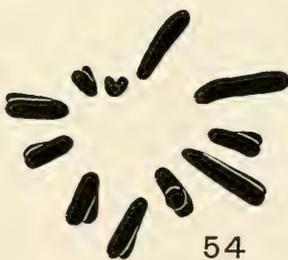
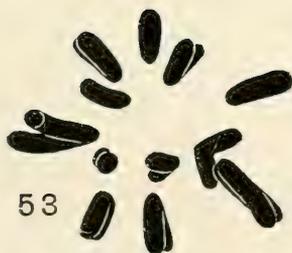
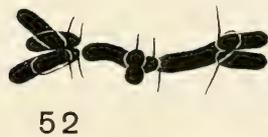
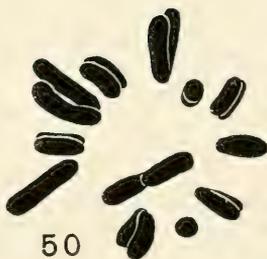
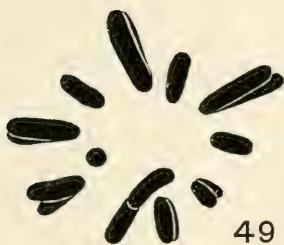
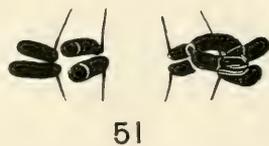
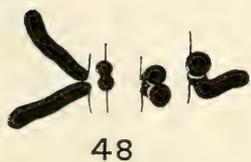
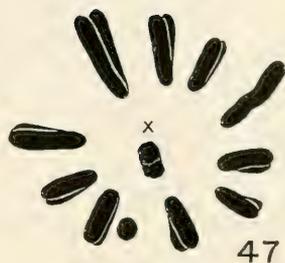
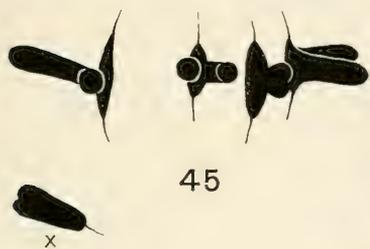


PLATE 5

EXPLANATION OF FIGURES

59 to 76 From the unidentified *Stenobothrus*-like form described in the text.

59 to 62 Polar views of spermatogonial metaphase. Of the twenty-one chromosomes (fig. 60 shows but twenty) twelve are the common rod-like forms with terminal attachment and the remaining nine have either median or submedian attachments.

63 Lateral view of first spermatocyte metaphase showing four ordinary shaped tetrads, a ring placed in the plane of the spindle axis, and, at the left, another such an element, but with the synaptic ends drawn out and bent to form a ring in the plane of the equatorial plate.

64 Various first spermatocyte chromosomes in metaphase or early anaphase. Tetrads formed from spermatogonial chromosomes with non-terminal fiber attachment shown at *a, b, c, d, g,* and *h*. At *e* and *f* are others produced by union of spermatogonial chromosomes with terminal fiber attachment.

65 Lateral view of the first spermatocyte metaphase showing four chromosomes of ordinary type and two with non-terminal fiber attachment.

66 Lateral view showing conditions as in figure 65 and the accessory chromosome which has a median attachment instead of terminal as in most species.

67 Polar view of first spermatocyte anaphase.

68 to 76 Polar views of second spermatocyte metaphase. The same series of sizes and forms apparent in the spermatogonium may now be seen in the granddaughter cells.

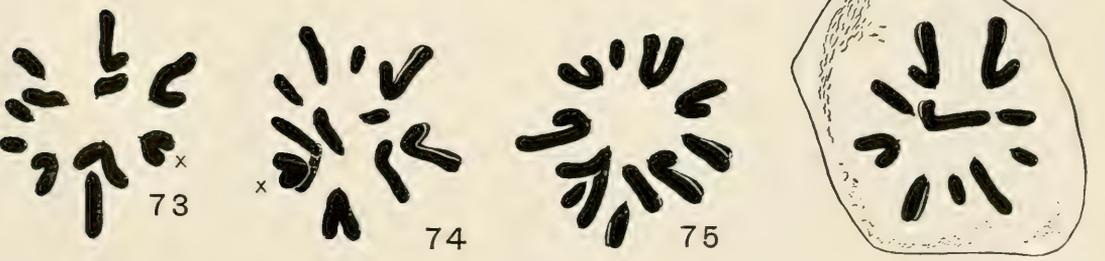
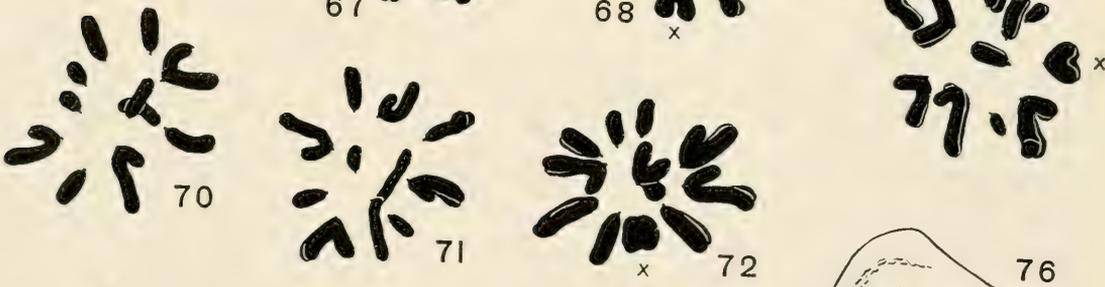
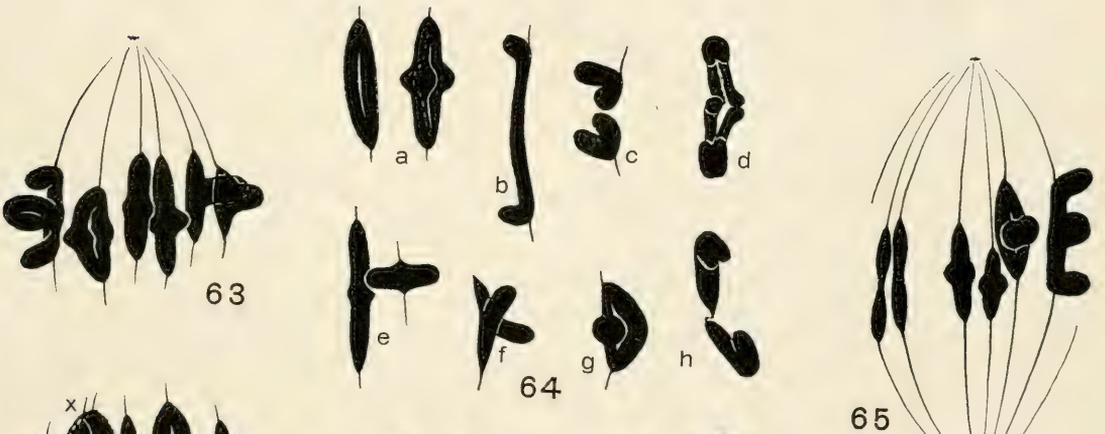
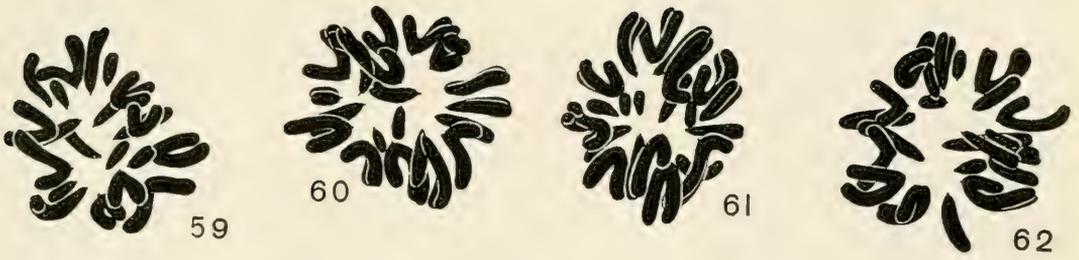


PLATE 6

EXPLANATION OF FIGURES

77 Polar view of spermatogonial metaphase of *Hesperotettix speciosus*. There are twenty-one rod-shaped chromosomes and a V with unequal arms. The subsequent history shows this to be a multiple chromosome consisting of the accessory chromosome and one euchromosome. While of the same shape as the chromosomes of *Stenobothrus* which have subterminal attachment it is of different composition.

78 Lateral view of a first spermatocyte metaphase complex of *Hesperotettix speciosus*, the multiple chromosome at the right with the accessory chromosome portion marked X.

79 Two multiple chromosomes from *Hesperotettix speciosus*.

80 Two multiple chromosomes from *Hesperotettix viridis*.

81 Two multiple chromosomes from *Hesperotettix pratensis*.

82 A multiple chromosome of *Mermiria* in prophase, the tetrad portion granular, the accessory chromosome homogeneous.

83 An oblique view of a first spermatocyte metaphase complex of *Hesperotettix pratensis* showing the eleven separate chromosomes with the accessory chromosome attached to one of the tetrads.

84 Multiple chromosomes in prophase, at *a* and *b* of *Hesperotettix speciosus*, at *c* of *Mermiria*. The accessory chromosome, X, is homogeneous and does not show its longitudinal cleft, while the tetrads are granular and cleft.

85 Polar view of second spermatocyte metaphase of *Boopedon nubilum*, including accessory chromosome.

86 Polar view of first spermatocyte metaphase of *Boopedon*, complete complex.

87 Lateral view of first spermatocyte metaphase spindle of *Hesperotettix pratensis*, the multiple chromosome in black.

88 to 91 Successive stages in the concentration and division of an unequal tetrad of *Phrynotettix magnus*. The shorter member, at the left in each figure, remains granular until complete separation in the metaphase. It is apparent that this is an equational division of each univalent part of the tetrad (see figs. 125 and 126 for photomicrographs of chromosomes drawn in fig. 88).

92 and 93 Polar views of first spermatocyte metaphase of *Mestobregma plattei*, showing variation in number of rings.

94 to 98 From *Chortophaga viridifasciata*.

94 and 98 Polar views of spermatogonial metaphases; in the former there are four tetrads, in the latter all the elements are separate.

95 Polar view of second spermatocyte metaphase of *Chortophaga* containing the accessory chromosome.

96 and 97 Lateral views of first spermatocyte metaphase complexes arranged in order of the sizes of their members. The variation in the form of the chromosomes with the stage of division shown. Chromosome 12 of figure 97 is a lateral view of a ring in which the contrasynaptic ends are extended like the synaptic.

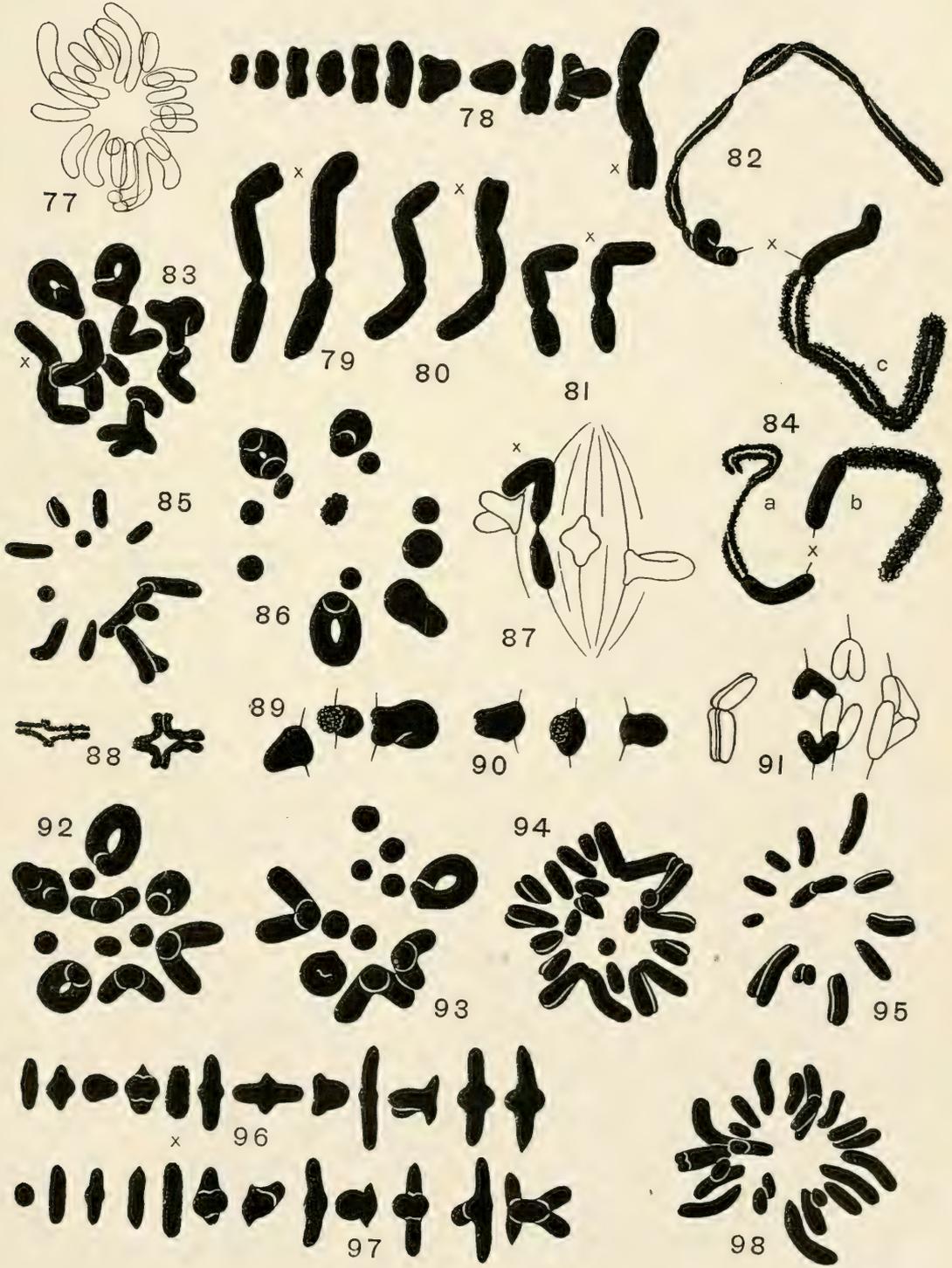


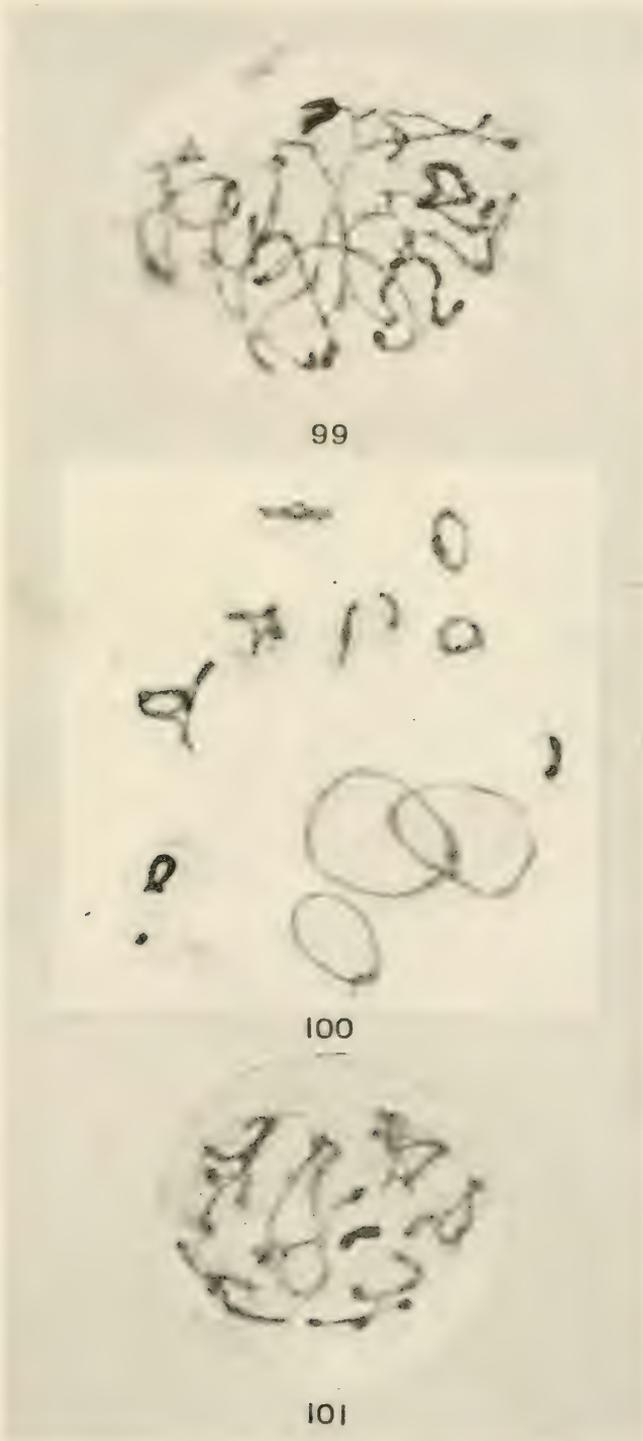
PLATE 7

EXPLANATION OF FIGURES

99 Photograph of a smear of first spermatocyte prophase of *Hippiscus*, showing the early longitudinal division of the tetrads, the early concentration of certain tetrads, and the homogeneous appearance of the accessory chromosome. $\times 875$.

100 Photomicrograph of a smear of a first spermatocyte prophase complex of *Trimerotropis maritima*. One of the twelve chromosomes, near the metaphase chromosomes, is very dim, due to spreading. Of particular interest are the four chromosomes on the left. The upper one is a typical rod; the second, a cross; the third, a ring. In each of these the structure is very clear. Just below the prophase ring is another from a metaphase complex. That the latter represents merely a more condensed condition of the former is very evident. The three lower rings are much extended by smearing, but still show their composition. There is no indication of crossed-over ends—they are true rings. $\times 875$.

101 Photomicrograph of a smear of a *Hippiscus* first spermatocyte prophase. The longitudinal division in the tetrads is very clear. $\times 875$.



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PLATE 8

EXPLANATION OF FIGURES

102 to 104 Successively later stages in the formation of the prophase chromosomes of *Brachystola magna*. The gradual opening out of the rings is here shown. Smear preparations. $\times 875$.

105 Prophase of *Mermiria*, with a ring near the center of the figure. $\times 875$.

106 and 107 Two views of the same first spermatocyte metaphase of *Mecostethus lineatus*. In figure 106 a chromosome elongated in the spindle axis is shown, in figure 107 another chromosome lying in the equatorial plate, with the synaptic ends extended; section. $\times 875$.

108 Late prophase of *Trimerotropis maritima*. The lower chromosome is a double ring showing the divergence of the halves of the primary ring (at the left) to form the secondary ring; smear. $\times 875$.

109 Polar view of first spermatocyte metaphase of *Mecostethus* showing the uniformity of chromosome form; this cell is also drawn in figure 34; section. $\times 875$.

110 View of similar stage in *Tropidolophus*; see also figure 29 for a drawing of this cell; section. $\times 875$.

111 to 113 Metaphase complexes of *Hippiscus* from smears. In this species one ring is always provided with the elongations terminating in knobs, from the synaptic ends, to which the fibers attach. $\times 875$.

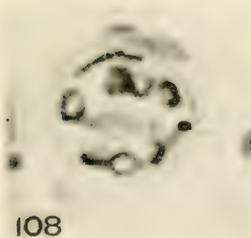
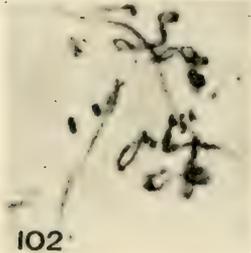


PLATE 9

EXPLANATION OF FIGURES

- 114 to 124 First spermatocyte prophase from *Mermiria*; 114 to 118, $\times 525$, from sections; 119 to 124, $\times 875$, from sections, except figures 122, 123.
- 114 Fragment of ring with the opening at the synaptic point.
- 115 A complete ring with the synaptic ends well drawn out and showing the extension of the ring cleft into these arms.
- 116 Another view of a ring chromosome.
- 117 A rod-shaped chromosome.
- 118 Oblique view of ring.
- 119 Oblique view of ring showing split extension.
- 120 View of a cross, *en face*.
- 121 Lateral view of a ring showing its division into super-imposed rings.
- 122 Rings and double rings; the lower chromosome presents clearly the structure of the double rings and shows that they are not merely looped structures.
- 123 Various rings in late prophase.
- 124 Lateral view of ring.
- 125 to 128 Prophase chromosomes of *Phrynotettix magnus*; sections. $\times 875$.
- 125 and 126 Show the unequal tetrad *U* at successive stages of development (see fig. 88).
- 127 Oblique view of a ring.
- 128 Bent rod.
- 129 to 133 Views of first spermatocyte metaphase complexes of *Mermiria*; sections. $\times 525$.
- 129 The upper cell shows a polar view of a complex with perichromosomal clear spaces, and with the centrally placed synaptic ends of the ring chromosomes dark.
- 130 Upper cell as in figure 129; in the lower cell a lateral view of one ring.
- 131 In the upper cell a rod shaped chromosome, *en face*, with central opening indicated.
- 132 Polar view of first spermatocyte metaphase of *Mermiria* showing clearly the rings and V's lying in the plane of the equatorial plate.
- 133 Oblique view of a ring in the upper cell demonstrating the symmetrical position of the synaptic extension with reference to the two sides of the ring (cf. fig. 127).
- 134 to 138 From the *Stenobothrus*-like form; sections. $\times 875$.
- 134 Polar view of spermatogonial metaphase showing chromosomes with terminal and subterminal fiber attachments.
- 135 Lateral view of first spermatocyte metaphase in which a ring composed of two spermatogonial chromosomes with subterminal attachments is separating into these two elements. The longitudinal cleft is apparent in the disjoined ends.
- 136 Lateral view, as in figure 135, showing other forms of chromosomes.
- 137 Polar view of second spermatocyte metaphase showing chromosomes with terminal and subterminal attachments.
- 138 Upper cell as in figure 137; lower cell, lateral view.

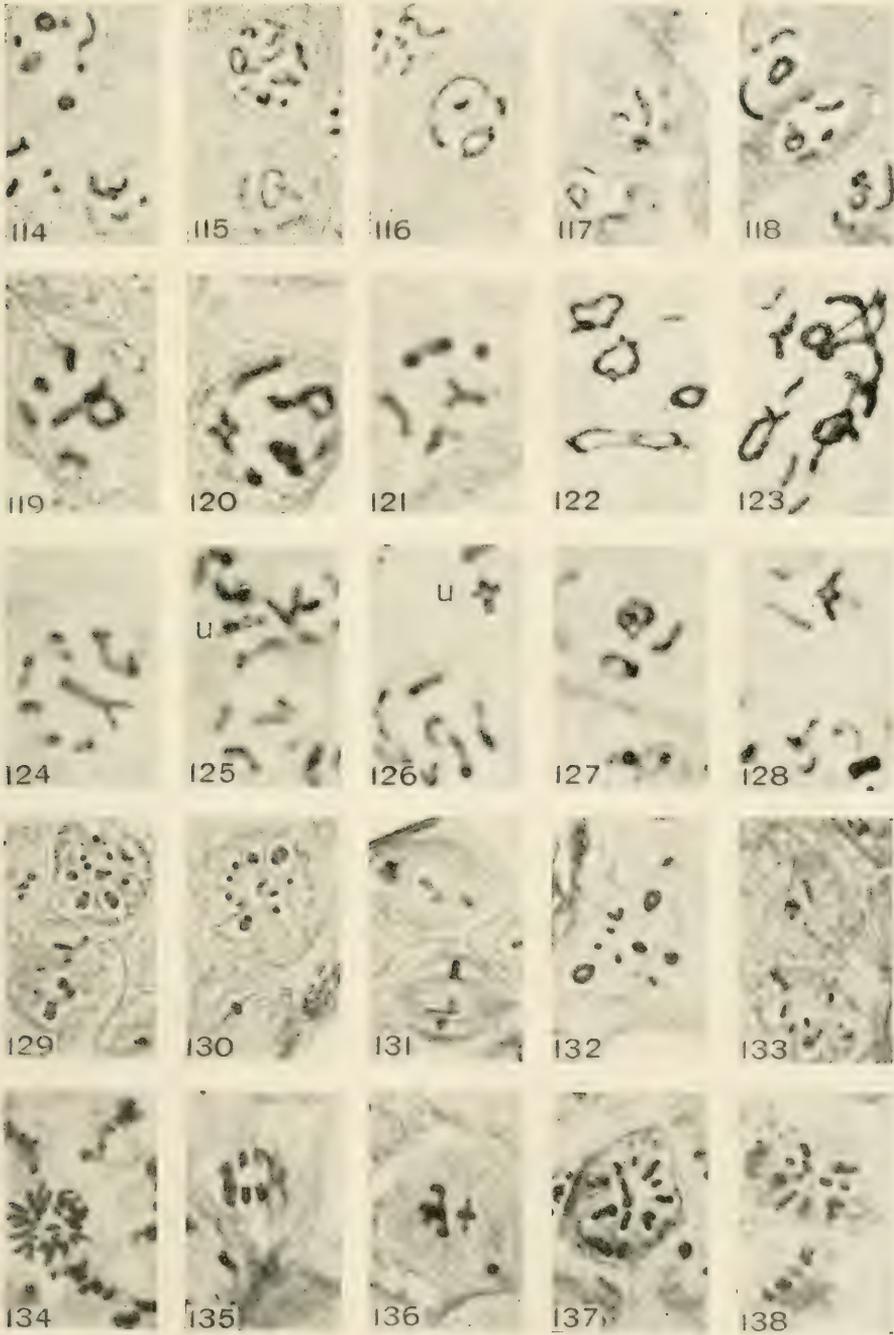


PLATE 10

EXPLANATION OF FIGURES

139 Copy of Davis' text figures *Oa, Ob, Oc*, rope models to explain his conception of the structure and method of division of the ring chromosomes. From these it will be observed that the rings lie in the plane of the spindle axis and that the longitudinal cleft is, therefore, not in the equatorial plate.

140 Photograph of a series of wax models of the chromosome complex of *Tropidolophus* shown in figures 29 and 110. These were prepared after a careful study of the group and they were arranged in the same relative positions in which they appeared in the cell. The central opening of the rings was purposely made larger in order to exhibit their structure more clearly. Compare these with figure 139 in order to see most clearly the difference between the conception of ring formation advocated by de Sinéty, Davis and Montgomery and that held by myself and students for the *Hippiscus* type.

141 Copy of Granata's text figure 1 illustrating his interpretation of tetrad formation in *Pamphagus*. His view of the formed tetrad is essentially similar to my own, even to the compound ring.

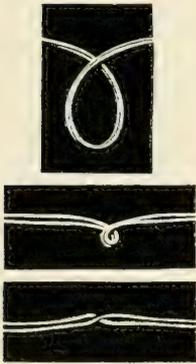
142 Copy of Morse's text figure 1, showing various interpretations. The series in the first row are copies of Sutton's figures and represent my own conception. The second row presents Davis' view, the third is Morse's explanation of the chromosome forms in *Periplaneta*, and the fourth is to illustrate the Schreiner's interpretation of *Tomopterus*. This series of figures represents clearly the various interpretations which I have discussed elsewhere.

143 Photograph of the ring- and cross-shaped wax models shown in figure 140. These demonstrate that such forms are indistinguishable from each other when viewed *en face*, and also that rings constituted as I have described them, when viewed obliquely, appear as loops with crossed ends.

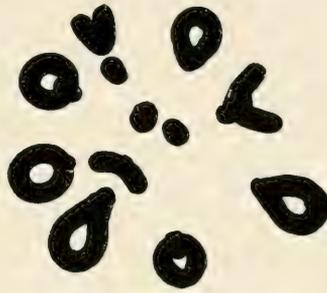
144 Diagrams by Otte to illustrate his conception of a double cross division, with chromosomes of similar appearance in first spermatocyte (E, F) and second spermatocyte (H, J). Compare these with the figures of these generations of cells in various Orthoptera given in this paper.

145 Figures 148 and 149 of de Sinéty to show the method of division in chromosomes with terminal (A), subterminal (B), and median (C) fiber attachment. Figures marked A illustrate the conception of ring division first advocated by this author and later supported by Davis (fig. 139) and Brunelli (figs. 146-147). Those marked A' are to explain chromosomes of the first spermatocyte metaphase in lateral view with diverging ends where the fibers attach and at the opposite side. These are in reality rings with the contrasynaptic ends extended.

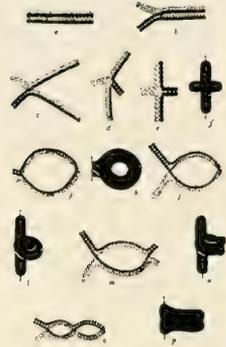
146 and 147 Copies of Brunelli's figures 11, 12, 14, 15, illustrating his ideas of chromosome division in the first spermatocyte. These are essentially like those of de Sinéty.



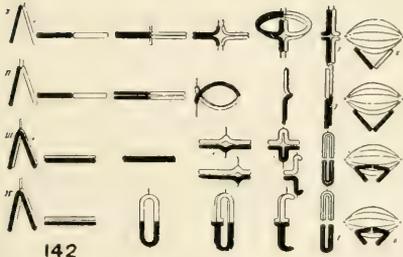
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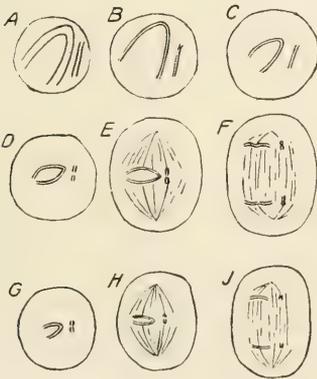
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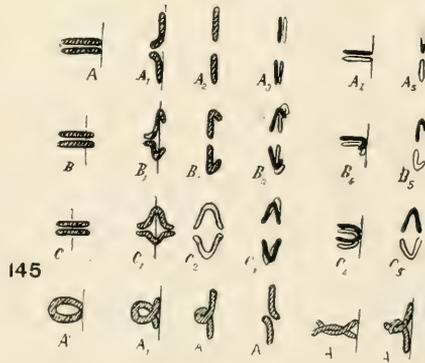
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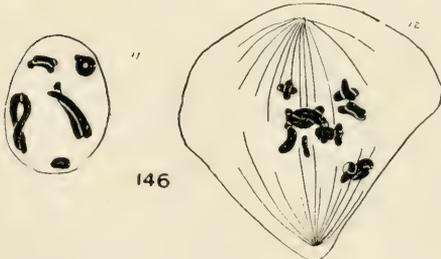
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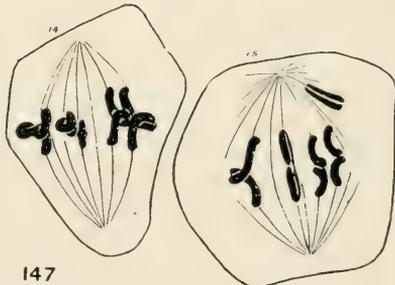
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